THE EFFECTS OF ANTIVIRAL THERAPY ON THE LEVELS OF NEUTRALIZING ANTIBODIES AND ANTIBODIES MEDIATING ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY IN HIV-1 SEROPOSITIVE PATIENTS

by

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The effects of antiviral therapy on the humoral response in HIV+ patients

ABSTRACT

This study consists of evaluating the effects of zidovudine or ribavirin treatment on the humoral response to human immunodeficiency syndrome (HIV-1) in a cohort of 36 HIV-1 seropositive patients. Viral neutralization antibodies were demonstrated against HTLV$_{IIIb}$ virus while titers of circulating antibody-dependent cellular cytotoxicity (ADCC) antibodies were measured against the HIV-1 envelope protein, gp120, using the vaccinia virus expression system which has been successfully used to express foreign viral proteins in target cells. Virologic (viral isolation) and immunologic ($CD4^+$ cells) parameters were also monitored pre and post antiviral therapy.

The results indicate that patients receiving zidovudine for 36 weeks, have a diminished anti-HIV-1-ADCC directing antibody response, while the levels of these antibodies in patients receiving ribavirin or placebo remain constant. The titers of neutralizing antibodies and CD4 counts remain stable regardless of the treatment except for patients receiving ribavirin, where a decline in CD4 cells is observed. The decrease in the HIV-1 specific ADCC during zidovudine treatment parallels with the decrease in the amount of viral burden. This suggests that the two effects are somehow correlated. The impact of a washout period was also assessed. An increase in viral burden during cessation of AZT was reported which may reflect the inability of the treated host to mount a rapid immune response. As a consequence, this may lead to the deterioration of the immune status and the progression of the disease. The implications of these findings will be discussed in this study.

RÉSUMÉ

Cet etude consiste d'évaluer les effets des traitements au zidovudine et au ribavirin sur la reponse humorale au virus de l'immunodeficience humaine (VIH-1) pour un groupe de 36 patients séropositifs au VIH-1. Les anticorps neutralisants viraux ont ete démontré contre la souche HTLV$_{IIIb}$ lorsque les titres d'anticorps d'ADCC ont eté mesure specifiquement contre les proteines de l'enveloppe. Ceci a éte possible grace au systeme d'expression du virus vaccinia. Le profile virologique (l'isolation du virus) et immunologique (cellules positives pour le marqueur CD4) a ete complété pour les periodes d'avant et après la therapie antivirale.

Les résultats indiquent que les patients recevant le zidovudine pendant 36 semaines ont une reponse diminuee d'ADCC dirigee contre VIH-1 tandis que les niveaux des ces mêmes anticorps des patients traités au ribavirin restent constants. Les titres d'anticorps neutralisants et de comptage de CD4 demeurent inchange qu'importe le traitement à l'exception des patients recevant le ribavirin où l'on a observé une diminution de cellule positives pour CD4. La réponse d'ADCC contre VIH-1 pendant le traitement au zidovudine diminue parallèment avec la quantite de virus presente. Ceci suggère que ces deux effets sont en quelque sorte lies. L'impact de la période d'arrêt du traitement a aussi ete examine. La discontinuation du zidovudine entraine une quantite virale accrue, à laquelle peut refléter l'incapacité de l'hôte d'eriger une reponse immunitaire rapide. En conséquent, ce fait peut entrainer la détérioration du système immunitaire et la progression de la maladie. Les implications de ces résultats vont être analysées dans cette étude.

PREFACE

The acquired immunodeficiency syndrome (AIDS) is characterized by severe immunodeficiency, life-threatening opportunistic infections, neoplasia, and a fatal outcome. Strategies for the treatment have focused on the development of drugs aimed at inhibiting the replication of the retrovirus and on agents that may restore immunity. Consideration of anti-retroviral therapy for the treatment of AIDS is based on the assumption that continued retroviral replication is involved in both the pathogenesis and progression of the disease.

Zidovudine or 3'-azido-2'-3'-dideoxy-thymidine (AZT) is the major antiviral drug currently used to treat HIV-infected persons. Other antiviral agents such as dideoxyinosine (ddI) and dideoxycytidine (ddC) have anti-HIV activity and are currently being evaluated. The nucleoside analogues function by inhibiting reverse transcriptase (RT) and viral DNA chain elongation. Clinical trials of AZT have shown that its use is associated with a decrease in the rate of progression to AIDS and possibly an improved survival time. Contrary to these results, important toxic side effects and drug resistant HIV mutants have also been reported.

Although AZT has been shown to be reasonably successful, few data are available concerning the *in vivo* immunologic effects that such treatment may have on the host's immune response to the infecting virus. The specific immunity (humoral or cellular) that is induced in humans in response to infection to HIV, is the normal means of containment and irradication of infectious agents. Other antiviral agents such as acyclovir (ACV) and rimantadine (α-methyl analogue of amantadine), which are used in the treatment of herpes simplex virus (HSV) and influenza A viruses respectively, have been shown to diminish the humoral response to HSV and decrease cellular T lymphocyte responses in mice infected with influenza A virus. Similar concerns regarding the effects of antibiotics prompted

several studies in the 1950s and more recently in the 1990s. For example, patients with group-A streptococcal pharyngitis who were treated with penicillin were shown to develop a diminished antibody response, especially to the M protein. In some reports, no adverse effects due to this treatment were documented, while in others, they discouraged the immediate use of antibiotics in order for the host to mount a substantial antibody response to the bacteria. Unlike these former examples, the epidemiology of HIV infection is different, and the interaction of the host immune response to infection and latency must be considered. Nevertheless, it is important to determine if anti-HIV-1 drugs diminish any of the immune responses to HIV and if so, what are the consequences of such effects (beneficial vs. deleterious).

In the present study, the therapeutic effects of zidovudine, ribavirin and placebo treatment on the induction of virus-neutralizing serum antibodies and antibodies mediating ADCC were examined in 36 seropositive patients. Virologic markers such as viral isolation and immunologic markers such as CD4+ cell counts were also monitored during treatment.

I would like to acknowledge those individuals who have contributed to this thesis. I would like to thank Dr. Tsoukas for providing the serum samples and his technician Debby for providing the CD4 cell counts for each of the individual patients. In addition , I would like to thank Dr. Wainberg and his technicians for providing the viral isolation results on the AZT study.

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CHAPTER ONE: INTRODUCTION

1. ACQUIRED IMMUNODEFICIENCY SYNDROME

1.1. History

The term Acquired Immunodeficiency Syndrome (AIDS) was coined to define clinically the various manifestations of this disease. The epidemiological data gathered from the first cases of AIDS, reported in 1981 and 1982, indicated that AIDS was a viral disease which could be transmitted from one individual to another 1). In 1983, Dr. L. Montagnier of the Pasteur Institute in Paris isolated the virus believed to cause AIDS from the lymph nodes of a patient, calling it LAV for Lymphocyte Associated Virus or Lymphocyte AIDS Virus (2). In 1984, Dr. R. Gallo of the National Institute of Health in Bethesda, Maryland, isolated a virus he called HTLV-III (Human T Lymphotropic Virus Type III) from an AIDS patient (3). It has now been demonstrated that these two viruses, LAV and HTLV-III are infact the same virus.

Following the isolation of HIV-1 in 1983, the isolation of a second serotype of HIV (HIV-2) from West Africa was discovered. In 1985, a simian lentivirus (SIVmac), closely related to HIV-2 was recovered from immunosuppressed macaques (4).

1.2 Epidemiology

The human immunodeficiency virus is transmitted primarily through sexual contact, exposure to blood or blood products and from mother to child during the prenatal period (5). The most direct means of HIV transmission is by the introduction of virus into the blood circulation . It is clear that body fluids other than blood and semen have viable virus. HIV has been isolated from tears, urine, saliva, ear secretions, vaginal or cervical secretions and breast milk . However, transmission of the virus in a free state seems just as likely than transmission by

infected cells but relatively low levels of infectious HIV particles are present in these body fluids (6).

Statistics indicate that 70% of individuals with AIDS in North America and Europe are homosexual men. 16% of cases are drug addicts, who have shared needles and syringes . Two percent of cases are patients who have received contaminated blood or blood derivatives, such as coagulation factors. With respect to the latter, in North America and in Europe, one is no longer exposed to this type of transmission of AIDS since blood from each donor is now systematically tested for the presence of the virus using specific markers for it. The remaining 12% are heterosexual men and women (which represent a growing segment), and new born infants.

1.3 Classification

Retroviruses are defined by their ability to reverse the normal flow of genetic information from genomic DNA to mRNA (7). Retroviruses, like other viruses, cannot replicate without taking over the biosynthetic apparatus of a cell and exploiting it for their own use. The morphology and composition of retroviral virions and the possession of a positive-stranded, positive-sense RNA genome are some of their distinguishing characteristics. Retroviruses have customarily been subdivided into three taxonomic groupings primarily on the basis of the *in vivo* and *in vitro* consequences of infection (8,9). The oncovirus subgroup includes retroviruses that are relatively benign and retroviruses that are able to cause neoplastic disease in the infected host animal. The lentivirus subgroup frequently induce cytopathic effects in infected cells and the disease they cause has a long incubation period resulting in immunologic disorders and neurologic disease . In particular, lentivirus genomes are large and contain several viral genes. Lastly, members of the spumavirus subgroup consist of the "foamy" viruses that induce

persistent infections without any clinical disease but cause vacuolization of cultured cells .

HIV-1 is classified as a lentivirus because of its characteristic genomic organization, slowly (lenti-) developing clinical sequelae and nontransforming biologic properties that are analogous to other lentiviruses such as simian immunodeficiency virus (SIV), visna virus and equine infectious anemia virus (EIAV) which infect different animal species (10,11) .

1.4 Structure and Genome

The mature virion of HIV forms an icosahedral sphere that is roughly 1000 angstrom units across (see figure 1). The particle is covered by a membrane, made up of two layers of lipid (fatty) material, that is derived from the outer membrane of the host cell. Studding the membrane are 72 spikes (glycoproteins). Each glycoprotein has two components: gp41 spans the membrane and gp120 extends beyond it. The core protein, p17, is found outside the viral nucleoid and forms the matrix of the virion, while p25 forms the internal core. The viral RNA is carried in the core, along with several copies of the enzyme reverse transcriptase, which catalyzes the assembly of the viral DNA .

HIV-1 has evolved an extremely economic use of its 9.5 kilobase (kb) of coding sequence. The HIV provirus contains two long terminal repeats (LTRs) flanking sequences coding for viral proteins (12,13) (see figure 2). Retroviral LTRs contain cis-acting elements which play a role in viral integration and transcription . RNA synthesis initiates in the 5' LTR at the junction between U3 and R regions, while the 3' LTR specifies the addition of poly-A tails to viral RNA molecules at the junction between R and U5 regions. Three transcriptive units code for the common retroviral structural proteins. The *gag* (group specific antigenic determinants) region encodes the viral core proteins. The core

FIGURE 1. The structural composition of the human immunodeficiency virus.

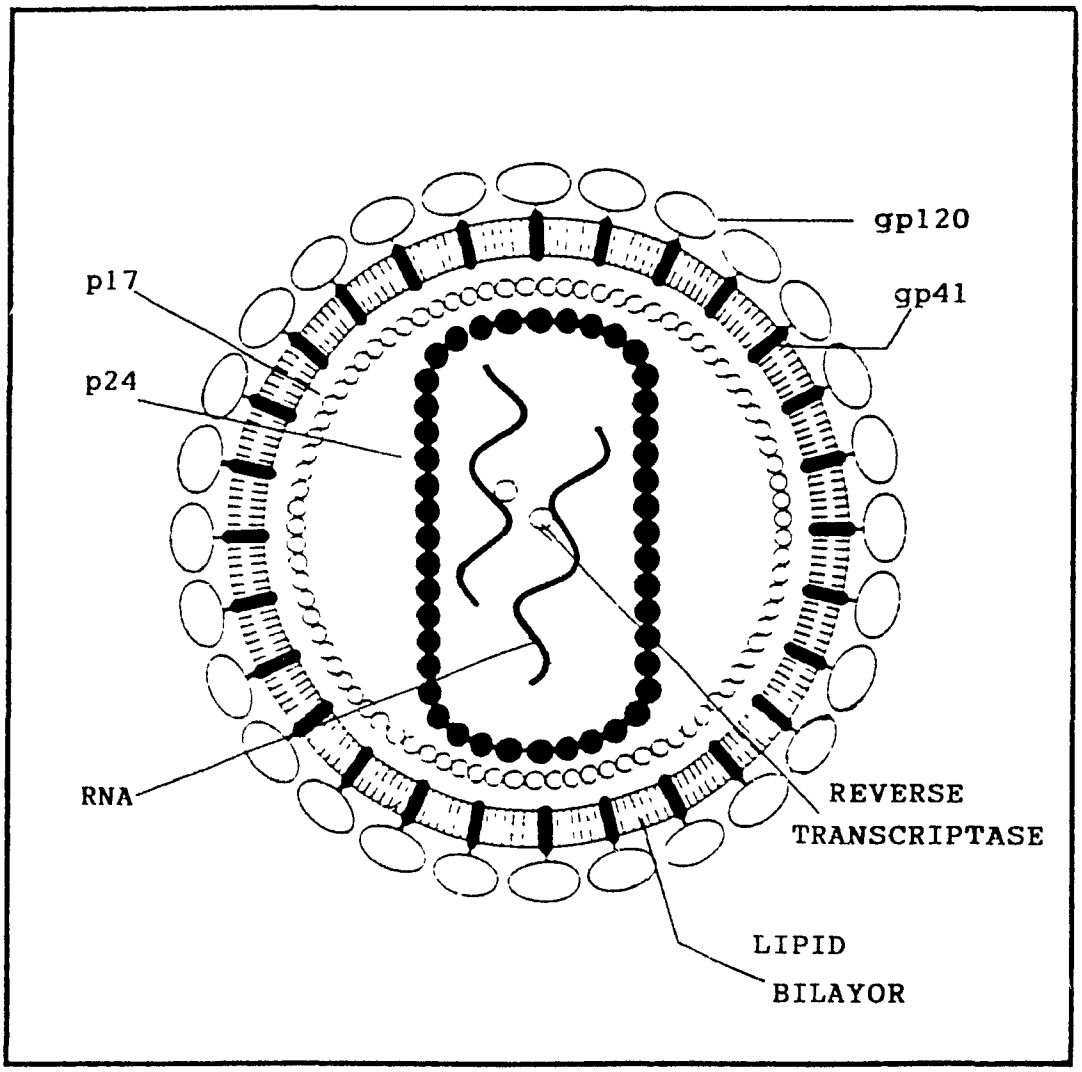
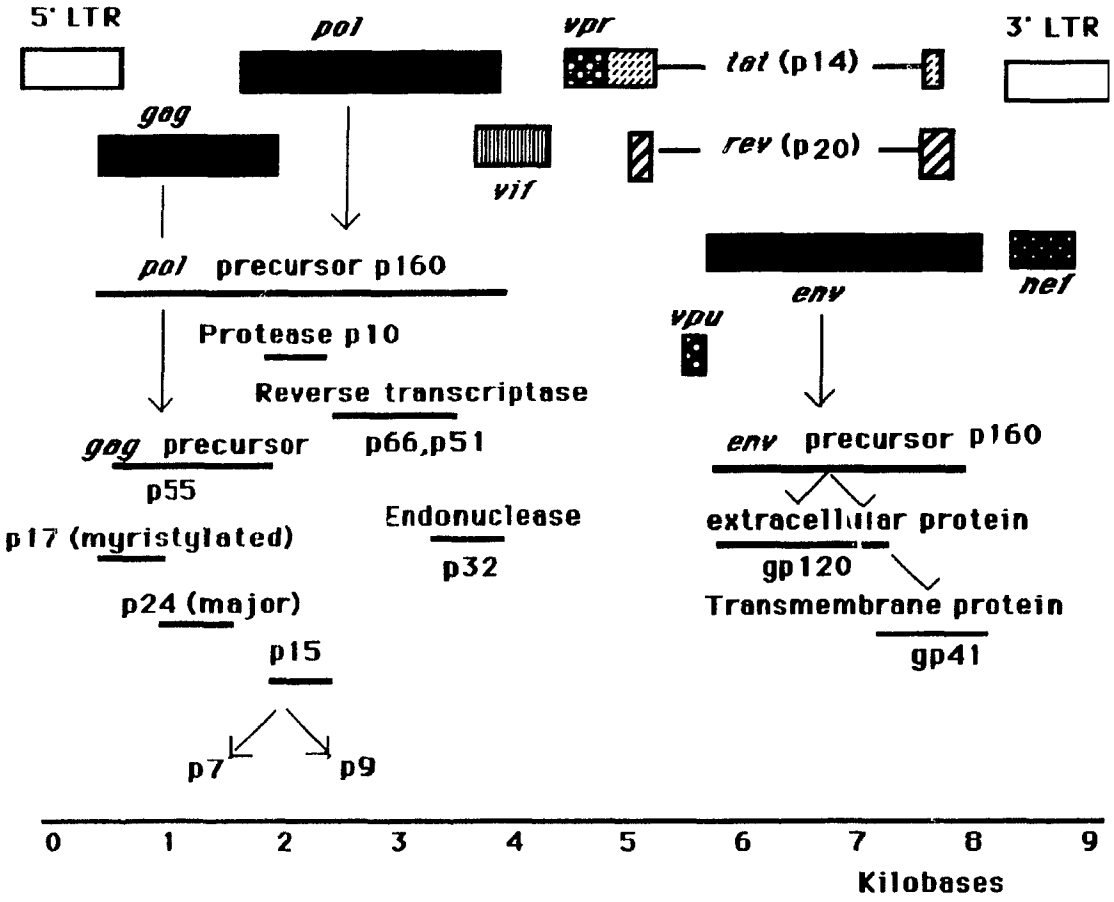

gp120
gp41
p17
p24
RNA
REVERSE
TRANSCRIPTASE
LIPID
BILAYOR

FIGURE 2. Proteins encoded by the HIV genome. Molecular sizes are in kilodaltons. *Gag, pol* and *env* genes encode precursor polyproteins which are cleaved during the maturation of virions as shown. *Tat* and *rev* are translated from spliced mRNAs which join two coding exons.


5' LTR
pol
vpr
tat (p14)
3' LTR
gag
vif
rev (p20)
pol precursor p160
env
nef
vpu
Protease p10
Reverse transcriptase
p66,p51
gag precursor
p55
env precursor p160
p17 (myristylated)
Endonuclease
p32
extracellular protein
gp120
p24 (major)
Transmembrane protein
gp41
p15
p7
p9
0 1 2 3 4 5 6 7 8 9
Kilobases

proteins consist of the nucleoid shell (p24) and several internal proteins (p9 and p7). The *pol* region encodes reverse transcriptase (or RNA dependent DNA polymerase), protease, and integrase. Common to other retroviruses, the polymerase (p66 and p51) enables the transcription of the viral RNA genome into a DNA copy (cDNA) that eventually integrates into the host cell chromosomal DNA via the action of integrase (p32). The protease (p10) then cleaves the polyproteins coded for by the *gag* and *pol* regions into active molecules. The *env* region codes for the two major envelope glycoproteins, gp120 and gp41 (14).

Besides these structural proteins, the HIV genome contains other genes important in the viral life cycle. The function of these genes are not fully understood, however, certain of these gene products have been implicated in the control of HIV-1 gene expression. Their presence reveals the "checks and balances" controlling HIV replication. Two major genes, *tat* (for transactivator of expression) and *rev* (for regulator of expression), affect events that enhance virus replication, whereas the *nef* (for negative factor) region down regulates virus replication . In addition to *tat, rev* and *nef*, there are three other known genes encoded in the HIV-1 genome that are less well characterized. The *vif* (for virion infectivity factor) region appears responsible for maturation of viral proteins at the time the virus bud from the cell . The *vpu* gene (for virion protein) and the *vpr* gene (for virion protein R) appear to function in the role of virion maturation and release (15). Some of these regulatory genes function through an interaction with cellular proteins that bind to the long terminal repeat and thereby influence viral replication

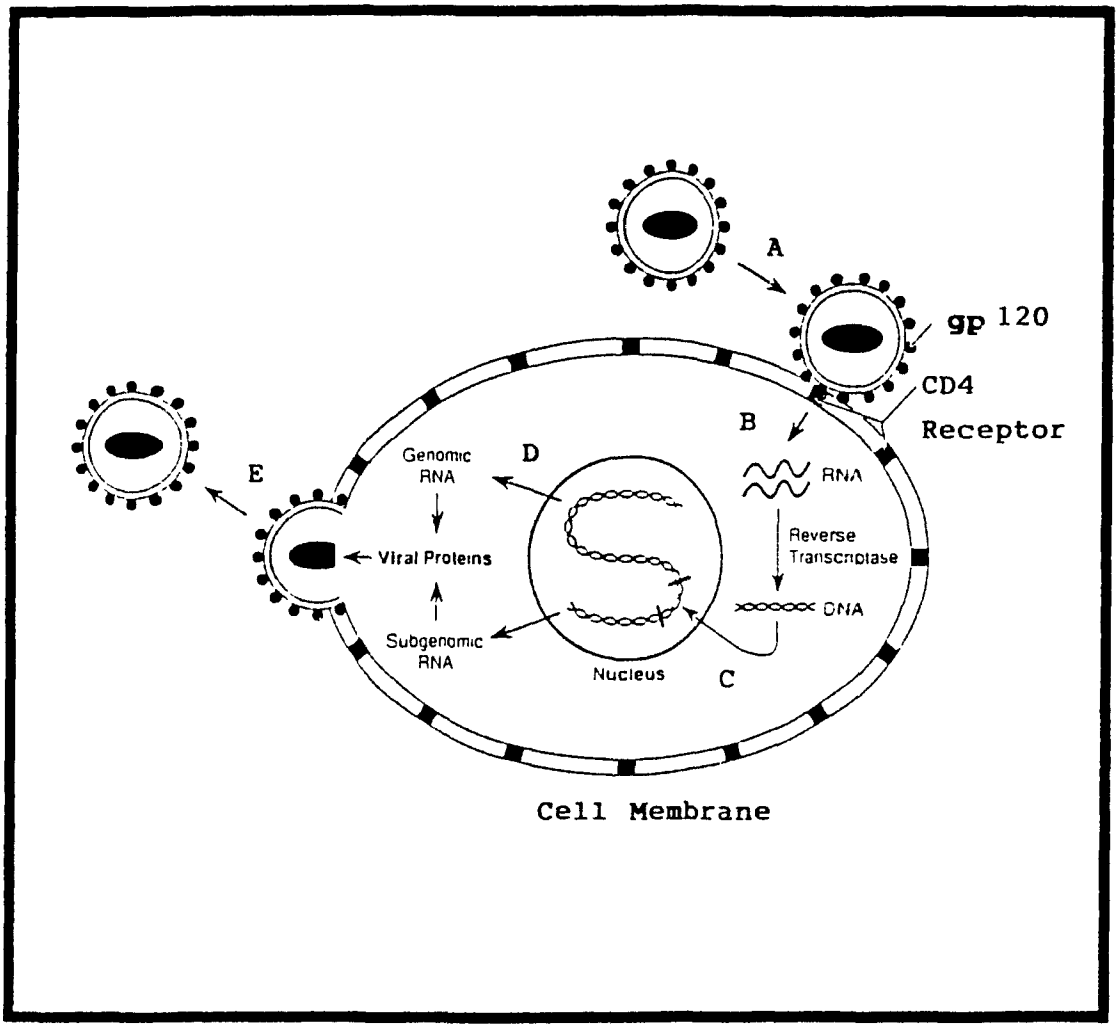
1.5. Replication Cycle

Replication in HIV is a complicated affair involving a large number of steps (see figure 3). The viral life cycle begins with a specific interaction between the carboxyl terminal portion of gp120 (16) present on the viral envelope and a specific cell surface receptor known as CD4 (17). HIV is internalized by either gp41 mediated fusion or receptor-mediated endocytosis (18). The virus is then propelled into the cytoplasm where it is uncoated. After HIV penetration, the virion-associated reverse transcriptase first produces hybrid RNA/DNA molecules and then converts these to double-stranded linear DNA molecules which contain two copies of the long terminal repeat. This linear HIV DNA (proviral DNA) is translocated into the nucleus where it is integrated into the host cellular DNA by the endonuclease activity of the viral integrase (14,19,20). Integrated as a provirus, HIV might remain latent for months or years , where very little RNA or protein is made and no infectious virions are produced (21). The provirus will duplicate together with the cell's own genes every time the cell divides. Thus, established infection is permanent. Following cellular activation and proliferation, the concerted actions of host cellular transcription factors and viral trans-activators lead to HIV replication and gene expression (22,23). Full length and singly spliced, long transcripts direct the synthesis of *gag* , *pol* and *env* polyprotein precursors which are assembled into viral particles together with two copies of single stranded genomic HIV RNA. The mature virion is then released from the cell surface where it can now infect other cells (14).

1.6 Cellular Receptors

When the AIDS virus was first recognized, its preferential replication in $CD4^+$ helper T lymphocytes suggested that the CD4 antigen was the receptor for viral infectivity. The definitive proof that the CD4 antigen was the receptor for HIV was provided by

FIGURE 3. Replication cycle of HIV showing a). binding of the viral envelope glycoprotein, gp120, to CD4 receptors, b). uncoating of viral RNA, and the reverse transcription of viral RNA into viral DNA, c). integration of proviral DNA into cellular DNA, d). production of viral mRNA and viral proteins, e). virus particle assembly and budding.


A
gp 120
CD4
Receptor
B
RNA
Reverse
Transcriptase
DNA
C
Nucleus
D
Genomic
RNA
Viral Proteins
Subgenomic
RNA
E
Cell Membrane

Maddon et al., (24) when they showed that CD4- cells (such as Hela), which are ordinarily not targets for HIV infection, could be rendered infectable by transfection of a cloned CD4 gene . The most important region for HIV binding has been mapped to a portion of the first amino-terminal variable domain (V1) of the CD4 receptor (amino acid residues 16-84) (25,26).

The CD4 molecule has been demonstrated to be the major receptor for HIV-1, however, certain studies have now suggested that other cell surface molecules may be important for viral entry. For example, expression of the human CD4 in murine cells was not sufficient to confer infectivity, suggesting that a second component present in human cells but not in murine cells was required for post binding events in the infection process (24). There has also been considerable investigation and discussion on the role of antibody mediated enhanced uptake of HIV into monocytes and macrophages as an additional method of attachment (27,28). In this model, antibody-dependent enhancement, is mediated by low levels of immunoglobulin which augment the uptake of HIV through the Fc or compliment receptors. Nevertheless, even when antibodies serve as an attachment system for HIV, the interaction with CD4 may be required for viral production either at the initial entry or at the stage of virus spreading in the culture (29,30).

1.7 Cells Susceptible To Infection

CD4+ lymphocytes are the main target of HIV infection and the decline in CD4+ cell concentration is the best predictive factor for progression to AIDS. In addition to T lymphocytes , the CD4 antigen or mRNA for CD4 can also be found on cells of the monocyte/macrophage lineage, and B cell lines. Infection of macrophages by HIV-1 is thought to be a very important reservoir for the virus in AIDS patients. Infected macrophages can travel throughout the body, even past the blood-brain barrier into

the brain, where it is thought that they could be involved in the neuropathological symptoms (31). In addition, infected macrophages *in vitro* do not appear to develop cytopathic effects, as do infected T cells. This suggests that the infected macrophage *in vivo* might persist, harboring the virus for long periods without being killed, allowing for a greater chance of virus spread. Other target cells include several cell types from the hematopoietic, neurologic and gastrointestinal systems (32-34). These cells do not express detectable CD4 cell surface molecules. Of particular interest is the recent demonstration that $CD8^+$ lymphocytes may also harbor HIV-1 (35). In conclusion, it is apparent that a wide range of cells types may be infected with HIV than was otherwise thought.

2. HUMORAL AND CELLULAR IMMUNE RESPONSES

It has been observed in HIV and other viral infections in humans and in non human species that humoral and cellular responses can contribute to antiviral immunity. Putative protective mechanisms include the following; antibodies that are neutralizing for virions or cytolytic for infected cells, natural killer (NK) cell lysis which can elicit a range of cytotoxic responses, cytotoxic T cells (CTL) that kill HIV infected cells and activated T cells that secrete antiviral cytokines or lyse cells in a major histocompatibility complex (MHC)-unrestricted manner. These mechanisms may work independently, competitively, or in concert with each other. Listed below are some of their properties.

2.1 Neutralizing Antibodies

The presence of antibody to HIV has been a reliable marker for exposure and probable current infection. Seroconversion has occurred from eight days to 10 weeks after the onset of acute

illness (36-38). On very rare occasions, certain individuals may "serorevert" (lose detectable antibodies while still carrying the virus). This makes diagnosis difficult and implies that the absence of antibodies does not completely rule out a HIV infection (39). Human immunodeficiency virus infection is serologically characterized by the development of antibodies against a variety of viral proteins: namely gp160, gp120 and gp 41 (encoded by the *env* gene); p 53, p24, p18, p15 (encoded by *gag* gene) and p64, p51 (encoded by the *pol* gene). The core protein p24 has been further studied with more quantitative methods such as enzyme-linked immunosorbent assay (ELISA).

Antibodies can destroy infected cells via several mechanisms. First, antibodies in combination with complement may react with virus infected cells, resulting in either reduction in virus production or destruction of cells. This activity, termed antibody-mediated complement dependent cytotoxicity (ACC) (40). Although ACC appears to be important for long-term protection from disease against murine and feline retroviruses (41,42), this activity is absent in HIV-infected humans (43). Secondly, antibodies can also mediate lysis of infected cells by antibody dependent cellular cytotoxicity (ADCC), which is discussed later in this introduction. Lastly, antibodies directed against the major envelope glycoprotein are responsible for eliciting neutralization in most infected individuals (44-46) or immunized animals (47-50). These antibodies can neutralize free virions or membrane bound particles by altering or masking critical sites on the virion membrane and impeding viral entry. Neutralizing antibodies do not, however, have access to intracellular virus which appears to be an important factor in cell-to-cell transmission and pathogenesis of HIV infection . HIV-neutralizing antibodies may develop as late as 1 year after primary HIV infection (51) or within a few weeks (52). Observations were made in a laboratory worker accidentally infected with the human T lymphotropic virus (HTLV-IIIb) have indicated that isolate-specific neutralizing antibodies developed

within months after infection (53). Similar results were observed in chimpanzees experimentally infected with HIV-1 (54). The principal neutralizing epitope has been determined and is located within the third variable region (V3) of the envelope glycoprotein gp120 (amino acid 307-330) (55-61). gp41 also appears to be a target for neutralizing antibodies (62).

The role of neutralizing antibodies in effective host immune surveillance is not yet clear. Correlations of antibody titer with disease progression have been identified by some groups (44-46,63-68) yet not confirmed by others (69,70). In general, neutralizing titers of HIV-1 and HIV-2 infected individuals are much lower than in other viral or retroviral infections. Antigenic variation might explain the apparently low level of neutralization of HTLV-IIIb by human sera. Evolution of antigenic variants which allow the virus to temporarily elude established host immune defenses is not particular to HIV infection. A mutation can affect a neutralization site in two ways: either by abolishing its function in virus neutralization or by modifying its antigenic conformation.

Infection with HIV-1 not only induces a humoral immune response that protects the host but one that also facilitates infection or promotes the virus pathogenicity. The presence of neutralizing antibodies might play a role in selecting *env* variants that exist in populations of virus within infected individuals, resulting in the propagation of variants that are resistant to neutralizing antibodies (71-73). This can explain disease progression in the presence of neutralizing antibodies.

Another phenomenon which could contribute to the pathogenesis of HIV is antibody enhancement of viral infectivity. It is a phenomenon that has been recorded for alphaviruses, poxviruses, banyaviruses, rhabdoviruses, coronoviruses, herpes virus and reoviruses. It has also been shown to occur in the serum or plasma of HIV infected individuals (27,28) and infected or

immunized animals (74). These antibodies could potentially increase the spread of HIV in the infected individuals and contribute to pathogenicity by expanding the host range. Although, enhancing antibodies are distinct from the neutralizing antibodies that block virus infection, they are also directed toward the HIV-1 envelope proteins. Antisera to several peptides from HIV-1 gp120/41 enhance HIV-1 infection (75). All sera may have an enhancing ability but it can also be masked by strong neutralizing activity. In essence, these antibodies may contribute to the pathogenesis of the infection and they are a concern in regard to the development of HIV vaccines because vaccination may induce such enhancing antibodies.

In addition, the increase in immunoglobulin production (hypergammaglobulinemia) in some individulas, may lead to an autoimmune syndrome. A widely accepted mechanism for viral-induced autoimmunity is molecular mimicry. It is thought that damage could result from an immune response to similar regions shared between virus and human cellular proteins. Specific antigens have ben identified as targets. Hoffmann et al. (76), believes that gp120/gp41 envelope glycoprotein mimics a conserved domain of MHC class II and class I. Anti-gp120 antibodies can block the interaction of CD4 with class II MHC. In addition a potential contributing factor to the loss of $CD4^+$ cells is the presence of antilymphocyte autoantibodies found in the serum of HIV-infected individuals (77).

Sequence homology has also been observed between HIV p17 and the thymosine alpha1 protein (78). This protein is produced by the thymus ans stimulates the production of IL-2 and interferon γ from T4 lymphocytes. Antibodies directed against thymosine-alpha1 may prevent the activation of T cells. In addition, several HIV-seropositive patients have antibodies against IL-2. The presence of such antibodies may be due to the homology between *env* protein gp41 and IL-2 (79). This may explain the immunosuppression observed in individuals infected

with HIV. Lastly, patients suffering from AIDS dementia complex were found to produce antibodies to gp41. This protein cross reacts with a 43-kDa CNS protein (80). In conclusion, if the self epitope has an important cellular function, an immune response generated to it would result in dysfunction and disease.

2.2 Natural Killer Cells

NK cells are a subclass of large granular lymphocytes (LGL) that comprise about 10-15% of peripheral blood lymphocytes (PBL) and 1 to 3% of total mononuclear cells (81). These effector cells can spontaneously lyse , on contact, a limited variety of target cells such as tumors and virally infected targets (82). The observed cytotoxicity does not show restriction related to the major histocompatibility complex (83,84). Single NK cells can participate in NK, lympocyte activated killer (LAK) and ADCC cytotoxic responses. Each response represents independent functions in which the recognitive structures and cytolytic mechanisms are distinct (84-86). NK cells bind without apparent specificity to target cells via ill-defined receptors.

Functionally active NK cells are heterogeneous and display a variable serologic phenotype (81,87). Greater than 90% of LGLs express the NKHI ($Leu19^+$) surface antigen. A significant proportion of total NK activity is mediated by NK cells which express CD16 ($Leu\ 11^+$) and complement receptor CD11b ($Leu\ 15^+$) surface antigens . Many NK cells (30-80%) also express HNKI ($Leu\ 7^+$) , however, the majority of NK cytotoxic function has been attributed to $HNKI^-\ CD16^+$ cells (88,89). They also contain cell-surface receptors for the Fc portion of immunoglobulin IgG1 and IgG3 (90). This latter quality allows them to bind antibody-coated target cells and mediate ADCC.

NK activity can be greatly augmented by interferon (IFN) , interleukin-2 (IL-2) and a variety of other agents, leading to

faster kinetics of lysis, an ability to kill a second or third time (recycling), and a greatly expanded target range (82).

Evidence suggests that NK cells offer potential resistance to viral opportunistic infections mediated by Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV) and HIV-1 associated disorders and other opportunistic infections (82,84,86,91). A number of studies have monitored NK function in patients with HIV. The results have shown that the NK function is frequently reduced in individuals at high risk for AIDS related disorders (92,93). This impairment of *in vitro* function is associated with defects in the ability of NK cells to rearrange their cytoplasmic organelles following formation of effector cell conjugates (94). This is associated with a concomitant loss of their ability to release substances that mediate lysis. In addition, there also appears to be a lymphokine imbalance in AIDS-related diseases which may contribute to this impairment but their partial restoration *in vitro* appears to be possible (95,96). Lately, evidence suggests that NK cells are also susceptible to HIV infection *in vitro* (97). Therefore, it appears that NK cells in AIDS patients are adversely affected in many different ways.

2.3 Lymphokine Activated Killer Cells

Lymphokine activated killer (LAK) cells are the *in vitro* generated counterparts of NK cells. LAK cells and NK cells are functionally and phenotypically distinct with different target cell reactivities. These cells can effectively mediate non-MHC restricted lysis of both NK-sensitive and NK-resistant targets without MHC restriction . They also display cytotoxic activity against a variety of autologous, allogenic, and xenogenic tumors. These cells lack markers typical of fresh NK cells (i.e., they have been reported to be $OKM1^-$, $OKT10^-$, $OK11^-$) and appear devoid of cytolytic activity before culture. It appears that both the

progenitors and effectors of LAK function are contained within the LGL NK pool (98,99).

Studies in animal models have demonstrated that cytotoxic LAK cells can elicit both tumor resistance and regression (83,84). The role of LAK cell therapy in AIDS patients has yet to be assessed, and few studies have documented *in vitro* LAK cell responses in seropositive individuals. One study demonstrated that the ability to recover LAK cells, following a 6 day exposure of PBLs to IL-2, was reduced by 20-25% in both HIV-1 infected asymptomatic and AIDS population, relative to healthy controls. Also, the overall lysis of targets by LAK cells from seropositive subjects can reach the same levels observed for controls but the relative lytic efficiency of individual LAK cells is reduced in seropositive individuals (100,101).

2.4 Antibody Dependent Cellular Cytotoxicity

Antibody-dependent cellular cytotoxicity involves the tripartite interactions between cytophilic antibodies (Abs), Fc expressing effector cells (NK) and sensitized targets. ADCC evokes a non-MHC cytolytic response to HIV-1 infected targets. Most ADCC killing in normal blood is mediated by Fc receptor positive,non-T, non-B cells with $CD16^+$ markers .

Two different but related forms of ADCC have been identified in infected patients. The first is termed direct ADCC or cell-mediated cytotoxicity (CMC). This assay measures the ability of cytolytic effector cells from HIV-seropositive individuals, bearing cytophilic antibodies to specifically lyse HIV-1 infected targets. The cytophilic antibodies present on these patient NK/K (killer) cells are solely those molecules that bind effectors *in situ* and are not derived from *in vitro* arming (87,102). Here, the cellular basis of HIV specific responses are monitored. A number of laboratories have monitored the ability of leukocytes derived

from HIV-1 seropositive individuals to mount ADCC-specific responses to HIV-expressing targets . Its activity as reflected in CMC assays has been found to decline markedly with disease progression (102,103). The nature of the cellular defect is less clearly defined but seems to be related to a general deactivation of NK/K cells that affects not only Fc-mediated ADCC but also spontaneous natural killer activity.

The second form of ADCC is termed indirect ADCC which is the classical serological form of ADCC. This conventional ADCC approach monitors the ability of sera from HIV-1-seropositive individuals to arm lymphocytes from healthy HIV-seronegative controls and thereby mediate lysis of HIV-infected targets. Unlike direct ADCC, indirect ADCC describes only the serological or humoral component of HIV-directed responses (104,105).

The selection of appropriate HIV-1 infected targets represents the most critical aspect of these ADCC assays. High background levels of NK cell-mediated cytotoxicity of many targets can mask their susceptibility to ADCC responses. Recall, NK cells can simultaneously participate in ADCC and NK responses. Optimally, targets are selected for NK-resistance and ADCC sensitivity in order to correctly determine HIV-directed ADCC. One of the methods used to characterize virus-specific cellular and humoral immune responses such as ADCC is by using recombinant vaccinia virus expression systems which express foreign viral gene products such as the HIV-1 envelope protein. Vaccinia virus (VV) vectors have been instrumental in delineating target antigens of virus-specific CTL in HIV (106) and other virus infections (107). Infection of susceptible cells such as B-lymphoblastoid cells (BLCL) with the HIV-1 env expressing recombinants result in normal synthesis, glycosylation, processing and membrane transport of the *env* polypeptide, which can then be recognized by serum antibodies from patients with HIV (108). BLCL represent ideal targets since they are NK-resistant and ADCC sensitive.

Rook et al., was the first to describe *in vitro* ADCC activity against HIV infected cells using sera from HIV antibody positive individuals (109). A number of important conclusions have emerged from these studies regarding the circulation, the titers and the specificity of ADCC Abs. ADCC-directing Abs are among the first antiviral immune responses to become detectable in sera from high risk donors before full seroconversion can be demonstrated in conventional enzyme-linked immuno-sorbent assays (ELISA) or radioimmunoprecipitation assays (104). In addition, ADCC Abs have been detected prior to the appearance of other functionally active antibodies, including those that neutralize , inhibit fusion and block gp120-CD4 interactions (83,104,110-112). The .ters of circulating ADCC Abs against HIV-1 proteins are in general higher than the corresponding neutralizing Ab titers in the same sera (83,103,110,111). Titers of ADCC Abs generally remain high throughout the course of disease . In most studies there does not appear to be a strong association between clinical status and ADCC levels (83,113,114). Other studies looking at smaller populations of patients have demonstrated that a slight decline in anti-HIV-1 ADCC may occur with disease progression (109,115-117) suggesting a protective role for this type of non-MHC-restricted cytolysis in seropositive individuals (118). Examination of a large cohort of patients infected with HIV-1 has revealed that between 40 and 80% have significant levels of Abs that direct broadly reactive anti-HIV-1 ADCC (109,113,115,116,119-122). HIV-1 ADCC Ab show broad reactivity against a variety of HIV-1 strains, including HIV-1IIIb, HIV-1IIIMN, and HIV-1IIIRF; however, there appears to be no detectable ADCC corresponding to HIV-2 (104,115). Thus, although some ADCC target epitopes have been shown to be conserved among HIV-1 isolates, cross reactive ADCC target epitopes between major HIV classes have yet to be identified.

A number of studies have attempted to address the epitope specificity of antibodies that mediate anti-HIV-1 ADCC. Comparative analysis of the neutralizing and ADCC-mediating

ability of patient sera in large groups of individuals revealed no correlation between these two reactivities suggesting that epitopes serving as targets for ADCC may be distinct from neutralizing epitopes. ADCC Abs are directed primarily if not exclusively against the HIV-1 envelope determinants gp120 and gp41 (102,113,116,118-121,123). Recent studies have mapped the fine specificity of ADCC-specific epitopes on gp160 by analyzing the ability of synthetic polypeptides to direct ADCC reactions. At least one epitope has been mapped to a region representing the carboxyl end (46 amino acids) of gp120 and the proximal amino terminus of gp41 (124). Peptides corresponding to determinants within the (gp41) portion of the HIV envelope glycoprotein may also be important . Although non-envelope structural antigens of HIV-1 have been shown to serve as targets for CTL in humans , there is little direct evidence to suggest that core proteins can also serve as ADCC targets. The reason for this is that the gag antigens on the surface of BLCLs may represent small amphipathic peptides presented in the context of MHC molecules which are recognized by T cell receptors and not by Abs (121).

The potential role for ADCC in the control of HIV infection has received an increasing amount of attention, primarily because this is one antibody-directed effector mechanism that has proven ability to attack HIV-infected cells (109,110,116,125).In view of the potentially protective role of ADCC, interleukin-2 and other NK/K cell modulators are currently under investigation to determine whether the cellular dysfunction in infected individuals can be reversed or partially compensated for by *in vivo* activation (126). Similarly, the passive infusion of anti-HIV-1 antibodies is being considered as a possible therapy to correct the defect occurring in individuals with low titers of ADCC-directing antibodies. Therapies designed to augment ADCC should proceed with caution. The phenomenon of ADCC, however, could conceivably accelerate disease progression via a process termed noninfectious lympholysis (102,113). This process may be a contributing factor to lymphopenia seen with HIV-1 infection,

which occurs when free gp120 binds to CD4 molecules on uninfected lymphocytes, rendering these coated cells susceptible to destruction by mechanisms of ADCC. Nevertheless, Abs that mediate ADCC may help remove from the circulation heterologous cells infected with a wide variety of viral isolates, providing to be a valuable adjunct to neutralizing antibodies.

2.5 Cytotoxic T Lymphocyte

The contribution of cell-mediated immunity to protection against virus infections appears to be sustained with respect to numerous viruses in both human and animal hosts, ranging from the relatively benign influenza viruses to oncogenic DNA viruses and retroviruses. It has been suggested that the occurrence of a measurable lymphocyte response can be a prognostic indication of the ability of the immunocompetent patients to overcome viral disease. CTL has been demonstrated to be one of the early host defenses generated in response to a variety of viral infections (127). For example, in murine infections with influenza A virus, virus-specific CTL are generated before the production of significant levels of antibody, and adoptive transfer of these cells to histocompatible mice challenged subsequently with influenza A virus, results in decreased viral titers and decreased mortality (128). In humans, effective virus-specific cytotoxic responses correlate with recovery from infection with both cytomegalovirus (CMV) (129) and influenza virus (130).

It appeared logical to search for the presence of HIV-specific CTL in HIV-1 seropositive patients based on the results obtained in murine experimental models concerning CTL-mediated protection against oncogenic retroviruses. Foremost among these reasons is the demonstration that the majority of HIV infected adults remain asymptomatic for years after infection. Unlike NK cells, cytotoxic T cells are antigen-specific. Characteristically, they are CD8+ and are HLA-restricted in that

they can kill only infected cells with which they share at least one HLA class I antigen (131).

Two parallel studies, published by Plata (132) and Walker (133) provided the first indications as to the reality of circulating HIV-specific CTL in seropositive patients. The vigorous CTL activity seen in the early stages of infection declined with disease progression suggesting that there was a correlation between the progression towards AIDS with the disappearance of circulating HIV-specific CTL (134,135). Subsequent studies (134,136,137), confirmed and extended these results. A striking feature of CTL cells is that they appear in many HIV infected persons in such high frequencies that their activity can be measured directly in fresh peripheral blood mononuclear cells (PBMC) (134,138,139). This situation contrasts sharply with that observed for other human viruses, where specific antigenic stimulation must first be provided *in vitro* to ensure expansion of the CTL population prior to detection. HIV-specific CTL has also been detected in the lungs of seropositive patients (132,140) and in cerebrospinal fluid of patients with neurological disorders (141).

Initial reports demonstrated that CTL activity in HIV seropositive patients was mediated by $CD8^+$ cells that were MHC restricted, recently, conflicting reports exist as to the nature of the effector lymphocytes in peripheral blood-mediating envelope-specific cytotoxicity. Koenig et al., (138) reported that both HLA-matched and mismatched target cells could be lysed by $CD8^+$ cells. Sethi et al., (141) using stimulated CSF T cells, obtained gp120 specific CTL clones that were both $CD8^+$ HLA class I restricted and $CD4^+$ -HLA class II restricted. In a study by Riviere et al., (142) using fresh blood from seropositive subjects, they observed that lysis of target cells expressing recombinant HIV-1 envelope glycoprotein was not MHC-restricted, leading them to conclude that the lytic activity was not mediated by T lymphocytes at all. The cell phenotype mediating lysis was thought to be NK cells which were "armed" *in vivo* with envelope-specific antibodies.

Non T cell-mediated cellular cytotoxicity has also been reported by Weinhold and colleagues (102,143). Lastly, Mc Chesney et al., (144) has recently demonstrated that both T-cell-mediated HLA restricted responses, as well as non T-cell-mediated cellular cytotoxicity are detectable with PBMC from seropositive patients.

Multiple HIV-1 proteins have been identified as target antigens. The first published studies regarding epitopes recognized by HIV-1 reactive CTL were performed in mice. Takahashi (145) found that H2d mice generated HLA class I restricted CTL which respond predominantly to a single immunodominant site represented by a 15aa synthetic peptide (aa308-322) from a highly variable segment of the *env* protein. A different envelope epitope was reported, contained within a 12aa peptide (aa381-392) from the C terminal portion of gp120 HIV-1 (135). Recognition of this epitope is highly conserved among sequenced isolates. In addition Clerici et al., (146) identified 4 new epitopes: two of gp120 (428-443 and 112-124) and two from gp160 (834-848 and 325-329). The CTL activity was mediated by T cells, predominantly MHC class I restricted and HLA-A2 was identified as a restricting element for all four peptides.

In addition to *env*-specific CTL, HIV-1 *gag* - and RT-specific CTL have also been detected in fresh peripheral blood from infected individuals (137,147). Again, this response has been found to be mediated by class I restricted CTLs which are of the $CD3^+$ $CD8^+$ phenotype, the predominant phenotype that mediates CTL activity in most other viral systems (148). With respect to *gag* epitopes, several CTL epitopes were defined. In one case (149) an HLA B27 restricted CTL epitope was found in the peptide composed of aa 265-280 of the HIV-1 p24 protein. Studies by Claverie et al., (150) revealed two epitopes within the p15 protein (aa418-413 and 446-460) and two within the p24 protein (aa193-203; 219-233). All were HLA-A2 restricted. Recently, a unique epitope on the *gag* protein located at amino acid 145-150

has been mapped using CD8+ CTL clones. The epitope is on a highly conserved region and it overlaps with a major B-cell epitope of *gag* . This has not been previously identified as a restricting element for human CTL responses (151).

In the case of RT, a total of five different epitopes were identified by Walker (147). The results indicated that multiple epitopes of a given viral gene product were recognized in conjunction with different host HLA class I antigens and that a single HLA antigen could serve as the restricting element for more than one of these epitopes. In addition, Hasmalin et al., (152) identified an HIV-1 RT epitope distinct from Walker. The epitope 203-219 is in a region highly conserved in evolution among RT genes of other viruses. Regulatory proteins also serve as CTL targets in infected people including *vif* and *nef* (142,153,154). In the case of the *nef* protein , a highly antigenic peptide corresponding to the central region of the *nef* aa sequence (aa113-128) was observed . An immune response directed against early expressed regulatory viral proteins such as *nef*, may allow elimination of infected cells before release of new viral particles would even occur. The *nef*-specific CTL have been demonstrated to be CD8+ and HLA class I restricted.

In addition to the HIV-1 specific cytolytic activity attributed to CD8+ cells, these cells from HIV-seropositive donors have also been shown to inhibit HIV replication *in vitro* (155,156). In one study, bulk CD8+ cells appeared to inhibit viral replication in an MHC-unrestricted manner, suggesting that more than one CD8+ subpopulation may participate in the regulation of HIV replication. This inhibition may occur by production of antiviral lymphokines whose structure is still unknown. The amount of cytokine produced varies among HIV-infected individuals with the highest amounts present during the early stages of infection.

Based on this fact and the analogy with animal model systems of virus infection, it is reasonable to postulate that HIV-1

specific CTL serve a protective role as a host defense. Identification of the target antigens recognized by these CTL are central to the design of vaccine strategies, and may provide insight in the immunopathogenesis of HIV-1 infection

2.6 Endogenous Cytokines

HIV has been shown to have remarkably intercalated itself into the normal immunoregulatory network of the human immune system. It uses for its self-propagation the very cellular and molecular mechanisms that the immune system uses for its homostatic regulation. Of particular interest is that HIV can utilize an elaborate network of cell-derived soluble factors or cytokines for its own replication advantage (157).

For instance, tumor necrosis factor-a induces HIV expression in chronically infected T-cell lines, by transcriptional mechanisms involving activation of NF-kb which is a pleotropic mediator of inducible and tissue-specific gene control (158,159). In accordance, plasma levels of tumor necrosis factor-alpha have been shown to be elevated in HIV-infected persons (160). Granulocyte/Monocyte-Colony Stimulation Factor and Interleukin (IL)-6 either alone or in combination also induce HIV expression by post-transcription mechanisms (161). Furthermore, increased levels of Interleukin-6 have been reported both in the serum and the cerebr0spinal fluid of HIV-infected persons (162,163).

While certain factors enhance infection, certain cytokines can actually down regulate the expression of HIV, and this, has potentially important therapeutic implications. One cytokine, interferon a, has already been shown in clinical trials to be an effective antiviral agent (164,165) by either decreasing virus expression *in vitro* or by blocking the budding of HIV from chronically infected cell lines (166).

3. ANTIVIRAL THERAPY

3.1 HIV Attachment and Entry

The first stage at which anti-HIV agents might intervene is during the binding of the viral envelope with CD4 receptors. Since all HIV isolates are tropic for $CD4^+$ cells, peptides derived from recombinant CD4 could be used to block viral infectivity and HIV induced cell fusion. This has been demonstrated *in vitro* using cell free virions (167). The results of early clinical investigations using soluble CD4 and truncated portions of CD4 have not been successful because of it's short half-life . In order to correct this problem, hybrid proteins between recombinant soluble rsCD4 and immunoglobulin Fc domains (immunoadhesins) were developed that had a prolonged serum half-life. Clinical improvement has not been associated with this treatment (168). Some of the limitations to soluble CD4 is that CD4 cannot prevent infected cells from generating viral progeny or eliminating such cells from the body. Secondly, it is also unlikely that rsCD4 preparations will be sufficient, as single agents, for virus control based on the studies that certain cells may become infected through other mechanisms. Lastly, theoretical concerns about the ability of rsCD4 to interfere with normal immune functions, to cross the blood-brain barrier or to induce an antibody response have been raised.

3.2 Reverse Transcriptase

The target that has received perhaps more attention than any other, is the next stage of viral replication which is the synthesis of viral DNA by the enzyme reverse transcriptase. Dideoxynucleoside analogues are molecules that closely resemble the nucleotides that serve as building blocks in DNA and RNA. It inhibits RT and causes premature chain termination. One such compound is AZT. It was originally formulated as an anticancer

drug (169), but now is the first effective anti-AIDS agent. A number of studies have shown that patients treated with AZT live longer than controls (170). Beneficial effects include a transient increase in $CD4^+$ cells, and a significant reduction in p24 antigenemia (171). Considerable AZT toxicity is also associated with such treatment (172). Other dideoxynucleosides that are active against HIV also appear to work by this mechanism. They include dideoxyinosine (ddI) and dideoxycytidine (ddC). Preliminary studies have shown similar results with less severe hematopoietic suppression (173).

3.3 Synthesis of Viral RNA and Proteins

The next target for therapy presents itself some time later in the cycle of HIV, when the cell begins to produce new proteins. Ribavirin, is drug currently used to treat respiratory syncytial virus-induced illness in children (174). This drug was first shown to have *in vitro* activity against HIV in 1984 (175), and clinical trials were initiated in 1985 in various infected subgroups. Ribavirin is thought to prevent the guanylation of newly made viral mRNA, and hence interfere with viral protein synthesis. Clinical trials have demonstrated that no clinical or laboratory benefits were detected (176) Ribavirin also appears to antagonize the activity of AZT *in vitro* (177).

Another approach to HIV-1 therapy is the use of synthetic oligonucleotides. The idea is to create short nucleotide sequences, that are complementary to a part of the viral mRNA. These antisense constructs probably function by obstructing the cell's ribosomes from moving along the RNA and thereby halting translation of RNA into viral protein (178). One disadvantage with oligonucleotides is that many of them can be degraded by enzymes in host cells.

Other strategies consist of inhibiting the activity of HIV-encoded proteases (179), which are essential for core protein

processing and viral infectivity, and inhibiting retroviral integrases which are necessary for integration of proviral DNA into cellular DNA. Another approach is to interfere with the addition of sugar residues to viral proteins(i.e. gp120) (180). If glycosylation does not occur, the proteins may be unable to function biologically.

3.4 Later Events in HIV Replication

Finally, the assembly and release of virus at the plasma membrane represents another stage at which effective intervention strategies may be useful. For instance, the budding may be stopped by interferon (IFN) an antiviral substance that is produced naturally in cells. Interferon alpha has shown to inhibit HIV replication *in vitro* (181) and render previously uninfected cells less susceptible to HIV. Clinical trials using interferon have shown to be beneficial in patients in earlier stages of infection. It also helps to suppress Kaposi's sarcoma . IFN might benefit certain AIDS patients by acting as both an antiretroviral and antitumor agent.

3.5 Combination Therapy

Combination antiviral chemotherapy may be required for prolonged control of HIV-1 infection because of the short-lived clinical benefits of AZT and the emergence of AZT resistance during chronic treatment (182). Many anti-HIV drug combinations have shown promise *in vitro* (183,184), that is synergistic virus inhibitory activity without enhanced toxicity. Some of these are now in clinical trials.

CHAPTER TWO: MATERIALS AND METHODS

MATERIALS AND METHODS

1. Patient population: zidovudine study. Sera was obtained from a Canadian Multicentre AZT study of patients (n=14) with HIV-1 associated disease. Subjects received 600mg/d of AZT for 18 weeks, 900mg/d for the subsequent 9 weeks, and 1200mg d for a further nine-week period. After 36 weeks on therapy, subjects did not receive AZT for a six-week washout period designed to establish the reversibility of the hematological effects of the drug. AZT was then reinitiated at a maximum dose of 1200mg/d for the following 42 weeks. This trial was planned at a time when the first results of AZT therapy where becoming known from the American Trial, which had indicated that in ARC and AIDS patients, zidovudine compared to placebo resulted in fewer deaths. It was considered unacceptable by the company to undertake a placebo-controlled trial. Therefore no placebo group is evaluated in this study, however, we were able to obtain sera from four HIV-1 patients that did not receive AZT for the same time frame.

2. Patient population: ribavirin study. Serum samples were obtained from a double-blind, placebo-controlled randomized Canadian multicentre trial with ribavirin. Patients received 15-16 mg/kg/d of ribavirin. Samples were obtained every 2 to 4 weeks for a period of 24 weeks. The patients were all antibody positive .

3. Recombinant vaccinia viruses: The viruses used for infecting target cells include the following: i). the negative control vSC-8 (NIH) which expresses the *Escherichia coli* lac *Z* gene product and ii). the vPE-16 (NIH) which expresses the entire *env* gene of HIV-1 (isolate HTLV$_{IIIb}$, clone BH8). It is regulated by the vaccinia virus p7.5 promoter. The *env* gene has been modified to eliminate cryptic vaccinia virus early transcriptional

stop signals without altering coding sequences. It expresses gp160 which is glycosylated , processed into gp120 and gp41, and then inserted into the plasma membrane.

4. Target cells: B-lymphoblastoid cell lines (BLCLs) from HIV-seronegative patients were obtained by infecting peripheral blood mononuclear cells (PBMC) with the Epstein-Barr virus (EBV). Log phase lymphoblasts ($3x10^6$) were mock-infected and infected with vaccinia virus recombinants at a multiplicity of infection (moi) of 5, and incubated at 37°C for 1.5 hrs. The cells were then washed and resuspended at a final concentration of10^6 cells/ml in RPMI 1640 (GIBCO, Lab. Grand Island N.Y.) containing 20% fetal calf serum (ICN, Flow), 2mM glutamine, 200 units/ml penicillin, and 200ug/ml streptomycin and 100mM Hepes for 14 hrs at 37°C. Fourteen hours later, the cells were washed and incubated with 100uCi of $Na_2(^{51}CrO_4)$ (ICN) at 37°C for 60 min. After incubation, the cells were washed three times in RPMI 1640 at 4° C and suspended after viability determination by trypan blue exclusion, to an appropriate concentration in RPMIcomplete. An NK sensitive cell line K-562 (tumor cell line originally derived from a patient with chronic myeloid leukemia in blast crisis) was labeled with Cr^{51} and run in parallel assays.

5. Sera: Plasma from heparinized venous blood was obtained from HIV-1 seropositive patients before AZT, ribavirin, and placebo treatment and at various time intervals thereafter. All serum samples were heat inactivated for 30 min. at 56°C and stored at -70°C.

6. Effector cells: PBMCs from a healthy HIV-seronegative donor were isolated from freshly drawn heparinized venous blood by centrifugation on Ficoll-Hypaque (Pharmacia, Uppsula, Sweden). They were then washed once and resuspended in

RPMIcomplete. PBMCs were obtained repeatedly from the same donor for each of the assays.

7. ADCC ^{51}Cr release assay: 50ul of target cell suspension containing 10^4 target cells were dispersed in 96 well round bottom microdilution plates containing 50ul of serum appropriately diluted in RPMIc (10^{-1}, 10^{-2}, 10^{-4}, 10^{-8}). After 30 min. at 37 C, 100ul of effector cell suspension containing $2x10^5$ cells were added to the test wells (effector to target cell ratio 20:1). Control wells with RPMIc in place of effector cells, serum, or both were included in all assays. Maximal release was obtained from targets incubated with 1% Triton X and spontaneous release was obtained from targets incubated in medium alone. The test plates were incubated for 4 hrs at 37 C in a humidified 5% CO_2 environment. The supernatant was harvested after 4 hrs using a Skatron harvesting frame (7072 SCS Skatron:OSI). The amount of radioactivity was counted in a LKB gamma counter. All tests were performed in triplicate. Percent cytolysis was calculated by the following formula: 100 x [(test cpm - spontaneous cpm) / (maximal cpm - spontaneous cpm)]. The percent ADCC was determined with the following formula: % ADCC = (% antibody-dependent lysis of vPE-16 infected BLCLs - % antibody-independent lysis of vPE-16 infected BLCLs) - (% antibody-dependent lysis of vSC-8 infected BLCLs - % antibody-independent lysis of vSC-8 infected BLCLs). Spontaneous ^{51}Cr release values from uninfected or vaccinia virus-infected cells varied from 10 to 35% of total incorporated label for a 4 hr assay. Experiments in which the mean spontaneous release exceeded 35% were discarded.

8. Expression analysis: Vaccinia-virus infection of BLCLs were assessed by FACS analysis using a polyclonal rabbit anti-vaccinia virus (Lee * Biomolecular) and FITC conjugate F(ab)2 goat anti-rabbit IgG (Cappel, Oragon, Teknika Corporation). In addition,

surface expression of HIV envelope glycoprotein was assessed by the ability of vaccinia virus-vector infected cells to form syncytia with MT-4 cells in a 24 hr co-incubation (ratio 1:3). Only cells expressing the envelope protein induce such effects.

9. Microneutralization assay: Levels of neutralizing anti-HIV-1 antibodies were determined via a microneutralization test (MNT). Serum samples were heat inactivated and two fold serially diluted when mixed with equal volume of $HTLV_{IIIb}$ virus stock (100-200 TCID50) in 96-well, flat-bottomed microtiter plates. The test was performed in duplicate. Virus-serum mixtures were then incubated for 1 hr at 4 C and seeded with $1x10^4$ MT-4 cells (100ul) per well. Titers were expressed as the reciprocal of the highest dilution found to inhibit HIV infection.

10. Statistical analysis: Results have been statistically analyzed for the various time intervals. The t-test for paired data was used to test the significance between a series of two measurements on the same individuals.

CHAPTER THREE: RESULTS

RESULTS

Expression analysis of vaccinia virus recombinants

We developed a B lymphoblastoid cell line (BLCL) by infecting peripheral blood mononuclear cells (PBMCs) with Epstein-Barr virus (EBV), as previously described. The production and processing of HIV proteins in BLCLs infected with recombinant vaccinia virus vectors was assessed by FACS analysis and syncytium formation.

The envelope glycoprotein of HIV, as expressed in recombinant vaccinia virus vectors is known to be processed and glycosylated normally and the end products become situated in the outer cell membrane in a manner which allows for spontaneous syncytia formation in the presence of CD4 bearing cells. Figure 4 demonstrates the ability of each target cell to form syncytia with MT-4 cells (cells expressing the CD4 cell surface receptor) during a 24 hour cocultivation. Only cells infected with vPE-16 were capable of inducing cytopathic effects such as ballooning or giant cell formation (figure 4d). No cytopathic effects were observed with cells which were either mock-infected or infected with the vaccinia control vSC-8 (figure 4b and 4c).

The percentage of cells infected with vaccinia were determined by FACS analysis (Figure 5). Figure 5a, represents the cell surface expression of vaccinia virus antigens on mock infected BLCLs (control). Only 1.8 % of cells fluoresced which represents non-specific binding. Figure 5b and 5c represents the percentage of cells expressing vaccinia antigens from vSC-8 and vPE-16 infected targets. Greater than 90% of these target cells were positive at a multiplicity of infection (moi) of 5. Infectivity was comparable over a wide range of moi's except that at higher moi's, spontaneous release values rose because of increasing vaccinia virus induced lysis of targets.

FIGURE 4. Expression analysis of vaccinia virus using a syncytium formation assay. Figure 4a represents MT-4 cells, b) mock infected BLCLs co-cultured with MT-4, c) vSC-8 infected BLCLs co-cultured with MT-4 and d) vPE-16 infected BLCLs co-cultured with MT-4 for 24 hours.

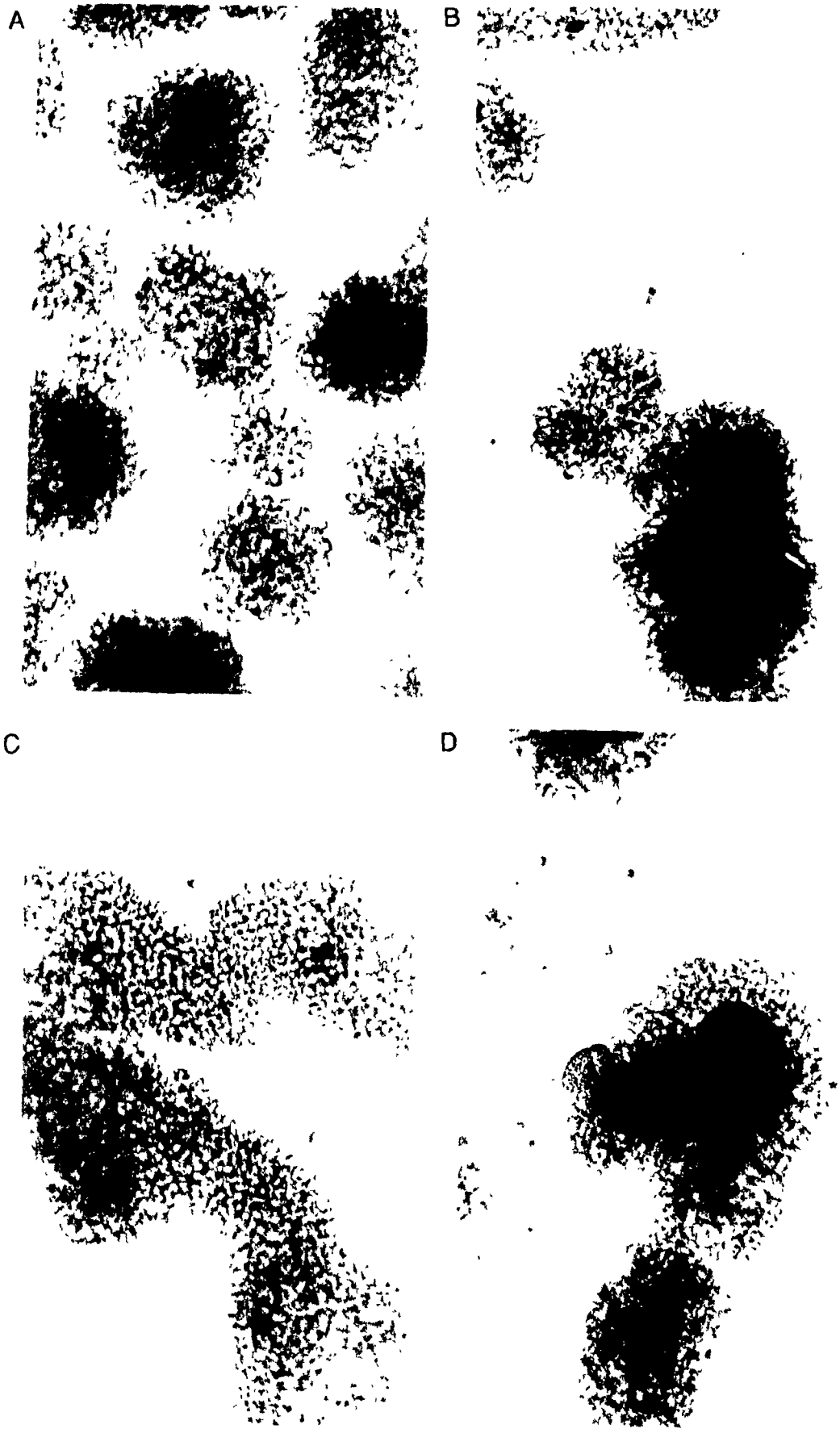
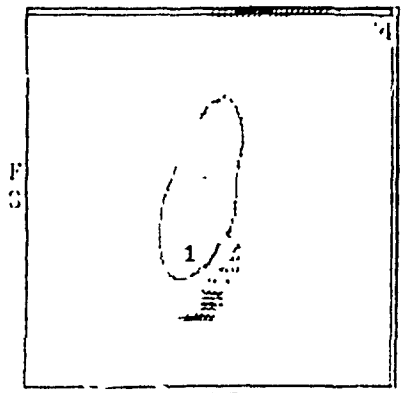
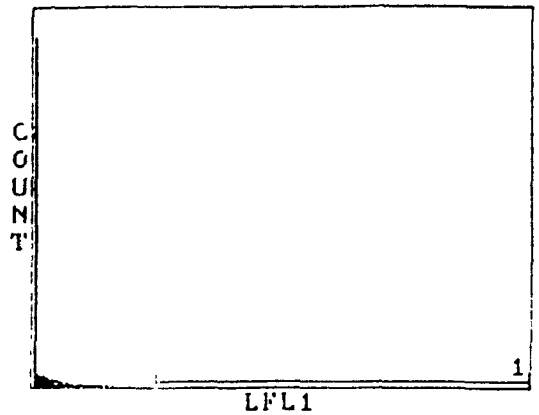

A
B
C
D

FIGURE 5. Expression analysis of vaccinia virus antigens using a fluorescent activated cell sorter (FACS) . Fluorescence was demonstrated using anti-vaccinia virus and FITC-goat anti-rabbit IgG F(ab)2. The figure represents a) fluorescence of mock infected BLCLs b) fluorescence of vSC-8 infected BLCLs and c) fluorescence of vPE-16 infected BLCLs at a moi of 5.

A

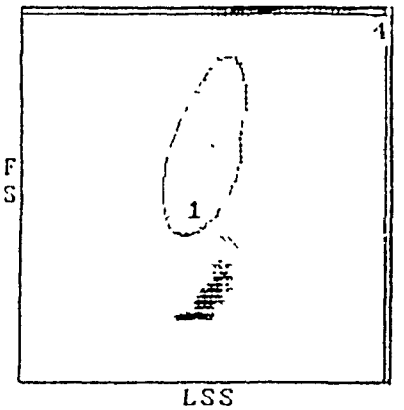


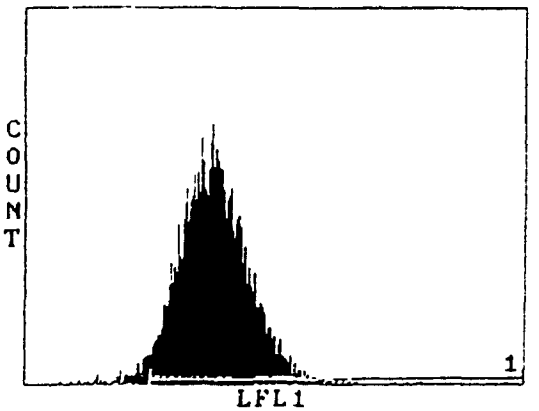


	MIN	MAX	COUNT	PERCENT	MEAN	SD	% RPCV
1 I 0	.102	886.0	2308	11.4	28.26	0.18	0.91
T	63	63					
3 I 0	.102	886.0	18136	88.5	19.46	0.19	11.6
T	0	62			26.5	13.5	27.7
4 I	886.7	1023.	0	0.0			
T	0	62					

	MIN	MAX	COUNT	PERCENT	MEAN	SD	% RPCV
1	1.024	1023.	92	1.8	1.552	0.1147	3.33

B

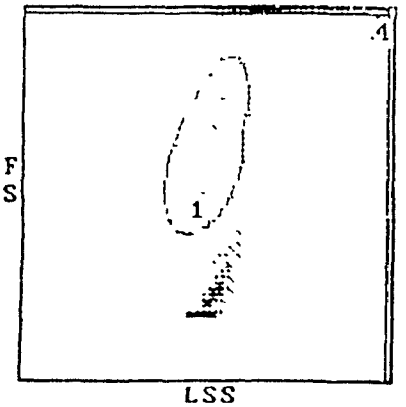


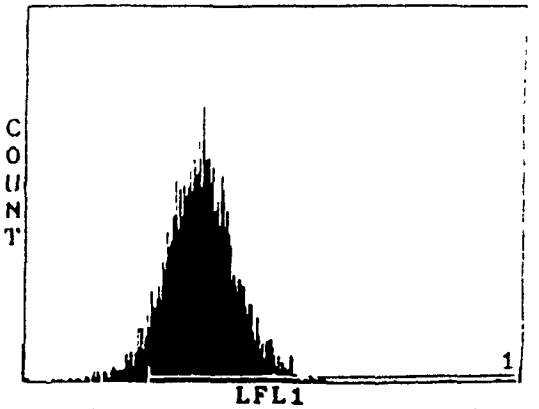


	MIN	MAX	COUNT	PERCENT	MEAN	SD	% RPCV
1 I 0	.102	886.0	889	13.7	33.46	0.19	9.32
T	63	63					
3 I 0	.102	886.0	10255	86.3	18.39	0.18	9.81
T	0	62			25.9	13.6	6.29
4 I	886.7	1023.	0	0.0			
T	0	62					

	MIN	MAX	COUNT	PERCENT	MEAN	SD	% RPCV
1	1.024	1023.	4838	96.6	1.454	0.192	14.8

C





	MIN	MAX	COUNT	PERCENT	MEAN	SD	% RPCV
1 I 0	.102	886.0	3474	19.4	32.40	0.19	8.56
T	63	63					
3 I 0	.102	886.0	14468	80.6	19.01	0.18	9.13
T	0	62			28.6	14.8	20.6
4 I	886.7	1023.	0	0.0			
T	0	62					

	MIN	MAX	COUNT	PERCENT	MEAN	SD	% RPCV
1	1.024	1023.	4648	92.8	2.929	0.192	15.1

Cytopathic effects of vaccinia virus

One of the drawbacks of this system is the lytic activity of vaccinia virus itself. Most targets have to be used within 24 hours of infection before overt cytopathology is observed. Figure 6 demonstrates cell viability post infection. Viability decreases markedly after 20-24 hours post infection.

Preferential lysis of HIV-1 envelope infected target cells

Sera from 36 HIV-seropositive donors were tested over a range of dilutions for their ability to mediate direct lysis of infected targets. Since HIV-1 infected patients may be infected with EBV or previously vaccinated with vaccinia virus, it is possible that they possess antibodies to EBV, vaccinia virus or both. Whether such antibodies obscure the serum HIV-specific ADCC activity was determined. Results from a representative experiment are shown in figure 7. In our screening, we found that HIV-specific ADCC was always present at higher titers than ADCC activity against uninfected or vaccinia virus-infected BLCL targets. Most of the seropositive patients tested, had an envelope specific cytotoxic response that was 5 to 20 times that observed for vaccinia virus vSC-8 infected cells. This allowed a clear delineation between these two activities. In only a few circumstances were the antibody titers to vaccinia per se elevated. Background cytotoxic responses to the EBV target cells were low in all serum samples tested. It is known that EBV-transformed BLCLs are latently infected with EBV and do not have surface expression of membrane antigens responsible for EBV specific ADCC (185). Direct NK activity, lysis in the absence of sera, was minimal against any of the targets. This attests their resistance to NK lysis. In addition, the graph also demonstrates that the optimum serum dilution is 1/100. Lysis of HIV-1 infected cells could also be demonstrated at dilution's exceeding 1/1 000 000.

FIGURE 6. Viability (%) of vaccinia infected BLCLs post infection

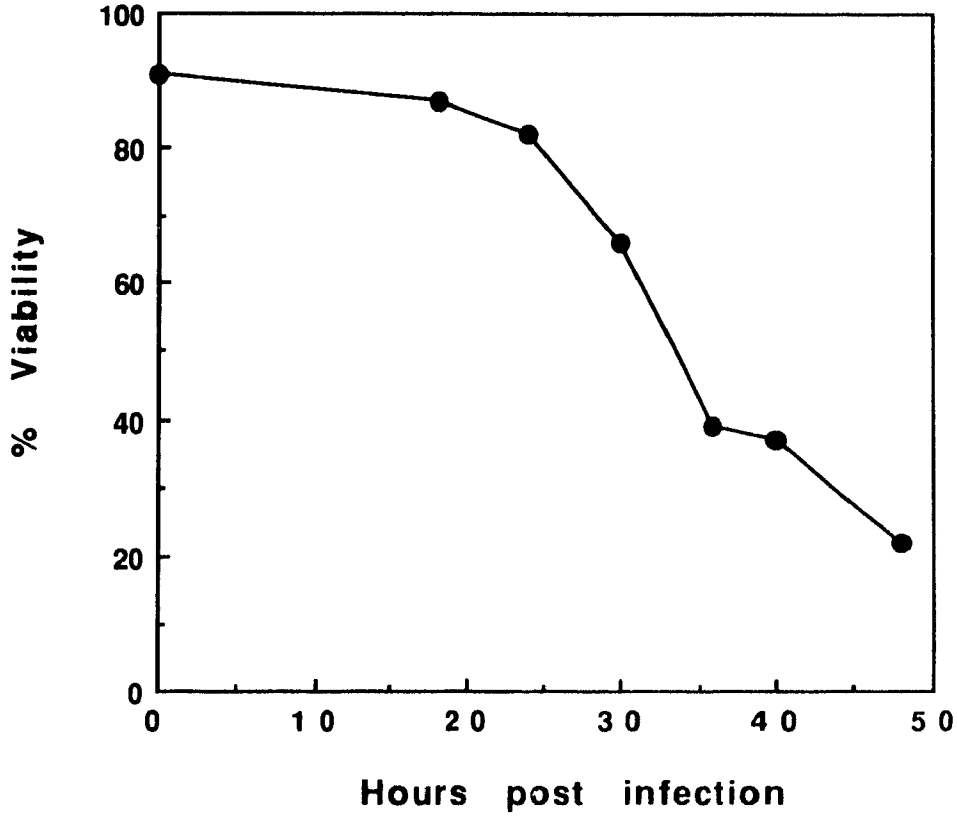
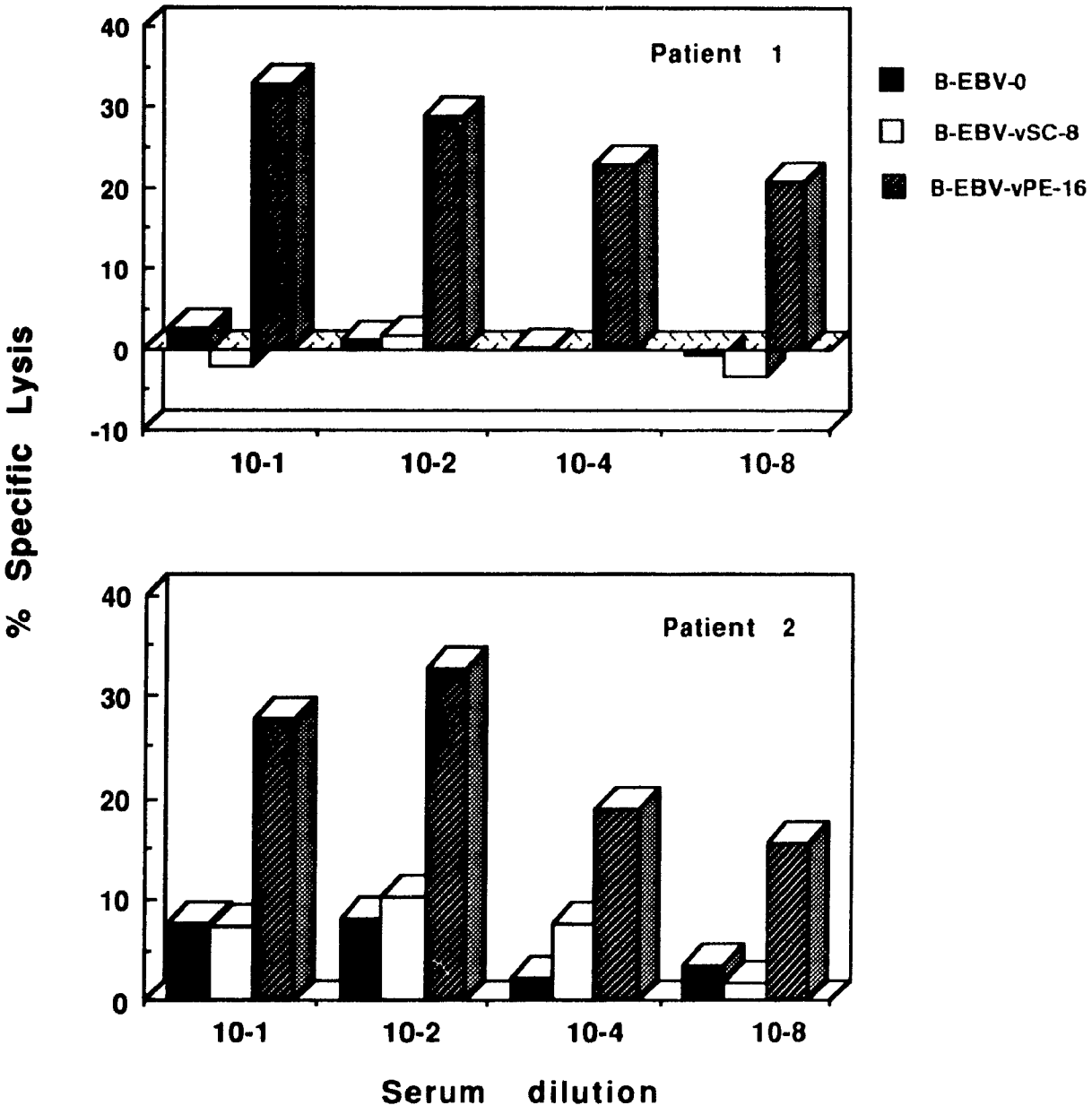

100
80
60
40
20
0
% Viability
0
10
20
30
40
50
Hours post infection

FIGURE 7. env-specific antibody-dependent cellular cytotoxicity (ADCC) patterns of lysis for two representative serum samples from HIV-1 seropositive patients assayed at various serum dilutions. The target to effector cell ratio is 20:1. The targets are indicated in the legend. (□ mock infected BLCLs, □ vaccinia virus control, □ env infected BLCLs)


Patient 1
B-EBV-0
B-EBV-vSC-8
B-EBV-vPE-16
% Specific Lysis
40
30
20
10
0
-10
10-1
10-2
10-4
10-8
Patient 2
40
30
20
10
0
10-1
10-2
10-4
10-8
Serum dilution

Longitudinal follow-up of HIV-specific ADCC in patients receiving AZT, ribavirin or placebo treatment

Having demonstrated appropriate HIV protein expression in vaccinia virus-vector infected BLCL and HIV-1 specific ADCC lysis, we then proceeded to assay serum samples collected just before initiating AZT treatment an regularly thereafter for their ability to mediate HIV-1 specific ADCC in this longitudinal study. Each serum sample was tested on two separate occasions utilizing the same set of normal effector cells and targets in order to minimize variability. In essence, the major test variable was the test serum. Figure 8 represents a summary of the effects of AZT (A) and lack of treatment (B) on the levels of ADCC activity. The percent ADCC tabulated for each serum sample represents the mean value of two assays. A significant difference in the levels of ADCC activity was observed in patients prior and after initiating AZT therapy. A decrease in the levels of ADCC activity ($p<.05$) was observed in most patients and this decrease occured as early as 18 weeks of therapy (see figure 9 and 10). In addition this decrease was dose-dependent. When comparisons among all patients were made , this decrease became more evident at 36 weeks of therapy. Patients receiving placebo or ribavirin treatment demonstrated constant or increasing levels of HIV-1 specific ADCC throughout the study trial (see figure 11). In essence, no definite pattern or fluctuations in the levels of ADCC were observed in the ribavirin study .

When AZT therapy was interrupted for a 6 week washout period (see figure 8), the level of HIV-1 specific ADCC, represented by the geometric mean, remained constant in the patients tested. However, if we look at each patient individually (see figure 9), we find that some patients demonstrated an increase in the ADCC activity during the washout period and even after AZT was reinitiated. Those that did show an increase, did not increase to levels comparable to the values obtained prior to the start of therapy. The ribavirin study did not include a washout period and comparisons could not be made between both studies.

FIGURE 8. Longitudinal follow-up of HIV-1 specific ADCC in patients treated with AZT (A) and patients not treated (B) for a period of 84 weeks . Each data point represents the mean value of two assays expressed as % ADCC. The geometric mean is indicated by the horizontal line.

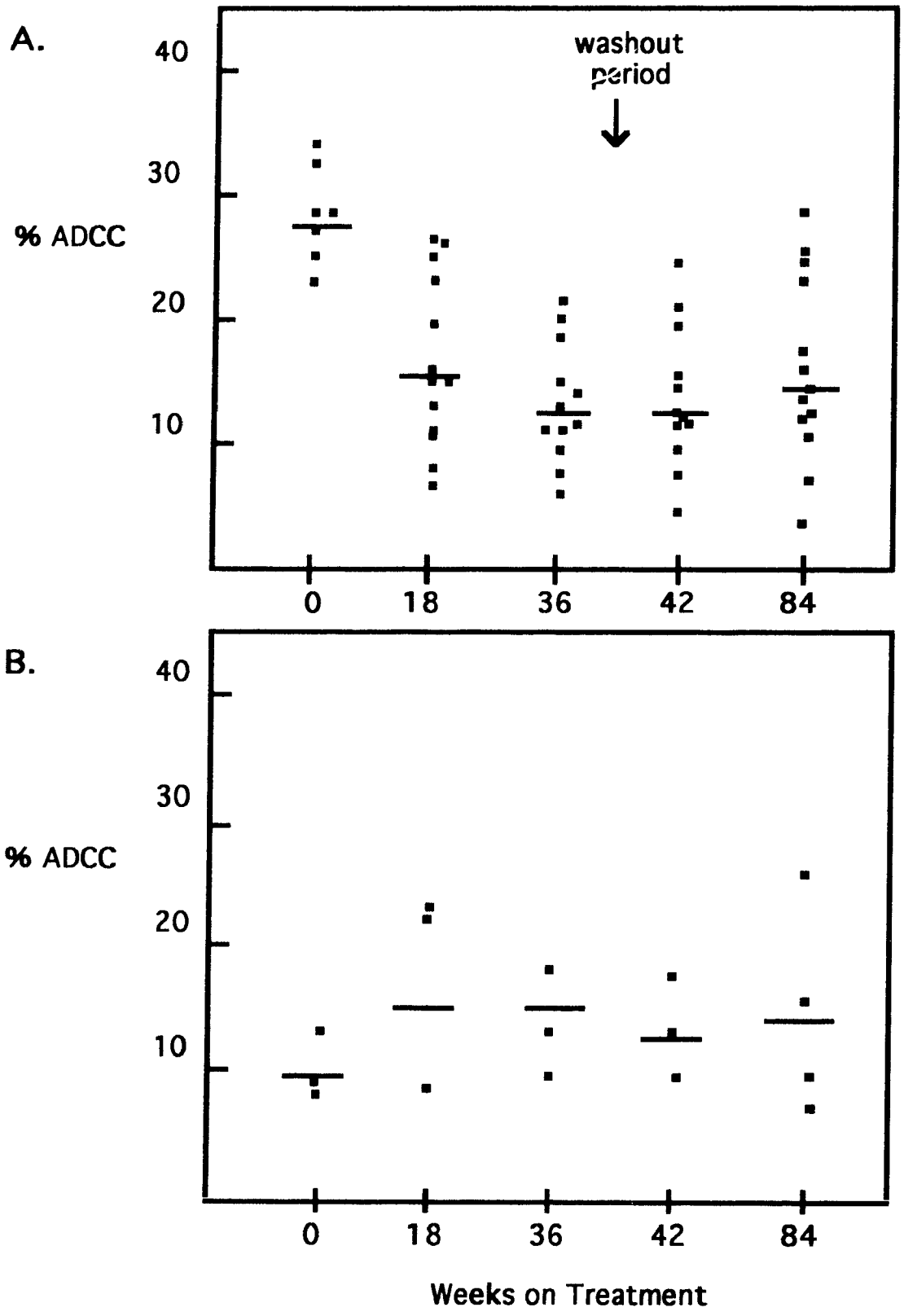

A.
40
30
20
10
% ADCC
washout
period
0
18
36
42
84
B.
40
30
20
10
% ADCC
0
18
36
42
84
Weeks on Treatment

FIGURE 9. An individual profile of the HIV-specific ADCC activity from patients randomized to AZT . The number in each graph represents one patient in which the *env* -specific ADCC activity was evaluated at each of the time intervals.

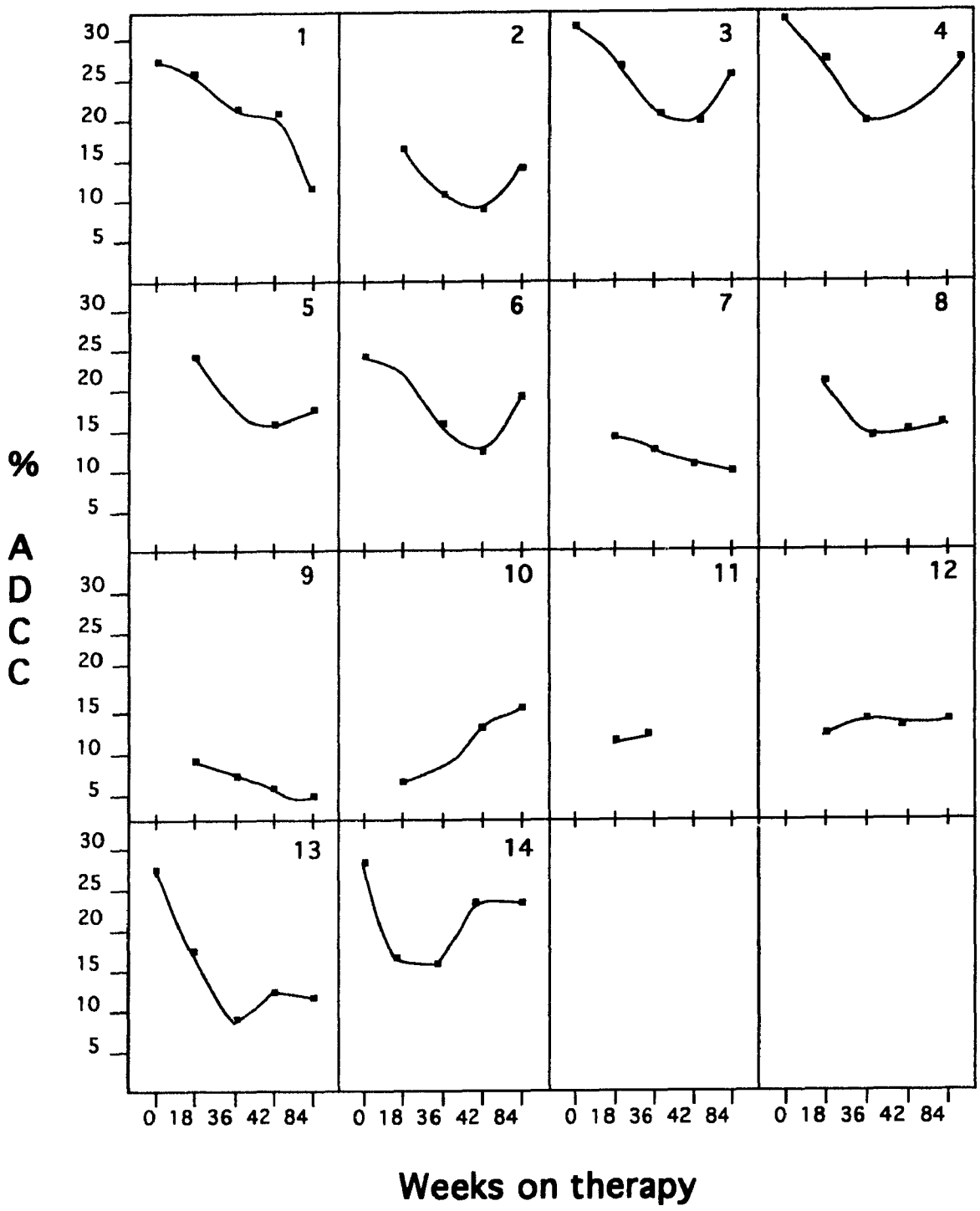

1
2
3
4
5
6
7
8
9
10
11
12
13
14
30
25
20
15
10
5
0 18 36 42 84
% ADCC
Weeks on therapy

FIGURE 10. Monitoring relative HIV-specific ADCC lysis from patients treated with AZT. (n=6)

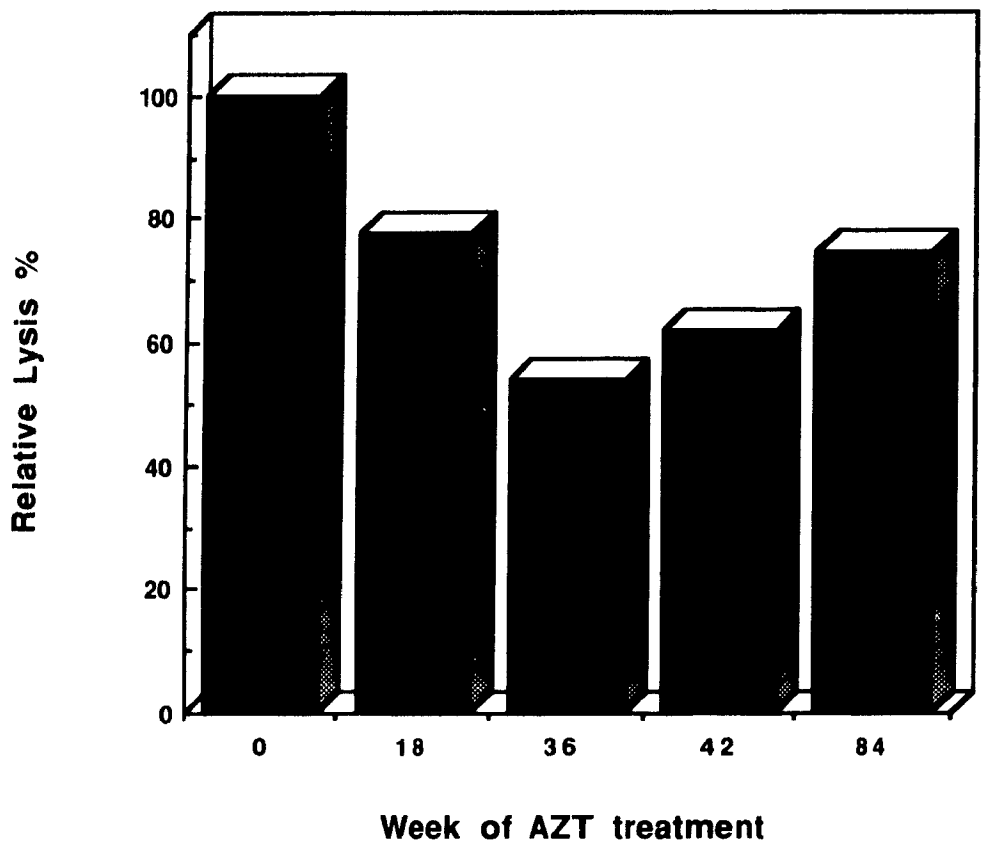
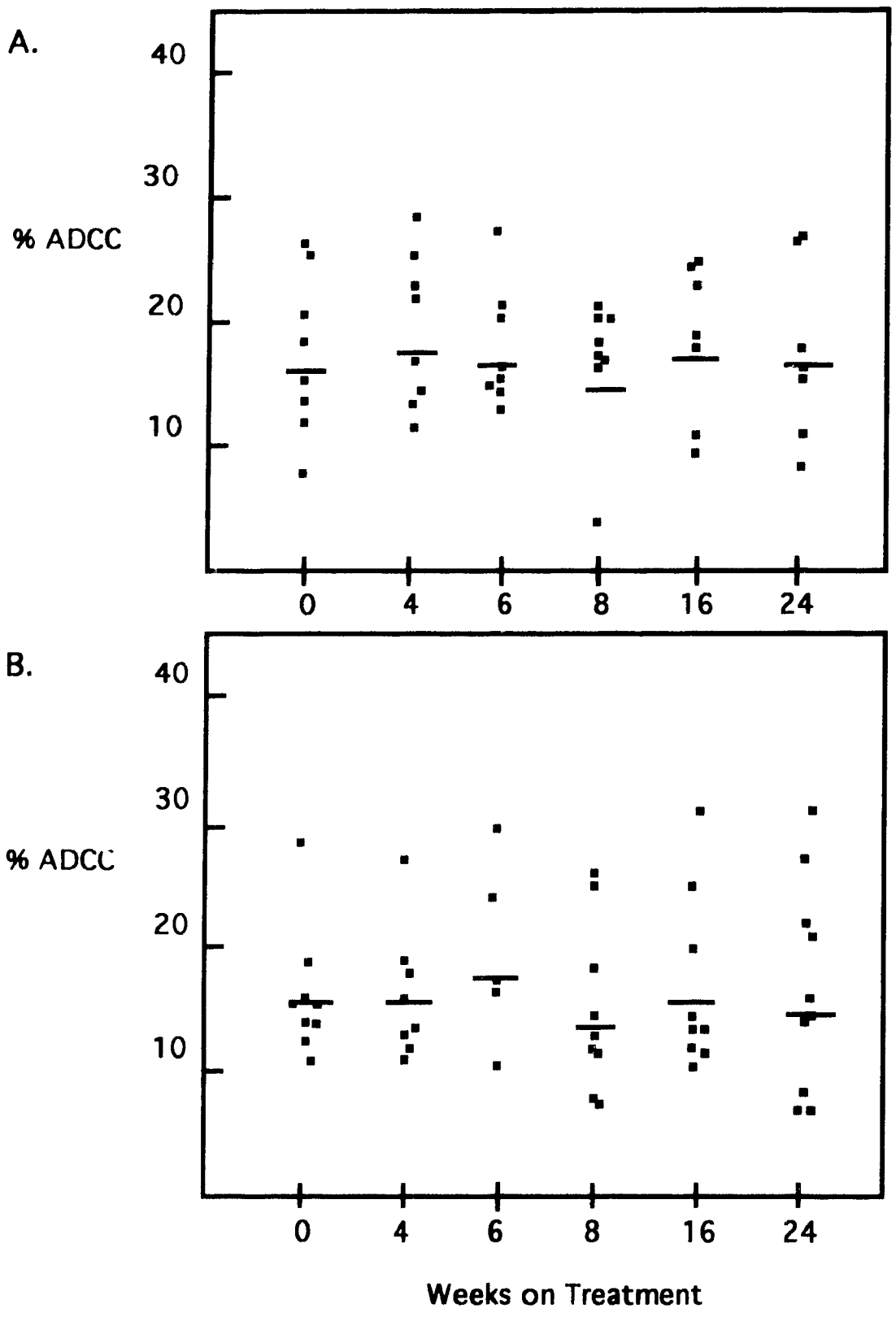

Relative Lysis %
100
80
60
40
20
0
0
18
36
42
84
Week of AZT treatment

FIGURE 11. Longitudinal follow-up of HIV-1 specific ADCC in patients randomized to ribavirin (A) or placebo (B) treatment for a period of 24 weeks. Each data point represents the mean value of two assays expressed as % ADCC. The geometric mean is indicated by the horizontal line.


A.
40
30
% ADCC
20
10
0
4
6
8
16
24
B.
40
30
% ADCC
20
10
0
4
6
8
16
24
Weeks on Treatment

Neutralizing antibodies

Neutralizing antibody titers were evaluated for each of the study trials (see table 1). No statistical variation was observed in the geometric mean for each individual randomized to ribavirin or placebo. Only in patients receiving AZT did we a observe a slight increase in the titers ($p<.05$). However, this increase was short lived. In general, neutralizing antibody titers were considerably lower than HIV-1 specific ADCC antibody titers. The neutralizing antibody titers ranged from 16 to 512.

Immunologic data

CD4 cell counts were also evaluated pre and post therapy . No statistical difference was observed in the levels of CD4 cells from patients randomized to AZT (see table 2). Data could not be obtained from patients not treated with AZT because blood samples were not available. A progressive reduction in the number of $CD4^+$ cells from baseline was reported in patients receiving ribavirin (see table 3). The reduction in CD4 cells from baseline occurred by week 4 and continued throughout the rest of the study. The reduction was significant at each week ($p<.05$). The median change of CD4 cells from baseline at week 4 is -98, at week 8 is -191, at week 16 is -187 and at week 24 is -212. Patients in the placebo group did not display any significant differences in CD4 counts measured during the same time intervals as the ribavirin treated patients.

Virologic data

Viral isolation provides information about the presence of HIV-1 in PBMCs, regardless whether such cells are latently infected or actively engaged in the production of progeny virus. Reverse transcriptase assays were performed on cocultured patient and phytohemmagglutinin-stimulated target cord blood PBMC at the

Table 1. Neutralizing antibody titers expressed as the reciprocal serum dilution required to inhibit HIV-1 cytopathic effects from patients randomized to AZT, ribavirin or placebo. (n=number of samples)

Patient	n	Week	Geometric mean of the neutralizing antibody titer ± s.e.m.
ZIDOUDINE	4	0	70.7 ± 9.5
	11	18	107.3 ± 8.9
	10	36	108.1 ± 13.0
	10	42	80.0 ± 8.9
	11	84	98.5 ± 14.3
RIBAVIRIN	9	0	70.6 ± 17.2
	6	2	40.3 ± 3.9
	8	4	45.2 ± 9.6
	5	6	32.0 ± 7.7
	9	8	90.5 ± 11.3
	9	16	40.3 ± 10.4
	10	24	33.6 ± 11.2
PLACEBO	9	0	69.7 ± 9.1
	4	2	45.3 ± 11.2
	8	4	64.0 ± 6.7
	5	6	32.0 ± 8.4
	9	8	34.6 ± 12.6
	9	16	40.3 ± 2.8
	10	24	42.2 ± 5.1

Table 2. CD4 cell counts (cells/mm3) from patients randomized to AZT

	Week of AZT Treatment				
Patient No.	0	18	36	42	84
A-1	1230	870	980	1010	1080
A-2	610	280	550	650	370
A-3	500	640	435	410	360
A-4	500	560	530	340	500
A-5	1580	870	N/D	825	950
A-6	920	N/D	690	670	785
A-7	380	280	345	240	260
A-8	1270	1130	870	2045	890
A-9	520	690	425	620	360
A-10	410	320	170	320	20
A-11	1320	850	N/D	1130	970
A-12	440	490	520	590	550

Table 3. CD4 cell counts (cells/mm3) from patients randomized to ribavirin or placebo treatment

	Week of Ribavirin Treatment				
Patient No.	0	4	8	16	24
R-1	551	437	360	368	315
R-2	340	342	297	320	360
R-3	399	357	150	255	234
R-4	825	667	198	528	336
R-5	559	378	180	272	N/D
R-6	680	650	735	468	425
R-7	546	448	391	437	357
R-8	420	434	525	322	340

	Week of Placebo Treatment				
Patient No.	0	4	8	16	24
P-1	703	595	N/D	550	648
P-2	504	416	432	520	408
P-3	322	320	288	391	304
P-4	798	832	756	609	572
P-5	224	525	391	357	231
P-6	567	567	N/D	551	432
P-7	663	594	540	676	630
P-8	520	408	345	336	390
P-9	N/D	792	660	546	600
P-10	510	570	450	567	550

Table 4. HIV detection from PBMC co-cultures. Recovery of HIV-1 was assessed by reverse transcriptase assays on material pelleted from phytohemagglutinin-stimulated co-cultures of patient PBMCs and cord blood lymphocytes. The time to HIV-1 recovery (days) is indicated in the bracket.

	Weeks on AZT Treatment					
Patient No.	0	18	36	42	72	84
A-1	+(3)	i	+(7)	+(6)	+ (3)	+(11)
A-2	- (12)	- (13)	- (8)	i	+ (3)	+(4)
A-3	+(7)	+(9)	+(6)	+(6)	+ (3)	+(1)
A-4	+(1)	+(2)	+(2)	- (1)	+ (1)	+(2)
A-5	+(8)	- (9)	- (7)	+(6)	+ (3)	+(4)
A-6	+(3)	+(6)	- (8)	+(4)	+ (6)	- (6)
A-7	+(6)	i	- (8)	+(7)	+ (7)	+(4)
A-8	+(6)	- (10)	- (9)	- (8)	+ (4)	i
A-9	+(3)	i	- (9)	+(3)	+ (3)	+(3)
A-10	- (14)	i	- (8)	+(5)	+ (4)	- (7)
A-11	i	+(1)	+(4)	+(3)	+ (3)	+(7)
A-12	i	+(5)	+(7)	+(2)	- (8)	+(3)

(+) HIV-1 Positive
(-) HIV-1 Negative
(i) Indeterminate

various time intervals. An anti-HIV effect was defined as two consecutive negative cultures, or sustained prolongation in days to positive culture. By these criteria, an anti-HIV effect was observed in patients treated with AZT (see table 4). HIV-1 was isolated at entry in 8 of the 12 patients (66%) receiving AZT with a median time of HIV recovery of 4.5 days. Only two patients had negative cultures at time 0. During AZT therapy, the ability to isolate virus decreased to approximately 40% with a median time of recovery of 5.5 days. The two patients that were negative prior to the start of therapy remained negative. No patient that was initially negative became positive while receiving AZT. Once AZT therapy was interrupted, the ability to isolate virus increased by 35% with a median time to recovery of 3 to 5 days ($p<.05$). The two patients that remained negative during AZT therapy, now became positive. The cultures remained positive even after AZT was reinitiated at a high dose of 1200 mg/d for an additional 42 weeks.

CHAPTER FOUR: DISCUSSION

DISCUSSION

The mechanisms involved in the immune response to HIV-1 infection are largely undefined. However, evidence suggests that deficient humoral or cellular immunity has been associated with an increase in the severity of infection and dissemination of the virus. Some factors influencing the development of humoral and cell-mediated immunity to HIV-1 infection are host specific and genetically determined (186,187). Other factors that modulate immune response relate to the level and type of antigenic stimulation and therapeutic interventions.

Because of the long latent period between infection with HIV and the development of AIDS, it is important that predictive markers, either viral or immunologic, be developed as tools in the management of HIV infected subjects. The most commonly used markers of disease progression today are; the absolute T4 counts, the p24 antigen levels, and the Beta-2 microglobulin concentrations (188-190). The need for these markers in studies of HIV are of particular importance because all candidate drugs to date suppress but do not eradicate infection. Although these markers are used to assess the effects of study drugs against HIV, little is known about the possible effects and consequences that antiviral therapy may have on the host's immune response. Some preliminary studies of immune functions have been reported for patients on therapy with AZT such as improvements in; skin hypersensitivity (191), T helper cell function (192) and HIV-specific CTL activity (193) .

Our retrospective study of humoral immunity and its response to antiviral therapy in a population of HIV-1 seropositive patients provided the first insight into the interpretation of the serological response as assessed by indirect ADCC and neutralizing antibodies to HIV. Two therapeutic modalities, zidovudine (targeted at retroviral replication) and ribavirin (which interferes with viral protein synthesis) were evaluated in this study. Recombinant vaccinia

virus-vectors expressing foreign viral genes have been valuable in defining immune responses in HIV and other viral infections and were chosen in this study for three reasons. First, HIV is known to infect BLCLs and B cells, allowing the assay results to have some reliance to *in vivo* infection . Second, the use of recombinant vaccinia virus expressing HIV genes allowed us to present HIV *env* in infected cells. Thirdly, BLCLs infected with vaccinia virus vectors expressing foreign viral proteins are known to be relatively resistant to NK cell lysis in the absence of antibodies. These targets along with patient sera and normal effector cells were used in the classical chromium release assays.

Our results have shown that sera from patients infected with HIV are active in ADCC and in the killing of HIV infected cells. Most interesting of all, our report indicates that AZT diminishes the levels of antibodies mediating ADCC. Note, the effect is only HIV-specific and does not imply a general decrease in antibodies to other antigens (i.e. recall antigens, influenza virus, *Tetnus toxoid*, etc.). Unfortunately, we were unable to evaluate other antibody responses. In the case of HIV, other antigens do not serve as targets for ADCC. This decrease was particularly evident at 36 weeks of therapy when a maximum dose of 1200 mg/d was given orally to these patients. The placebo or ribavirin treated group did not exhibit similar patterns. This decrease in HIV-1 specific ADCC antibodies parallels closely with the decrease (by 25%) in the ability to culture virus during AZT treatment. The reduction in the ability to culture virus during prolonged AZT therapy (36 weeks) is indicative of its antiviral activity. Viral isolation results from patients not receiving AZT or from the ribavirin study were not available. Nevertheless, if we look at similar studies, we find that no decrease in HIV isolation is reported in patients who were not treated with AZT (194). They report that their rate in virus yield is 40% at each of the time intervals. In addition, another study has observed no difference in HIV isolation from patients randomized to ribavirin or to placebo during similar time intervals (195). They report that prior to the start of therapy 75-83% of patients had

positive cultures and remained so thereafter. These observations indicate that ribavirin had no demonstrable beneficial effect on virologic HIV surrogate markers.

It is unlikely that these observations exhibited during AZT therapy are due to the natural progression of the disease because the significant decline in the levels of antibodies mediating HIV-specific ADCC in patients treated with zidovudine contrasts with the failure to observe such a difference in patients receiving placebo or ribavirin treatment. In addition, the decrease observed can not be attributed to the lymphopenic effect of AZT or HIV because, according to flow cytometric analysis, quantitative alterations in the levels of CD4 cells were not detectable among circulating PBLs cells from patients receiving AZT. Generally, other reports (196) have shown transient increases in the level of CD4 cells during AZT treatment. The increase occurs as early as 4 weeks and persists through 16-20 weeks of therapy. Since the absolute numbers of CD4 cells generally increase within the first few months in many subjects, it is possible that the beneficial effect was overlooked based on the time intervals chosen for this protocol. In addition, no correlation could be observed between the proportion of CD4 cells and the level of HIV-1 specific ADCC at anytime. We observed that even patients with severe T helper cell depletions maintained high-titer serum. Others have also reported that the level of HIV envelope-specific ADCC noted in HIV-seropositive sera did not differ significantly between individuals with near normal (>400ul/ul) numbers of T helper cells and those with severely decreased (<200/ul) number of T helper cells (121). In contrast, a progressive reduction in the number of CD4 cells from baseline was reported in patients receiving ribavirin and not placebo. The per-patient change from baseline was significant ($p<.05$) . These results are in accord with those of larger multicentre trials (197).

Based on our results and those reported by others, we can

speculate that the decrease observed in the levels of antibodies mediating HIV-specific ADCC is somehow correlated with the decrease observed in the virus load. AZT may directly or indirectly decrease this aspect of the humoral response to HIV-1 infection by decreasing the quantity of viral antigen. We feel that the antiviral effect of AZT is a more likely explanation of our results than is a direct immunosuppressive effect of the antiviral drug. One way to exclude the latter is by infecting an ideal animal with an AZT-resistant mutant strain and observing the effects of AZT therapy. If AZT were to decrease the humoral response to AZT resistant strains, then we would assume that the depression, as measured by indirect ADCC, is not dependent on inhibition of the virus but due to direct immunosuppression. Due to the lack of an optimal HIV animal model, it is difficult to draw such conclusions.

Neutralizing HIV antibody titers did not differ significantly between each time interval regardless of the treatment. Thus, it is most likely that viral replication modulates antibody production to some HIV glycoproteins without necessarily affecting neutralizing antibody titers.

The impact of a washout period was also analyzed. The ribavirin study did not have a washout period, therefore comparisons could not be made. Analysis of serum samples obtained 6 weeks after treatment had been discontinued showed that most but not all the AZT treated patients appeared to have developed a full compliment of ADCC antibodies. Only 7 out of 14 patients showed a significant increase. This increase was not comparable to values prior to the start of therapy. For those that did not demonstrate a significant increase, it is possible that if enough time elapsed between cessation and reinitiation that a increase could have been observed. Cessation of AZT therapy had no effect on the levels of CD4 cells and neutralizing antibodies. An observable difference was noted in the ability to isolate virus from patients receiving AZT. Our results and those by Wainberg et al (194), in which a larger population of patients were studied , both reported

that cessation of chronic administration of AZT therapy, for reasons of drug toxicity or patient noncompliance , was followed in most instances by an increase in viral isolation and a decrease in time to culture positivity . The rate of viral isolation during the washout period, significantly exceeded the rate during the initial 36 weeks of treatment and that prior to the start of therapy. Although the outcome of these results are statistically significant, it is important that these results are also of clinical significance . The long-term clinical implications of these findings are as yet undefined however, this increase in viral burden observed during the interruption of AZT treatment may reflect the inability of the treated host to mount a significant immune response needed to control HIV replication. As a consequence, this may result in an increase in the rate of replication which may lead to an increase in the generation of HIV-1 resistant mutants over a long period of time. This may explain why the rate of HIV isolation and ADCC antibody levels are just as high or higher in most individuals even weeks after AZT is reinitiated. Finally, the generation of these mutant viruses will ultimately evade the immune response and create a viscious cycle which culminates in the rapid deterioration of a patient.

Besides the risks involved, one advantage of a diminished humoral response may be by limiting the amount of auto-antibodies which have been attributed to the depletion of CD4 cells and other disorders. Many reviews have shown evidence that HIV may trigger a self destructive immune response, for which appropriate models may be found in graft-versus-host disease, certain autoimmune disorders, and some animal viral infections. If this hypothesis gains support, there are profound implications for prevention and treatment.

The lack of a consistent increase in antibodies mediating ADCC associated with a decrease in virus load (p24) argues against the concept of drug-induced immunologic restoration. The findings also argue against recent reports of improved T helper cell function and enhanced HIV-specific cytotoxic T lymphocytes during AZT

treatment. However , these benefits are short lived and occur only in the first weeks of therapy. On the other hand, the effects observed with AZT are similar to those effects observed with other antiviral agents such as acyclovir (198) or rimantidine (199). As mentioned earlier, acyclovir and rimantadine are associated with a decrease in the humoral response to HSV and a decrease in cellular T lymphocyte responses in mice infected with influenza A virus respectively.

The knowledge gained by these results provides information essential to make rational therapeutic decisions in individual patients. It is known that the nature of each patient may differ for a variety of reasons including the stage of the epidemic. Therefore each patient responds differently to a given regimen. As a result, the precise tailoring of dosage to the needs of a particular patient would be required. These results suggest that lower doses of AZT (300-600 mg/d), unlike those utilized in this study, would be just as effective at preventing clinical deterioration while being less toxic or suppressive. The degree of inhibition or suppression may relate to the rate at which patients progress to HIV disease. If the degree of inhibition is limited, this would allow a restoration of a "normal" immune if cessation of AZT should occur. These results suggest that the evolution of ADCC function may be used as an indicator of progression towards HIV-induced disease. Although AZT might not be able to cure HIV, its the only drug shown to be reasonably effective . This study does not in any way disfavor the use of AZT. Nevertheless, it does suggest that its administration or cessation should proceed with caution . Most of the major problems arise as a consequence of either terminating or interrupting AZT therapy. It would be of interest to study the effects of other antiviral agents such as ddI or ddC to determine if they follow a similar pattern.

In conclusion, whether a decrease in the ADCC antibody response to HIV is beneficial or detrimental is debatable. One has to question the role of ADCC in HIV infection. If ADCC plays a major role in the defense against HIV, then suppression of this response

may contribute to the progression of the infection. On the other hand, if AIDS is an autoimmune disease directed at the immune system and triggered by HIV, then the effects of AZT may prove beneficial by limiting the amount of autoantibodies.

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