Asymptomatic Infection Of The Human Immunodeficiency Virus

Kimberly L. Booke
Lake Forest College
ASYMPTOMATIC INFECTION OF THE HUMAN IMMUNODEFICIENCY VIRUS

by Kimberly L. Booke

Advancements in modern medical technology seemed to have virtually wiped out the threat of worldwide infectious disease epidemics. By the early 1980's, however, scientific communities around the globe began to gather evidence that would quickly shatter the earlier conceptions that we had effectively won the battle against infectious diseases. The isolation of the human immunodeficiency virus (HIV) in 1985 brought the threat of an infectious disease epidemic back to the forefront throughout the world and spurred a massive effort by scientific communities to understand the virus and its ability to spread, with the hope of gaining control over the epidemic.

The human immunodeficiency virus (HIV) is a retrovirus that attacks the immune system, specifically cells expressing the CD4 receptor such as T helper cells. This attack on the immune system eventually leads to severe immune suppression and causes a person to become open to life-threatening opportunistic infections. Suppression of the immune system by HIV results in a condition called acquired immune deficiency syndrome (AIDS), and it is from AIDS, not the virus itself, that the infected person dies.

HIV is passed from person to person primarily through sexual contact and/or IV drug use. Once infected with HIV, a person may remain asymptomatic for many years. This period of asymptomatic infection is a major cause for the rapid and silent spread of HIV throughout the world. Thus, a major concern of researchers is the ability of HIV to "hide" from the immune system. How does HIV effectively avoid attack and destruction from the immune system, and what triggers the shift from a latent unproductive viral state to a state of active viral production? An understanding of these questions could lead to the development of vaccines and treatments that would provide immeasurable benefit to both those people already infected and others at risk of infection.

In order to understand the mechanisms of HIV latency, it is first necessary to understand the molecular aspects of the virus, specifically its structure and life cycle. When HIV is passed from person to person through body fluid contact, the virus will come in contact with the immune cells of the newly infected person. The use of a host cell is absolutely necessary for viral replication and survival. The virus consists of envelope proteins, glycoproteins called gp120 and gp41. The gp120 protein protrudes out from the
viral membrane and binds to target cells that exhibit a CD4 molecule on the membrane surface. Binding of gp120 to the CD4 receptor results in a conformational change that allows the gp41 protein to become exposed. The gp41 protein attaches to the host membrane, and the two membranes fuse together (1). The virus core, which consists of the viral genetic material, is then injected into the host cell. With successful entry into the host cell, the life cycle of HIV can begin.

The first step of the HIV life cycle rests on the ability of the virus to use reverse transcriptase to convert the viral genetic information encoded in the viral RNA to a complementary DNA strand. The RNA template is destroyed in unison with reverse transcription of the RNA to DNA. DNA polymerase is used to make the second copy of the DNA strand (1). The double stranded DNA consists of redundant DNA sequences at each end of the DNA strand, which are referred to as long terminal repeats (LTR’s) (2). The DNA is spliced into the host genome by the action of the enzyme integrase. The incorporated viral DNA (provirus) can then be replicated along with the host’s DNA and can either remain latent within the host’s genome or begin assembly of new viral particles.

If the virus is activated to produce new viral particles, then sequences within the LTR’s regulate the host cell enzymes in such a way that transcription of the provirus to RNA and activation of the viral genes occurs. The RNA directs the synthesis of new virions (1). The regulatory genes of the virus—tat, rev and nef—regulate their own expression and the expression of the structural genes. Tat is a positive regulator and triggers the other genes to become activated or “turned on.” Rev is a differential regulator and triggers structural gene expression while suppressing all other genes. Nef is a negative regulating gene and suppresses the action of other genes. It is important to understand that latency and activation of cellular components are precisely controlled and connected. If cellular activation does not occur, then the regulatory proteins, especially the positive regulator tat, are prevented from directing assembly of new viral particles, and the virus remains latent (2).

In order to completely discuss the concept of latency, it is first necessary to define what is meant by the term. A latent infection is characterized by the integration of proviral DNA into the host genome so that periods lacking viral expression exist (2). Latent infections should be distinguished from productive persistent infections which can occur in three different states. A steady state infection refers to viral resistance to cell death which results from an established equilibrium between the virus and the cell. A carrier state infection refers to resistance that may be a result of cell type, defective viral forms,
or cell cycle. Intracytoplasmic persistence is an infection that results from ineffective viral production despite viral spread from cell to cell (2).

A resting state form of HIV latency occurs when the virus integrates into a host cell that is in the $G_0$ phase. This phase lacks all of the activation and proliferation factors required for cell replication (2). Bednarik and Folks (2) have found that HIV tends to resist integration into a host genome when it is in the $G_0$ phase, but if it does become integrated into a host cell at this phase, reverse transcription will occur only incompletely. Thus, a cell may become infected, but it will serve primarily as a storage space for the virus until an activation signal is encountered and viral replication can continue. The activation signals may come from specific antigens, antibodies, mitogens, or cytokines binding to receptors on the cell surface (2).

Latent HIV infection may occur in different states of viral expression, all of which could be induced into the active viral state according to Bednarik and Folks (2). They identified five different forms of viral infection. Type I viral expression results from an extrachromosomal form of HIV expression where reverse transcription is incomplete. Type II refers to a wild type form of HIV which is capable of full expression but is uninduced. Type III is also a wild type form of HIV but is latent due to viral regulation that results from incomplete production of regulatory proteins. Undetectable or sub-detectable levels of RNA or proteins characterize type IV viral expression. Type V viral expression represents the complete or absolute latency, and viral expression is most likely controlled by cellular factors.

Latency may also be a result of differences or variations from cell to cell (2). Bednarik and Folks (2) have found that under uninduced viral conditions, the viral genome exists almost exclusively in the full length state. In this form of latency, the CD4 receptor molecules remain expressed on the outside of the host cell membrane. It is only when the cell is activated with cytokines that the cell will express viral antigens.

Bednarik and Folks (2) have proposed six models of transcriptional repression of the proviral DNA. These models include: 1) cellular or viral repressor DNA-binding proteins; 2) chromatin conformation; 3) DNA hyper-methylation; 4) pre-integrative viral species; 5) mediation of transcriptional transactivation by cellular nuclear transcription factors; 6) HIV regulatory proteins. The cellular or viral repressor DNA-binding protein model suggests that a repressor may bind to sites of initiation along the long terminal repeats. Binding to the initiation site would result in structural restrictions that prevent transcriptional elements from binding and, therefore, transcription from occurring. Bednarik and Folks (2) do not elaborate on or present a mechanism for the chromatin
conformation model. Chromatin is a nucleic acid-protein complex which is found in chromosomes (3). I speculate that during DNA replication, when the chromatin begins supercoiling and becomes more dense, the initiation sites, as in the repressor model, become blocked and prevent transcription.

According to Bednarik and Folks (2), gene expression and control is regulated primarily by the methylation of cytosine to 5-methylcytosine in mammalian DNA. This mechanism has also been detectable in the retrovirus, murine leukemia virus (MuLV). Inactivation of transcription in MuLV can be achieved when the LTR becomes hypermethylated, following integration into host chromosomal DNA. Suppression of transcription could also be reversed after treatment with mitogens or DNA methylation antagonists (2). Based on this information as well as other previous studies on hypermethylation, Bednarik and Folks (2) suggest that the provirus may become integrated in a region of the host's DNA that is hyper-methylated. If factors that reverse the methylation processes are introduced, then the barriers against transcription are removed, the viral regulatory proteins can begin to accumulate, and initiation of viral production occurs.

The HIV regulatory protein nef is a negative regulator of transcription and replication (1). Guy et al. (4) found that the nef gene down-regulates the DNA binding factor, Factor A1, which belongs to a group of cellular activation factors. They also found that factors affecting nef, such as mutations, will result in an alteration of viral production. Viral production is increased with the expression of rev, which acts as a down regulator of nef (4). Therefore, viral production can be controlled by factors that affect both rev and nef, and they can potentially induce a switch between a state of latency and viral transcription (4). Malim and Cullen (5) have found that productive viral infection can only occur if a critical level of rev is expressed. When rev protein is at low levels, the rev gene is almost completely non-functioning, and this may be a cause for latent infection (5).

Kato et al. (6) have found a cellular negative regulatory protein (LBP-1) that interacts with the HIV promoter region that includes the TATA element necessary for transcription. The binding site on the promoter region consists of a low affinity site (site I) and a high affinity site (site II). At high concentrations of LBP-1, both site I and site II are covered by LBP-1, whereas at low concentrations only site I is covered. According to Kato et al. (6), binding to site I resulted in increased transcription, while binding to site II resulted in suppressed transcription. Therefore, LBP-1’s binding specificity could act as a regulator of transcription, which may have implications for latency.
The pre-integrative viral species model refers to the process of incomplete reverse transcription. Cells in this state maintain this form of HIV in a non-integrated state until HIV is stimulated by cellular activators (2). Bednarik and Folks (2) also noted that this non-integrated DNA is unstable but is abundant because of continual reinfection.

Kalebic et al. (7) examined the effects of glutathione (GSH), glutathione ester (GSE), and N-acetylcysteine (NAC) on HIV expression, using a monocytic U1 cell line. U1 cells exhibit low levels of viral expression and can be activated or suppressed by providing the appropriate stimuli. According to Kalebic et al. (7), transcriptional activation can be induced by factors such as phorbol 12-myristate 13-acetate (PMA), tumor necrosis factor (TNF-α), and interleukin 6 (IL-6). They also found that GSH, GSE, and NAC could produce a suppressive effect in induced cells. Their results indicate that the level of TNF-α in the blood plasma of AIDS patients is increased with an associated decrease in GSH. Therefore, levels of transcriptional suppressor factors are decreased in patients with AIDS, which may indicate that these factors play an important role during latency by effectively suppressing activation factors. Pomerantz et al. (8) noted that almost no RNA is produced in infected cells during the early stages of infection, but when induced by factors such as PMA the infected cell quickly progressed to later stages of infection with significant viral production and RNA synthesis. The control of transcription is due to the levels of Rev which code for singly-spliced mRNA's or multiply-spliced mRNA's. The multiply-spliced mRNA's code for regulatory proteins, and the singly-spliced mRNA's code for structural proteins (8). If the singly-spliced mRNA's are not available to code for structural proteins, then synthesis of new viral particles cannot occur, and the virus must remain latent. Thus, mediation of the transcription inducers could provide a treatment with, perhaps, a remission into latency for AIDS patients.

Now that the mechanisms for HIV latency have been explored, it is worthwhile to examine the effects of latency on the HIV infected person. "Asymptomatic" is not an accurate phrase to use when discussing the latent period of HIV infection. This period of infection can be identified by a number of signs and symptoms that affect the living quality of each infected person. If a person in the latent phase of infection, which may last (on the average) ten years, is compared to a person with full blown AIDS, the former does indeed appear "asymptomatic." The person is free from life-threatening opportunistic infections, his immune system is not severely suppressed, and his life continues as normally as possible.

Hoover et al. (9) examined the signs and symptoms of asymptomatic infection in homosexual men for the multicenter AIDS cohort study. At six month intervals, 916 ho-
homosexual/bisexual men who were HIV seropositive and AIDS free were evaluated by personal interviews, physical examinations, and laboratory tests. Also enrolled as controls were 2,161 HIV seronegative men. Mean values were calculated for comparison at visit 3 and visit 7. The seropositive subjects reported 0.2 more symptoms, including fever, thrush, headaches, new rashes, and unusual coughs compared to the seronegative subjects. Skin rashes showed an increase of four percent for the seropositive men versus the seronegative men, indicating that this condition may be highly correlated with HIV infection in the asymptomatic stages (9). Hemoglobin levels and T-helper cell levels were significantly lower in visit 7 compared to visit 3 for the seropositive subjects. Seropositive subjects also had a lower body mass index at both visits than the seronegative controls. Hoover et al. (9) noted that diarrhea and skin discolorations did not differ between the two groups, which may indicate that these symptoms are more indicative of the later stages of infection, including AIDS related complex. Based on the results indicating that seropositive men report 0.20 more symptoms, it was calculated that these men see an increase of 5.6 days per year of discomfort.

Hoover et al. (9) postulated that the increase in reported headaches for seropositive asymptomatic men may be due to the effect of HIV on the central nervous system. Sinforiani et al. (10) specifically examined cognitive abnormalities in relation to disease progression in asymptomatic seropositive subjects. In this study, forty-one asymptomatic seropositive homosexual/bisexual men from an age range of 19-60 and an educational level range from 3-17 years, and forty-one seronegative men with a similar age and educational level range, underwent neuropsychological evaluations. The evaluation included nine tests which assessed short term verbal memory and attention, logic memory, visual reproduction, non-verbal intelligence and visuo-spatial abilities, logic abilities, visuo-motor and attentive functions, selective attention, word fluency, and visuo-perceptive abilities. Sinforiani et al. (10) found that there was no significant difference in cognitive, neurological, and neuropsychological abilities between the asymptomatic seropositive group and the control group. One drawback of this study is that it was a cross-sectional study, and a change in a person's cognitive ability over time was not examined. I was unable to find a longitudinal study on the effects of HIV on the CNS that follows a person from diagnosis of HIV and the asymptomatic stage through AIDS, but I believe that such a study would provide a great deal of insight into this area of research.

Lifson et al. (11) examined long term HIV infection in asymptomatic homosexual/bisexual men, in order to determine immunologic and virologic characteristics. The
study examined twenty-four men who had been seropositive for up to 10.5 years but had not yet developed AIDS, AIDS related complex, or low CD4+ levels (non-progressors). Two control groups were also used, men with AIDS and men seronegative for HIV. The study found that the non-progressors had increased levels of \( \beta \)-2-microglobulin compared to the seronegative controls but levels below that of the AIDS controls. \( \beta \)-2-microglobulin levels are used as a predictor of HIV progression, and the increased levels in non-progressors indicates that the immune system is affected in some manner. The increased levels may indicate viral replication, although maintained at very low levels, or it may represent another mechanism controlling viral replication so that a latency state is preserved. The non-progressors also differed from both control groups in that their CD8+ levels were higher. Lifson et al. (11) cited a number of studies to help explain the elevated CD8+ counts. One study found that elevated CD8+ counts signified development toward AIDS and declines in CD4+ levels, while the other study indicated that elevated CD8+ counts inhibited HIV replication. The latter is supported by the work of Lifson et al. (11) which presents data that implies that the elevated CD8+ levels serve as a suppressor and may help to control viral replication.

Based on the clinical differences between asymptomatic HIV patients and AIDS patients, it is not unreasonable to postulate that the virus may be both biologically and genetically different in the two stages of infection. Balachandran et al. (12) explored this premise and found that viral isolates from asymptomatic patients were clearly different from the isolates taken from AIDS patients. Both phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes (PBL) and H9 cells were not infected with asymptomatic isolates. Isolates from asymptomatic men, who remained asymptomatic for two to three years, were tested in a longitudinal study and found to be incapable of infecting H9 cells. On the other hand, a longitudinal study of asymptomatic men who later developed AIDS showed that they were later able to infect H9 cells. This change in the ability of the isolates to infect H9 cells may be caused by infection of an HIV strain that is more biologically and genetically similar to the strain found in AIDS patients, or the individuals may have been more susceptible to a more virulent strain of HIV (12). Balachandran et al. (12) also found that AIDS isolates were more likely to induce syncytia than the asymptomatic isolates. The ability of the AIDS isolates to replicate was found to be much greater than in the asymptomatic isolates, and AIDS isolates produced higher levels of viral DNA than the asymptomatic isolates.

Genetically, AIDS isolates and asymptomatic isolates differ in the restriction site Bg/II in the gag region of the genome, according to Balachandran et al. (12). In their
study of the Bg/II restriction site, six out of six asymptomatic isolates contained the site, while only twelve of the twenty-eight AIDS isolates contained the site. Although the significance of this site is unknown, it remains important to the study of HIV.

Although CD4+ cell counts remain the dominant predictor of disease progression for HIV and serve as the defining factor for separation of AIDS patients from HIV infected individuals, this is not the only method for prediction of disease progression. Dehydroepiandrosterone (DHEA) is a steroid hormone that enhances synthesis of interleukin-2 (IL-2) by activated T cells (13). Patients infected with HIV exhibit decreased levels of IL-2. In a study of DHEA as a predictor for progression, Mulder et al. (13) found there is a decline in DHEA levels from the seroconversion for HIV to the development of AIDS. In addition to DHEA, anti-p17 antibody levels can be used as a predictor of disease progression. P17 is an envelope protein that is displayed on the surface of infected cells so that antibodies are produced against it (1). Anti-p17 antibody tends to have a neutralizing effect against HIV, and Choudhury et al. (14) found that in a study of fifty-six asymptomatic subjects and forty-six AIDS subjects, the number of subjects not exhibiting anti-p17 antibodies increased as the disease progressed from CDC stage II/III to CDC stage IV. They also noted that the asymptomatic patients exhibited a strong antibody response, while the symptomatic patients exhibited little or no antibody response, and concluded that the decline of anti-p17 antibodies favors advancement towards AIDS.

All of the studies presented thus far have used homosexual/bisexual men as their subjects, but Deschamps et al. (15) examined the effects of childbearing on women with HIV. They conducted an observational study of forty-four women who became pregnant or were pregnant during the study. All women were seropositive and asymptomatic patients from Haiti. The study found that pregnant women developed signs and symptoms of HIV before nonpregnant women. Of the nonpregnant women, nineteen percent developed AIDS during the study, while thirty-two percent of the pregnant women developed AIDS. Deschamps et al. also found, however, that twenty-seven percent of the nonpregnant women died from AIDS during the study, as opposed to fifteen percent of the pregnant women. Thus, pregnancy was not found to reduce AIDS free survival time. Based on the results, the study theorized that pregnancy increased the rate of depletion of CD4+ cells but the effects of pregnancy still remain unclear.

Many different models and mechanisms for latency have been presented as well as a variety of studies on asymptomatic infection. This paper only touches the surface of the