

## Review

# Human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis*: A collaboration to kill

Jason L. Haskins, Jonathan Ladapo and Veronica C. Nwosu\*

Department of Biology, North Carolina Central University, 1801 Fayetteville Street, Durham, NC 27707, USA.

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HIV continues to be a serious problem in developing countries. It is estimated that these countries account for about 35% of all reported HIV cases in the world. Most of these countries, which are plagued by poverty and poor sanitation, have begun to see an increase in the number of tuberculosis (TB) infected cases. In decades leading to the 1990s, tuberculosis had been on a decline in the world and was thought to be on its way to elimination. However, during the early to mid 1990s health officials began to see a rise in the reported cases of TB, especially in developing countries. Even more astounding was that out of those reported TB cases, about 30% of the affected individuals also tested positive for HIV. These findings prompted a deeper look into the relationship between HIV and tuberculosis to better understand the pathogenesis of HIV and *Mycobacterium tuberculosis* and their combined capability for enhanced virulence.

**Key words:** HIV, tuberculosis, lymphocytes, macrophages, dendritic cells, CD4 receptors, chemokine receptors, alveoli cells, reverse transcriptase, protease inhibitors, rifampicin, rifabutin, HAART.

## INTRODUCTION

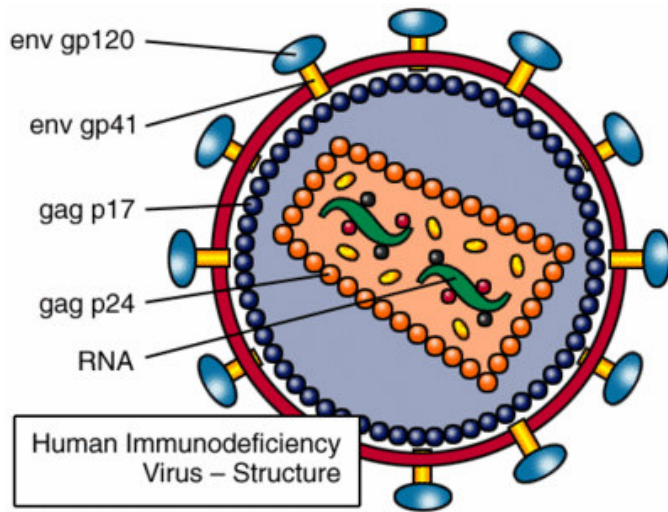
HIV and tuberculosis are the two leading causes of death in developing countries (Baltussen et al., 2005). About one-third of all reported HIV cases have also tested positive for tuberculosis (Dye and Williams, 2003). HIV is an enveloped human retrovirus found in the Lentivirus family (Figure 1), and was discovered in the early 1980s. Two strains of this virus have been identified; HIV-1 and HIV-2. HIV-1 consists of three groups, M, N and O while HIV-2 consists of seven subtypes, A – G with a possible eighth group, H (Marx, 2005). HIV-1 is more virulent,

while HIV-2 is found mostly in the western part of Africa (Coico et al., 2003). The reduced virulence in HIV-2 remains unclear, however, the glial cells in the primary central nervous system are susceptible to infection from HIV-2, but not HIV-1 (Albert et al., 1999). HIV-2 is genetically closer to Simian Immunodeficiency Virus (SIV) found in chimpanzees than HIV-1. HIV-1 is better studied and characterized than HIV-2. In particular, relative to the chemokine co-receptors, CCR5 and CXCR4, little is known about HIV-2 and its interaction with the chemokine co-receptors (Albert et al., 1999). The primary host cell for HIV is the class of T-lymphocytes containing the cell surface receptor protein CD4. The CD4 cells are subdivided into two functional population subsets: T helper cells 1 and 2 (T<sub>H</sub>1 and T<sub>H</sub>2). T<sub>H</sub>1 cells participate in cell-mediated immunity and are responsible for recruiting and activating phagocytic cells of innate immunity. T<sub>H</sub>2 cells stimulate B lymphocytes to activate antibody production via humoral immunity. Thus CD4 cells are critical immune system cells hence the devastating consequence of their role as hosts for HIV. The virus also infects macrophages and dendritic cells.

Tuberculosis caused by the bacterium *Mycobacterium*

\*Corresponding author. E- mail: [vcnwosu@nccu.edu](mailto:vcnwosu@nccu.edu). Tel: (919) 530-6170.

**Abbreviations:** HIV, Human immunodeficiency virus; TB, tuberculosis; SIV, Simian immunodeficiency virus; T<sub>H</sub>, T helper cells; RT, reverse transcriptase; Gp, glycoproteins; CXCR4 and CCR5, chemokine receptors; cDNA, complementary DNA; Rev/RRE, Rev responsive element; CTE, constitutive transport element; HAART, highly active anti-retroviral therapy, RNA, ribonucleic acid; MDR, multidrug-resistant; CYP-450, Cytochrome P450.

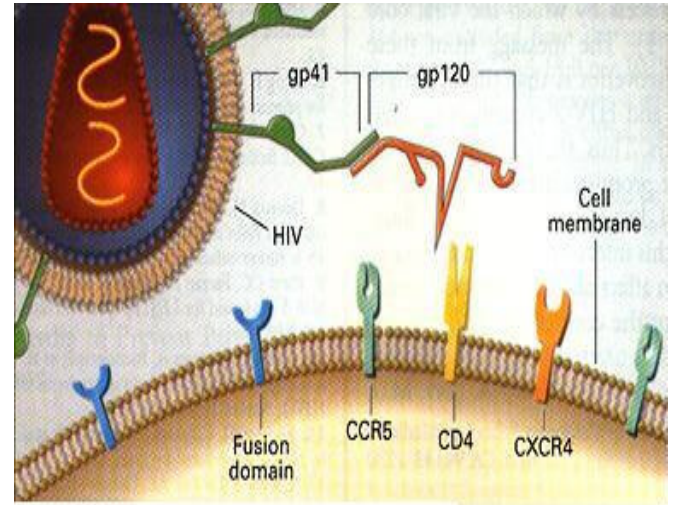


**Figure 1.** Schematic representation of the structure of Human Immunodeficiency Virus (HIV). The genome of the virus consists of two single-stranded RNA molecules. The virus has minimal viral proteins essential for maintaining its life cycle (From Chen et al., 2004).

*tuberculosis*, is believed to enhance the progression of the HIV disease. This enhancement of the virus has been suggested to be a result of the generalized immune activation seen in tuberculosis patients (Paton et al., 2005). The immunosuppression caused by HIV, creates a mutualistic interaction with *M. tuberculosis* and modifies the clinical presentation of TB. This allows anti-tuberculosis drugs to interfere with the anti-retroviral drugs inhibiting their effects (Aaron et al., 2004). Because of this interaction between the different drugs, special attention is placed when the antiretroviral drugs should be administered to individuals with these coinfections (Dlodlo et al., 2005). This paper reviews literature on HIV and tuberculosis in order to understand the pathogenesis and global impact of both diseases, the collaborative nature of the diseases and future trends in the management of the diseases.

## HIV ARCHITECTURE

The HIV architecture as schematically depicted by Chen et al. (2004) is shown in Figure 1. The HIV genome consists of two single-stranded RNA molecules. The virus also contains three enzymes that are critical to its life cycle and infectious process: integrase, protease and reverse transcriptase (RT). Whereas the integrase facilitates incorporation of viral genome into that of the host cell, the protease and RT play major roles in the mutation of the virus, a factor that leads to the drug resistance of the virus and ineffectiveness of single drug treatment regimen for HIV disease. The viral envelope is created from the host cell membrane and contains viral

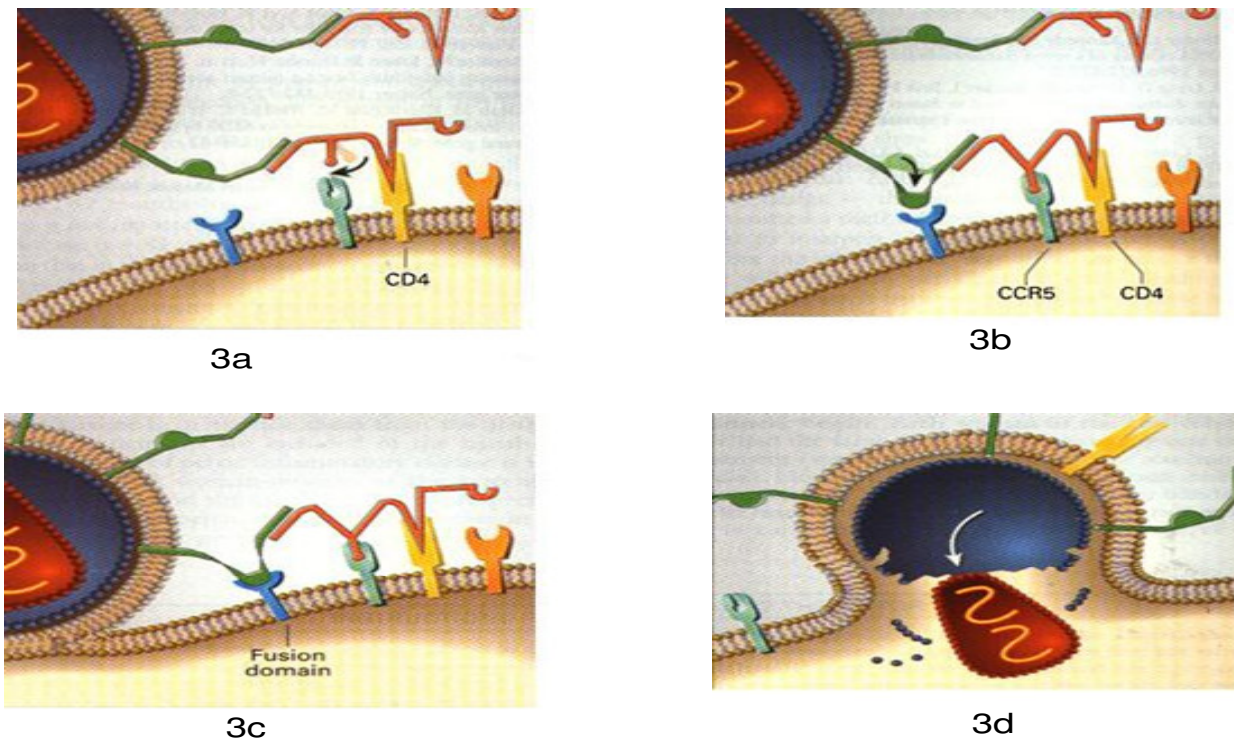


**Figure 2.** HIV and its glycoproteins gp 120 and gp 41 essential for binding to the CD4 receptor on the host cell with CD4. Gp41 is a viral transmembrane protein that anchors the viral gp 120 and facilitates binding to the CD4 receptor. Also shown are chemokine coreceptors CCR5 and CXCR4 that facilitate bind and fusion of the virus with the host cell (From Chen et al., 2004).

glycoproteins (gp). Two non-covalently bonded gps become key factors in the infection of HIV: gp 120 and gp 41 (Figure 2) with gp 120 having a high affinity for CD4 cells. Gp41 is a viral transmembrane protein that anchors gp 120 facilitating the binding of gp 120 to the CD4 receptor on the host cell (Figure 2). This enables fusion to occur between the viral envelope and the cell membrane of the host leading to the entry of the virus into the cell.

## VIRAL ENTRY INTO HOST CELL

The entry of HIV into host cell (Figures 3a - d) begins with the binding of gp 120 to CD4 receptor on the host cell. Because of the variations in gp 120, several chemokine receptors can act as co-receptors for the molecule. These include the chemokine receptor CCR5 on macrophages and dendritic cells and CXCR4 present on T cells. The binding of gp 120 and the CD4 receptor causes gp 120 molecule to undergo a conformational change resulting in the binding of a co-receptor. The involvement of co-receptors is the determining factor that dictates which CD4 cells will be targeted and the resultant type of HIV: macrophage tropic versus lymphotropic HIV. Macrophage tropic HIV is mediated by the chemokine receptor CCR5 while lymphotropic infection is mediated by the chemokine receptor CXCR4. Whereas macrophage tropic HIV does not require a high level of CD4 to be present on the host cell, lymphotropic HIV requires high levels of CD4 to be present (Coico et al., 2003). CCR5 is proposed to be the major co-receptor responsible for



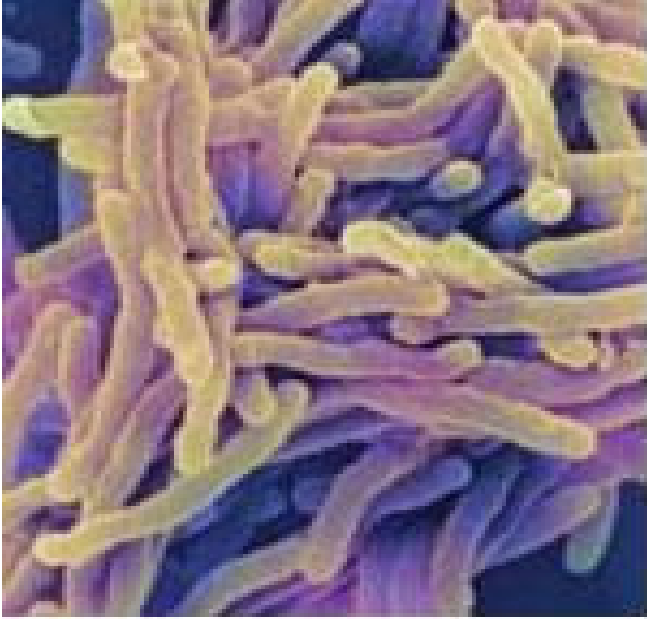
**Figure 3a-d:** Interaction of gp120 with CD4 on cell surface. **3a:** As gp120 comes in contact with the CD4 receptor on the host cell it undergoes a conformational change allowing the binding of the co-receptor CCR5 or CXCR4. **3b:** Conformational change of gp120 that allows gp 41 to undergo a change and move closer to the fusion domain of the host cell. **3c:** Second attachment brings virus and cell closer together allowing fusion between the viral envelope and the cell membrane of the host. **3d:** Viral genome enters the host cell (From Chen et al., 2004).

primary infection due to HIV because it is present on macrophages and dendritic cells which serve as reservoirs for the virus. These reservoir cells are not destroyed by the infection. They circulate and re-seed the virus with appropriate stimulus in the infected individual. The reservoir macrophage is stimulated upon encountering an antigen and viral replication is activated. The replication of the virus occurs and release of newly replicated viruses causes a switch to the lymphotropic form thus further spreading the virus to the lymphoid tissues of the infected individual (Coico et al., 2003). Once in the host cell, the viral RNA is replicated to complementary DNA (cDNA) by RT. The cDNA can remain in the cytoplasm or enter into the nucleus where the viral integrase assists in incorporating the virus into the host's genome as a provirus. This allows HIV to replicate slowly in a latent phase potentially for several years. The initial stage of HIV infection for most people is asymptomatic (Lipman and Nelson, 2006). During this period the virus replicates slowly, but there is a continuous decline of CD4 cells. Antibodies and cytotoxic T cells are generated to eliminate the virus from the host by the destruction of the viral infected cells which are the immune system CD4 cells. The patient becomes

seroconverted and expresses antibodies for HIV proteins and the infection can be detected with appropriate immunoassay (Lipmann and Nelson, 2006).

## HIV REPLICATION

Retroviral replication is highly dependent on post-transcriptional regulation because a single primary transcript directs synthesis of many viral proteins (Chen et al., 2004). Retroviruses have evolved fascinating ways to deal with their host cell environment and to make use of cellular transport pathways, allowing nuclear export of intron containing RNAs which are normally restricted to the nucleus. Two post-transcriptional regulatory systems, Rev responsive element (Rev/RRE) and constitutive transport element (CTE) have been identified and characterized for HIV (Chen et al., 2004). What is revealed is the efficient use of the host's cellular transport pathways by retroviruses to achieve successful reproduction of viral progeny. The host's CTE normally used for export of cellular proteins and RNAs, is redirected by the viral Rev protein for the replication of the virus. The Rev/RRE system of HIV-1 consists of the viral Rev pro-



**Figure 4.** *M. tuberculosis*, causative agent of tuberculosis, a gram-variable filamentous rod with mycolic acid in its cell wall (From Dwivedi et al., 2005).

tein which binds to its target sequence on incompletely spliced RNAs and channels these into the host's CTE export pathway. The CTE directly recruits a cellular mRNA export receptor to the viral RNA. Specific signals on the viral RNAs recruit key factors of cellular export, bypass restrictions on RNA export and ensure efficient viral replication (Chen et al., 2004). Once a stimulus, such as another virus or a bacterium activates the provirus, it is transcribed into a polycistronic viral mRNA and viral genome RNA. The process of the mRNA exiting the nucleus is assisted by the host enzyme, RNA helicase, DDX3, which unwinds the viral RNA before it leaves the nucleus. This enzyme, along with the viral Rev protein and the host protein CRM1, assist the viral RNA in evading splicing. Upon reaching the cytosol, the polycistronic viral mRNA is translated to viral proteins in the form of a polyprotein. The viral protease then cleaves the polyprotein into functional viral proteins. The viral proteins and genomic RNA are assembled and packaged into new virions. The glycoproteins, gp 120 and gp 41 attach to the viral envelope surface and the new virions are budded off from the host cell (Chen et al., 2004). From the extracellular space they circulate throughout the body to infect more host cells and to repeat the life cycle.

#### HIV MUTATION

The central dogma of molecular biology is that DNA serves as template for its replication and for the synthesis of messenger RNA that is translated into proteins. The HIV defies this central dogma in that their genetic infor-

mation is encoded by RNA rather than DNA. HIV contains RT which is an RNA dependent polymerase that directs the synthesis of a DNA from the viral RNA genome template. HIV is notorious for its ability to mutate and thereby evade the effects of drugs. Many drugs used to treat HIV interfere with the virus' RT, slowing the viral growth rate. Mutations in the RT gene of HIV produce mutated RT that is uninhibited by the drugs, allowing the virus to begin growing again (Beniowski et al., 2006).

#### TUBERCULOSIS

Tuberculosis (TB) is caused by *M. tuberculosis* (Figure 4). The pathogen is a slow growing bacterium that is believed to have evolved from soil bacterium more than 10,000 years ago (Dwivedi et al., 2005). Among communicable diseases, TB is the second leading cause of death worldwide, killing nearly 2 million people each year (Dwivedi et al., 2005) with most cases occurring in developing countries. Of all the new tuberculosis cases reported each year, 95% occur in developing countries. It is a respiratory disease affecting nearly 32% of the world's population (Dye and Williams, 2003). Among the infected individuals, approximately eight million develop active TB and almost two million of these die from this disease (Corbet et al., 2003). Approximately, one million young women in these countries are diagnosed with this disease. The occurrence of TB is linked to dense population, poor nutrition and poor sanitation (Byarugaba, 2004). A pathological and anatomical description of the disease was clarified in 1882, when Robert Koch discovered *M. tuberculosis* to be the causative agent of the disease. In 1906, French bacteriologists, Dr. Albert Calmette and Dr. Camille Guerin, created the vaccine for *M. tuberculosis*, Bacillus Calmette-Guerin (BCG). This vaccine has been shown to be somewhat effective in children, but ineffective in adults.

#### INFECTION OF THE HOST

The infectious process of TB was reviewed by Dwivedi et al. (2005). The disease is spread by airborne droplet nuclei, particles of 1 – 5  $\mu\text{m}$  in diameter that contain the pathogen. The relatively small size of these particles allow them to remain airborne for several hours after being expelled as droplets from people with pulmonary or laryngeal tuberculosis during coughing, sneezing, singing, or talking. The infectious droplets are inhaled by non-infected individuals and lodged in the alveoli of their distal airways. *M. tuberculosis* is then taken up by alveolar macrophages, initiating an immune reaction (Dwivedi et al., 2005). When the inhaled microorganisms multiply to a sufficient extent, an antigen-antibody interaction is evoked by the cell-mediated T-lymphocytes. This immune response that is created causes tubercles to form because of the accumulation of macrophages at the site of

infection. These events result in either successful containment of the infection or progression to active disease. After being ingested by alveolar macrophages, *M. tuberculosis* replicates slowly, but continuously and spreads via the lymphatic system to the hilar lymph nodes. In most infected individuals, cell-mediated immunity develops 2 – 8 weeks after infection. Activated T lymphocytes and macrophages form granulomas limit further replication and spread of the organism. The development of cell-mediated immunity against *M. tuberculosis* is associated with the development of a positive result in the tuberculin skin test (Dwivedi et al., 2005).

## HIV AND TUBERCULOSIS

Over the past decade, tuberculosis incidence has increased in several countries in Africa. Available data suggest that this increase is mainly as a result of the burden of HIV infection in those countries. The epidemic of *M. tuberculosis* has infected about one-third of the world's population creating an adverse impact socially and economically in developing countries (Malen et al., 2006). Chaisson et al. (2004) reported that HIV infection appears to be a key component in the development of active TB by rapidly increasing its progression. Although very little substantial evidence is currently available, *in vitro* studies have shown that *M. tuberculosis* induces HIV replication increasing the viral load in patients with TB (Chaisson et al., 2004). The frequency with which HIV and *M. tuberculosis* infection occur together is determined by the epidemiology of each disease in a given population. The highest rates are found in the developing countries. The incidence of TB in HIV-infected persons is more than 100 times that of the general population. In untreated HIV-infected persons who have a positive tuberculin skin test, the risk of active TB is about 8% per year (Coico et al., 2003). CD4 cell-mediated immunity and macrophage function are essential in the control of *M. tuberculosis* infection. During primary infection of an immunocompetent host, cell-mediated immunity usually develops and arrests progression of disease. About 5% of patients whose primary infection is controlled have reactivation years to decades later. In another 5% of patients, infection is not contained and primary pulmonary, extrapulmonary, or disseminated TB can occur. The hallmark of HIV infection is progressive deterioration and depletion of CD4 cells, coupled with defects in macrophage and monocyte function (Aaron et al., 2004). There is evidence that the immune response in patients with TB might enhance HIV viral replication and accelerate the natural progression of HIV infection (Ferrari, 2004). The risk of TB developing in an HIV-infected patient who is latently coinfecting with *M. tuberculosis* approaches 10% per year, as opposed to a 10% lifetime risk in an immunocompetent host (Frieden,

2005). Patients with more advanced HIV infection (CD4 count, <200 cells/mm<sup>3</sup>) who are newly infected with *M. tuberculosis* may lack the ability to contain the primary infection, which can progress rapidly and is fatal if not treated (Cheng et al., 2003).

## TREATMENT AND DRUG INTERACTION

Antiretroviral drugs are used for the treatment of infection by retroviruses, primarily HIV. Different antiretroviral drugs act at various stages of the HIV life cycle. Various combinations of three or four drugs are known as highly active anti-retroviral therapy (HAART). These drugs include nucleoside reverse transcriptase inhibitors, protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). HIV has a life span that can be as short as about 1.5 days. The virus itself lacks proofreading enzymes to correct errors made when it converts its RNA into cDNA via reverse transcription. Since the life time of HIV is short and the DNA copies are wildly diverse due to a high error rate, HIV mutates very rapidly. Combinations of antiretroviral drugs reduce viral load, limit viral mutations thus reducing the chances of a superior mutation by keeping the number of offspring low. If a mutation arises that conveys resistance to one of the drugs in the cocktail, the other drugs will help suppress reproduction of mutant virus. With rare exceptions, no individual antiretroviral drug has been demonstrated to suppress an HIV infection for long. The drugs must be taken in combinations in order to have a lasting effect. As a result, the standard of care is to use combinations of antiretroviral drugs (The Antiretroviral Therapy Cohort Collaboration, 2005). Even though HAART has been effective for many HIV patients, one of the most threatening features of TB in HIV-infected patients has been the spread of multidrug-resistant (MDR) organisms (Aaron et al., 2004). Some of the PIs and NNRTIs used for the treatment of HIV have been shown to interact with the major anti-tuberculosis drugs, rifampicin and rifabutin. These effects are a direct result of the drug-drug interaction that cause changes in the metabolism of the antiretroviral agents and rifampicin in regards to the hepatic cytochrome enzyme system (CYP-450) (Aaron et al., 2004). Cytochrome P450 haemoproteins are found mainly in the liver and intestinal tissues, where endogenous and exogenous substances are metabolized. The drug efflux pump system, P-glycoprotein, found in the liver decreases the efficacy of PIs by decreasing the intracellular disposability of drugs. Rifampicin induces CYP-450 activity thus effecting decrease in the concentrations of PIs and NNRTIs to low therapeutic levels. Rifabutin is less potent than rifampicin in respect to inducing CYP-450 and can be used with antiretroviral drugs under strict observations. Thus a therapeutic approach weighing drug-drug interactions must be recommended for patients under treatment for the coin-

fections of HIV and TB (Aaron et al., 2004).

Drugs needed to contain HIV and to treat infected patients are very costly and almost beyond the reach of most infected individuals. In developing countries alone, over 200 million dollars is needed to create an adequate work environment to house research for HIV. The antiretroviral therapy cohort collaboration (2005), an international collaboration of study groups, showed that patients that received HAART were 70% less likely to contract tuberculosis. Infected individuals in Sub-Saharan Africa have a higher mortality rate from HIV than those in the more developed countries. Moreover, they also have a relatively low rate of use of anti-retroviral drugs. This is a problem that must be addressed in a global way.

## CONCLUSION

There continues to be an increase in the number of individuals affected with HIV and now HIV/TB in developing countries (Badri et al., 2005). It is estimated that by the year 2020 a billion people will be co-infected with HIV/TB (Chaisson et al., 2004). New and sustained efforts must be made to contain this co-infection, prevent HIV transmission and expand efforts to implement ways of delivering health care to developing countries. The President's Emergency Plan for AIDS Relief (PEPFAR), an HIV initiative started by former President George W. Bush of the United States of America (USA) and approved by the Congress of the USA designated \$15 million to be used over 5 years to conduct research on HIV treatments as well as drug research involving HIV and tuberculosis. The plan also supported capacity building relative to development of laboratory resources in those countries with high incidence of HIV/AIDS. Former President William Jefferson Clinton has also developed an organization, Clinton HIV/AIDS Initiative (CHAI) that supplies money through grants, to the developing countries to assist with the care of children living in these countries with HIV/AIDS. Through their foundation, Bill and Melinda Gates have also donated millions of dollars to sponsor HIV research in developing countries. Some of this money has been used to obtain drug treatment for affected individuals. The health care facilities in developing countries are bombarded with patients suffering from HIV and HIV/TB which outnumber the staff by a ratio of 3:1. Due to limited space and available resources, this number will continue to grow until additional funds are provided to support adequate public health research in these countries. Research on how to control the mutation of HIV will be very helpful in breaking the infectious process of the virus and to lay the foundation for the production of a stable vaccine against the virus. There have been bioethical concerns around the current proposal of making HIV testing mandatory during yearly physicals. On the positive side, proponents feel that this will increase awareness of the disease as well as ensure treatment for individuals that may have the

disease and not know that they are infected. However, the privacy issue continues to be a major concern for most people. Research to create a vaccine for this disease continues in many laboratories throughout the world. There are two types being developed, preventative vaccines, are for HIV negative individuals which will try to prevent infection of HIV, and therapeutic vaccines, which will try to improve immune system function for individuals that are HIV positive. Although, no vaccine has been approved for use thus far, both preventative and therapeutic vaccines are being tested in clinical trials.

## REFERENCES

- Aaron L, Bouchaud O, Calatroni I, Dupont B, Launay O, Lortholary O, Marchal G, Memain N, Valeyre D, Vincent V (2004). Tuberculosis in HIV-infected patients: a comprehensive review. *Clin. Microbiol. Infect.* 10: 388-398.
- Albert J, Bjoradal A, Bjoring E, Fenyo E, Inove R, Kewalramani V, Littman D, Morner A, Thorstensson R (1999). Primary Human Immunodeficiency Virus Type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in Coreceptor usage. *J. Virol.* 73: 2343-2349.
- Badri M, Lawn SD, Wood R (2005). Risk Factors for tuberculosis among HIV-infected patients receiving antiretroviral treatment. *Am. J. Resp. Crit. Care Med.* 172: 123-127.
- Baltussen R, Dye C, Floyd K (2005). Cost effectiveness analysis of strategies for tuberculosis control in developing countries. *Brit. Med. J.* 10: 1136.
- Beniowski M, Clumeck N, Dragsted U, Lazzarin A, Ledergerber B, Lundgren JD, Mocroft A, Phillips A, Podlekareva D, Vetter N, Weber J (2006). Factors associated with the development of opportunistic infections in HIV-1 infected adults with high CD4 cell counts. *J. Infect. Dis.* 194: 633-641.
- Byarugaba D (2004). A view on antimicrobial resistance in developing countries and responsible risk factors. *Internat. J. Antimicrobial Agents.* 24: 105-110.
- Chaisson R, Charalambous S, Churchyard G, Cock K, Day J, Fielding K, Grant A, Hayes R, Moloi V, Morris L, Puren A (2004). Does tuberculosis increase HIV load? *J. Infect. Dis.* 190: 1677-1684.
- Chen L, Lee C, Pertina A (2004). Positive selection detection in 40,000 HIV Type 1 sequences automatically identifies drug resistance and positive fitness mutations in HIV protease and reverse transcriptase. *J. Virol.* 55: 3722-3732.
- Cheng R, Currie C, Dye C, Williams B (2003). Tuberculosis epidemics driven by HIV: is prevention better than cure? *AIDS.* 17:2501-2508.
- Coico R, Sunshine G, Benjamini E (2003). *Immunology: A short course;* 5<sup>th</sup> edition. John Wiley & Sons, Inc., Hoboken, New Jersey. pp. 245-247.
- Corbett E, Dye C, Maher D, Raviglione M, Walker N, Watt C, Williams B (2003). The growing burden of tuberculosis. *Arch. Int. Med.* 163: 1009-1021.
- Dlodlo RA, Enarson DA, Fujiwara PI (2005). Should tuberculosis treatment and control be addressed differently in HI-infected and uninfected individuals? *Eur. Resp. J.* 25: 751-757.
- Dwivedi N, Tewari N, Tiwari V, Tripathi R (2005). Fighting Tuberculosis: An Old Disease with New Challenges. *Med. Res. Rev.* 25: 93-131.
- Dye C, Williams B (2003). Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. *Sci.* 301: 1535-1537.
- Ferrari MJ (2004). Eleven-million adults co-infected with AIDS, TB. *Can. Med. Assoc. J.* 171:437.
- Fielding K, Glynn J, Godfrey-Faussett P, Shearer S, Sonnenberg P (2005). How soon after infection with HIV does the risk of tuberculosis start to increase? *J. Infect. Dis.* 191: 150-158.
- Frieden T (2005). Tuberculosis control: Critical lessons learnt. *Ind. J. Med. Res.* 121: 140-142.
- Lawn SD, Wood R (2006). Tuberculosis control in South Africa-will HAART help? *S. Afr. Med. J.* 96: 502.

- Lipman M, Nelson M (2006). Management of advanced HIV disease in patients with tuberculosis or hepatitis co-infection. *International J. Clin. Pract.* 60: 976-983.
- Malen H, Mustafa T, Riise AMD, Wiker HG (2006). Vaccine approaches to prevent Tuberculosis. *Scandinavian J. Immunol.* 64: 243-250.
- Marx PA (2005). Unsolved questions over the origin of HIV and AIDS. *ASM News.* 71(1): 15-20.
- Paton N, Tan G, Teo L, Villacian J (2005). The effect of infection with *Mycobacterium tuberculosis* on T-cell activation and proliferation in patients with and without HIV co-infection. *J. Infect.* 51: 408-412.
- The Antiretroviral Therapy Cohort Collaboration (2005). Incidence of tuberculosis among HIV-infected patients receiving highly active antiretroviral therapy in Europe and North America. *Clin. Infect. Dis.* 41: 1772-1782.