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Therapeutic Potential for Inhibition of HIV Activation

Camille Belzil
University of Lethbridge
Lethbridge, Alberta, Canada

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Introduction

The Human Immunodeficiency Virus (HIV) is as simple as it is successful. Consisting of only relatively few elementary molecules, it is exceedingly hard to detect and has proven both difficult and expensive to treat. Treatment modalities are limited by the simplicity of its life cycle and the already compromised immune systems of patients. Current antiretroviral treatments are reasonably effective, but far too expensive for widespread distribution. Similarly, education can only go so far in combating such a virulent disease that is infectious in the absence of symptoms. This scenario presents an epidemiological dilemma: The highest rates of HIV prevalence are in underdeveloped and uneducated parts of the world. The areas worst afflicted lack the finances and infrastructure to initiate effective treatment programs.

This paper discusses the pathway for activation of the HIV provirus, and proposes the use of inexpensive plant extracts to slow rates of transmission and the progression from HIV infection to AIDS. It also addresses the growing problem of HIV/tuberculosis co-infection and use of combined treatments to slow infection rates of both diseases.

Origin of the HIV Virus

To find the origin of the AIDS epidemic, one need only follow the trail of devastation: The pandemic radiates around south and central Africa. While the HIV virus is specific to humans, it is undoubtedly of zoonotic origin [54]. It bears striking homology to the Simian Immunodefiency Virus (SIV) found in the common chimpanzee [60], which is native to these areas. However, the original crossover from primates to humans remains unknown and is a point of academic interest. The most common notion is that it was transferred to humans via consumption of bush meat. The African diet is notoriously low in protein, and it became a profitable enterprise to collect and sell wild animals for food. This theory of transmission holds that one of these animals collected was infected with SIV and brought back to the human population. Consumption of the infected animal is an unlikely source of transmission, but improper slaughtering or contact between a wound and the animal's blood could have accounted for its entry into a person's bloodstream. At this point the SIV virus adapted to the human immune system, and began to spread. Estimates on when HIV began infecting humans vary greatly, going as far back as the 1800s [60]. Most estimates center on a zoonotic transmission occurring in the 1930s [54, 60]. The first verified case of HIV was in the Democratic Republic of Congo in 1959 [59]. After that there were sporadic reports until the 1980s, when AIDS reached pandemic status [58].

Epidemiology of HIV/AIDS

Sub-Saharan Africa bears the brunt of the HIV pandemic, with two thirds of AIDS cases worldwide [52]. In 2003 an estimated 25 million people were infected in this region, with a growing prevalence in congenitally infected children [27, 52, 58]. Infection rates among adults have reached up to 38% of adults in Swaziland [52], and continue to rise throughout the area. AIDS is also becoming an issue in Asia, with the second highest prevalence worldwide and 7 million AIDS cases since the original outbreak [58].

Groups at highest risk of infection are intravenous drug users, infants of infected mothers, and those involved in the sex trade [27,58]. Migrant workers especially help to transmit the disease from areas of high prevalence to new areas. Similarly, international travel has been important in spreading the disease to nearly every country around the world. The recurring problem with HIV is that it can be transmitted even when an individual is asymptomatic and may be unaware that he/she is infected. Given these conditions, awareness, education and preventative measures are still the best available method of combating transmission.

Immunology of HIV/AIDS

HIV is a diploid retrovirus, containing 2 copies of only 9 genes [28,54]. The viral capsid itself is enclosed in a lipid bilayer with interspersed gp120 proteins [32]. Immediately following infection, the HIV virus targets a variety of immune cells. Entry into the cell involves binding to the CD4 surface receptor by the gp120 protein, followed by a membrane-mediated internalization [13,54]. The viral contents are liberated of the lipid coat, and the capsid proteins insert the viral genome into the host DNA using the enzyme reverse transcriptase [2]. At this point, the viral proteins become obsolete as the virus exists only in the host DNA. Replication and manufacture of new viral proteins makes use of exclusively host replication machinery [2]. Immediately following infection there is a brief period of illness corresponding with a widespread viraemia as the virus spreads throughout the host tissues [59]. As the virus spreads signals are sent to CD4 cells to undergo apoptosis, compromising the host's immunity [35,44]. The infection then subsides, and the remaining CD4 cells harbour the latent virus in their genome. Evidence suggests that macrophages in particular resist undergoing apoptosis, acting as reservoirs that periodically release newly synthesized viruses [42].

Following the original viraemic episode, the patient undergoes seroconversion: The body begins to manufacture antibodies against proteins in the viral capsid. These antibodies are, however, ineffective at neutralizing the virus, as the capsid is protected by a lipid bilayer [13]. The HIV diagnosis is geared at detecting the presence of these capsid antibodies rather than the virus itself [27], which is not continually present in the blood. The immune system cannot successfully neutralize the gp120 surface protein due to its extreme variability [13]. In order to bond, gp120 forms heterotrimeric spikes with the gp41 protein, which is recessed and unavailable for antibody targeting [13,32]. The gp120 protein is exposed, but consists of several variable domains and is in variable arrangements with other proteins [13]. On top of this, gp120 has a relatively low affinity for the viral envelope and even if antibodies were to bind to it, it is unlikely that the entire virus would be neutralized. This combination of factors has made the search for an antibody based HIV treatment unproductive, but still a promising target.

Scattered release of new viruses into the blood occurs at irregular intervals, but results in a slow decline in CD4 cell count lasting up to a decade. Once CD4 cell count falls below a threshold point there is a mass propagation and viraemic episode that lasts indefinitely if not treated [58]. When white blood cell count falls below 200 cells/μL the patient has the full AIDS complex and shows characteristic symptoms [36].

Common Opportunistic Infections

The immune function of an AIDS patient is so severely compromised that any malignancy propagates in the host. While the body normally detects and destroys carcinomas, they are allowed to spread unchecked in an immunocompromised patient. One of the characteristic symptoms of AIDS is the rare cancer Kaposi's sarcoma [27,59]. This malignancy of the connective tissue is a disease that was previously endemic to only a small portion of the elderly men, exclusively of Mediterranean or Jewish descent. However, when the AIDS pandemic began, cases of Kaposi's sarcoma began to show up in younger people in areas where it was previously not seen. Certain rare types of lymphomas were also diagnosed in unusually high prevalence in the younger population [27]. These originally indicated the presence of a new disease and hinted at immune deficiency.

In addition to cancers a plethora of viral, bacterial and fungal infections proliferate in AIDS patients. The most common bacterial parasites are *Mycobacterium tuberculosum* and *M. avium*, followed by a variety of oral parasites [27,59]. Though *M. tuberculosum/avium* are typically pulmonary pathogens, in AIDS patients they can spread to the skin, gastrointestinal tract and even cause meningitis [59]. There is also a high incidence of viral infections in AIDS patients such as herpes zoster/simplex and cytomegalovirus. In advanced viraemia the HIV virus causes its own set of symptoms, typically characterized by flu-like symptoms and one or more cutaneous rashes [59]. There is also a loss of cortical mass in the brain, isolated mainly to the parietal and frontal cortices [9]. There is also ventricular expansion and thinning of the corpus callosum [50]. It is unclear whether this loss of brain matter is due to the HIV virus itself, a potentially associated immune response, peripheral infections/malignancies, or drug treatments. The loss of cortical function can result in diminished motor function, coordination and instances of HIV-related dementia [9,59]. Fungal infections that are normally easily contained by the immune system also spread in patients with advanced AIDS complexes, including cryptococcosis, histoplasmosis, aspergillosis, and coccidioidomycosis [59].

The infections that afflict AIDS patients are subject to chance of exposure, stage of the disease, and the natural endemicity of pathogens. A typical patient may acquire any or all of the aforementioned infections/malignancies depending on living conditions, region, and access to health care. Receiving treatment for HIV increases the chance that the immune system will be able to fend off opportunistic infections, thereby increasing the life expectancy of treated individuals. Lack of treatment both compromises the patient's immune system making them susceptible to infections and can increase transmission of opportunistic infections to other individuals. An AIDS patient is the perfect host in which parasites can breed, recombinate, and then transmit to new hosts.

Activation of the HIV Provirus

Theoretical Approach

The targets for treatment of HIV increase as we continue to elucidate different aspects of the HIV virus' life cycle. One area in particular that has confounded researchers is how the latent provirus is activated and becomes infectious. During the asymptomatic phase, cells infected with HIV do not contain any viral proteins, allowing them to evade detection by the

immune system [27]. The provirus exists only as an extension of the host's DNA. Like any other host gene, the viral genes require a trigger in order to be expressed. A change in cellular environment directs the host cell to transcribe both its own genes as well as the embedded viral genes, allowing the virus to begin synthesis of new viral proteins and spread to other cells. The HIV virus' problematic choice of infecting immune cells affords it the ability to use the host's system of detecting concurrent infection in order to coordinate replication. The receptors that the HIV virus uses are critical to host pathogen recognition, the Toll-like receptors [4].

Toll-Like Receptor (TLR) Structure and Function

The Toll receptor was first classified as a developmental protein in *Drosophila*, implicated in determining dorso-ventral embryonic polarity [4] and mediating cardioblast development [56]. In maturity, the Toll receptor plays a key role in antiviral and antibacterial immunity [61]. The homologous receptor in mammals is a family of transmembrane receptors consisting of an extracellular domain which recognizes various pathogen-associated molecular patterns (PAMPs), a transmembrane domain, and an intracellular Toll/IL-1 identity region (TIR) [23]. The extracellular domain contains several leucine rich repeats to facilitate PAMP binding [23]. The intracellular domain is homologous to the interleukin-1 (IL-1) receptor [23] and begins similar transcriptional changes that ultimately lead to the production of inflammatory cytokines [2].

TLR Expression on CD4 Cells

The pathogenic patterns recognized by the various receptors include viral, bacterial and fungal ligands, though there are also binding sites for heat shock proteins and fibrinogen [5]. Ligands for TLR 3 & 9, dsRNA (double stranded viral RNA) and CpG oligodeoxynucleotides (unmethylated bacterial DNA), stimulate TLRs on the endosomal compartments to initiate a signaling pathway that leads to CD4 cell proliferation [24,40]. TLR detection of viral single stranded RNA has also been implicated in stimulating dendritic CD4 cells after infection [7]. The stimulation of TLRs induces the release of the cytokines such as type I interferons (IFN-α & β), tumor necrosis factor alpha (TNF- α), IL-18 and cyclooxygenase-2 (COX-2) [51]. The release of IFNs is important in creating a cordon of uninfectible cells around the infected cell via RNA degradation, and acting as a chemotaxin for cytotoxic cells [6]. TNF- α, IL-18 and COX2 induce the synthesis of vasoactive substances, fever, and changes in other immune cells. More importantly, these cytokines promote CD4 cell survival and proliferation, supporting the cellular immune response [7,24].

TLR Mediated HIV Proliferation

After HIV infection and reverse transcriptional encoding into the host genome, there is an asymptomatic period during which the virus makes periodic excursions into the blood [2]. The mechanism of this sporadic viral replication is mediated by the stimulation of TLRs. Ligands for TLR 2 & 9 (gram-positive/mycobacterial cell wall components and CpG ODNs, respectively) induce proliferation of the HIV virus in an additive fashion [4,18]. TLR 4 ligands (lipopolysaccharides from gram-negative bacteria) have also been shown to induce viral replication in transgenic CD4 cells [4,18,22]. Levels of viral p24 proteins in the blood correlate in a positive and linear manner to concentrations of TLR 2,4 and 9 ligands [18,19]. This mechanism suggests that this viral proliferation is dependent on the degree of TLR stimulation. From the perspective of the HIV virus, stimulation of TLRs has two key benefits: The proliferation (and subsequent higher concentrations) of CD4 cells, and the presence of a co-infection. Both of these constitute ideal conditions for the HIV virus: High levels of infectable CD4 cells that are circulating to a variety of infectable host tissue, and an immune system preoccupied with a co-infection.

Cellular Interactions of HIV

The HIV virus requires a primary binding site, the CD4 receptor, as well as the co-receptor CCR5 [2]. Unfortunately these receptors are found in conjunction only on immunocompetant cells, especially T-helper, dendritic and mast cells [29]. The co-expression of Toll-like receptors as well as the primary binding receptors results in the viral proliferation. This in turn leads to virally induced CD4 cell apoptosis and a decline in immunocompetancy. The assays of HIV responsiveness to TLR 2 stimulation were conducted by adding soluble tuberculosis factor (a solution containing mycobacterial membrane components) to a colony of infected monocytic cells [18]. TLR mediated HIV proliferation and progression to AIDS in humans is likely associated *in vivo* with stimulation of TLRs by the intracellular parasites *Mycobacterium tuberculosum/avium* [25]. These strains of Mycobacteria predominantly infect alveolar macrophages, which also express the CD4 surface protein [29]. This parasite stimulates both TLR 2 (via mycobacterial cell wall components) and TLR 9 (via CpG ODNs) on these macrophages. As in lab models, this would quicken CD4 cell apoptosis, the progression of HIV infection, and the onset of AIDS. *In vivo* transgenic mice studies support this model [4].

Interestingly, these factors that induce replication are not part of the immune reaction to the virus itself. The fact that Toll-like receptor stimulation with bacterial ligands induces HIV replication suggests that not only do opportunistic bacterial infections occur concurrently with HIV infection, but that the normal immune response to bacterial infections causes AIDS in HIV infected people.

The Signaling Pathway

The TLRs implicated in causing HIV replication (TLR 2, 4 & 9) share an associated protein known as myeloid differentiation factor 88 (MyD88). The MyD88 protein consists of a C-terminal domain that associates with the TIR intracellular domain on the TLR, and an N-terminal death domain [11]. The structure of the MyD88 protein allows it to act as a medium between the TLR family of proteins as well as the IL-1-associated kinase (IRAK) family [11]. The IRAK family proteins are serine/threonine kinases that are stimulated after MyD88 activation to continue the signaling cascade. This cascade transfers signals through various pathways, mostly involving MAP kinase kinases (MKKs), into the nucleus via Nuclear Factor-κB (NF-κB) [11,29]. Most inhibitors of TLR signaling, such as SIGIRR transmembrane protein or the MyD88s cytosolic protein, target the MyD88 protein thereby preventing the signaling cascade at its source [11,55].

HIV Activation

The mechanism of transcription of the HIV provirus is not yet fully understood. The long terminal repeat (LTR) region on

the HIV type 1 (HIV-1) virus has a binding site for NF-κB [28,45,49]. Current evidence suggests that the binding of NF-κB to the LTR region is sufficient to induce replication of the HIV-1 provirus [47]. Conversely, experiments involving chemical inhibition of NF-κB have demonstrated that NF-κB binding is also necessary to induce transcription [20]. While the presence of NF-κB in the nucleus normally mediates the production of inflammatory mediators and cytokines, it also transactivates HIV replication via binding to the LTR on the viral DNA [18]. It is in this fashion that the latent HIV virus detects the presence of bacterial infections and begins to replicate. Other transcription factors have been isolated as well. Notably, small extracellular concentrations of viral proteins Tat and Vpr have been shown to induce transcription [8,53]. The assays using synthetic Vpr to induce replication have also shown increased levels of NF-κB and other TLR mediated signaling proteins [53]. This evidence indicates that the replication signals (viral proteins) are not acting directly on NF-κB or nuclear proteins. While the matter is still under investigation, it is probable that the viral proteins induce replication by binding to sensitized surface proteins that then activate Toll-like receptors at the beginning of the signaling cascade.

A relatively recently suggested mode of HIV treatment involves exposing patients to synthetic CpG ODNs in order to stimulate TLR9, promoting the production of interferons [1,4,6] . While this pathway would indeed activate interferon production, it would also transactivate HIV replication. Given what in vitro studies have shown about HIV activation from bacterial ligands (such as CpG ODNs), this treatment should be done cautiously or not attempted at all.

Synopsis

Infection with the HIV virus is independent of the Toll-like receptor system, targeting only CD4 immune cells. However, the expression of Toll-like receptors on activated CD4 cells is a necessary condition for viral propagation. The HIV provirus relies on the stimulation of TLRs to begin a signaling cascade that induces both the production of immune cytokines as well as viral proteins. The TLR pathway acts as a medium to signal the presence of bacterial co-infection, which entails a perfect breeding environment for the HIV virus. The HIV virus has embedded itself in the TLR signaling pathway, and is reliant on TLR signaling to begin replication.

This proposition has several implications. Without stimulation of any of the TLRs, the HIV provirus is unable to replicate. If a patient is theoretically isolated from any endogenous or exogenous TLR ligands, they will not only fail to develop the full AIDS complex, but they will survive indefinitely *with a fully functional immune system*. A more realistic option is to target the TLR pathway with specific drugs, thereby holding the HIV virus in genomic stasis and allowing immune system to recover.

TLR Inhibition Therapy

Harmala Extracts

Harmala compounds are chemically simple organic molecules originally isolated from the plant *Peganum harmala* [16,39]. These extracts are β-carbolines consisting of 3 rings, an aromatic indole and an affixed hybridized six-membered ring [30]. Natural harmala extracts contain short additions to these rings, resulting in a plethora of compounds with similar biological functions. These extracts are fairly common in a variety of plants worldwide, and have a long history of use in ethnic medicine. In Iran, seeds from the *P. harmala* shrub have been used for centuries to treat cancer and infections [16,39]. Controlled studies have confirmed the potent cytotoxicity of harmala on carcinoma cell lines [16,33]. Other varieties of harmala-containing plants are native to India, southern China, South America and recently the southern United States [30].

Recently, harmala compounds have also been found to act as monoamine oxidase inhibitors (MAOIs) with antidepressant properties [21]. MAOIs can activate dimethyltryptamine (DMT), resulting in hallucinogenic effects [34]. Certain South American shamanistic practices involve the use of yagé (ayahuasca), a mixture of harmala compounds and DMT [34]. Anecdotes from drug-related literature have reported a strange phenomenon when using yagé - a perceptible drop in body temperature [12]. Regulation of body temperature is critical in immune function, and few mechanisms could be accountable for a drop in body temperature. One of the most likely explanations of this mechanism is the antagonism of pyrogenic receptors. In this scenario, membrane proteins typically responsible for binding inflammatory molecules would be blocked. This would result in decreased production of pyrogenic cytokines and a drop of overall body temperature. Investigation into the compound responsible for this phenomenon led to molecules with a specific area of action on the immune system, and therefore considerable therapeutic value.

Harmala Compounds and TLR Inhibition

Tests have shown that harmine hydrochloride blocks certain TLRs in a concentration dependent manner [37]. Specific pathogenic ligands responsible for activation of the TLR signaling cascade are blocked from binding, preventing the production of inflammatory cytokines. These findings indicate that harmala compounds are responsible *in vivo* for the drop in body temperature associated with using yagé [39]. More importantly, it demonstrates that raw, unrefined plants containing harmala compounds block TLRs and the production of certain pyrogenic cytokines . Blocking the TLR pathway prevents the production of inflammatory cytokines at the source, targeting a very specific aspect of immune function. The potential for blocking this signaling cascade carries enormous therapeutic potential in the treatment of both external pathogens and autoimmune diseases. The potential for TLR inhibition as a method of treatment also extends to new applications, especially the prevention of HIV activation.

Contemporary HIV Treatments

Most current HIV treatments target the protein reverse transcriptase, a component of all retroviruses [36]. This protein is solely responsible for encoding the viral genome (RNA) into the host DNA [2]. These antiretroviral treatments (ARVs) are remarkably effective in reducing symptoms and decreasing serum levels of the virus. There are, however, serious side effects associated with the recommended dose. Patients on ARVs often suffer from anaemia and bone marrow depletion, as well as less serious side effects such as nausea, headaches and skin discolouration [41]. Classic ARVs are either nucleotide analogs or are metabolically converted into nucleotide analogs by the cell. The side effects seem to be associated at least in part to the fact that they not only block viral reverse transcription, but also native DNA polymerase to

a lesser degree [41]. The dose dependent inhibition of DNA polymerase could account for the loss of function in the parietal (motor) cortex of AIDS patients [9], an area requiring cellular reorganization and hence high levels of polymerase action. A separate class of drugs, the protease inhibitors, blocks synthesis of viral proteins by preventing cleavage of the larger and unprocessed transcriptional product [41]. Again the side effects can be severe, even deadly. A treatment consisting of two types of reverse transcriptase inhibitors and a protease inhibitor is entitled Highly Active Anti-Retroviral Therapy (HAART). The causative agent is still unclear, but it is possible that HAART may also be responsible for the loss of cortical mass, associated diskinesia and AIDS-related dementia [41]. HAART is also prohibitively expensive, with American patients paying around 6000$ US per year of treatment in the asymptomatic HIV infected phase [36]. Individuals living in the United states with AIDS pay well over 8000$ US per year for medication alone [36]. While ARV treatment is effective, it has dangerous side effects and could not be realistically distributed to places in the world with the highest prevalence of AIDS. Impoverished areas such as sub-Saharan Africa could not support the financial burden of sustaining widespread ARV treatment, even with significant assistance from international organizations. The problem is growing increasingly difficult: AIDS is considered a chronic disease, and a growing number of people need long-term treatment. To compound the problem further, infected individuals tend to belong to the economically productive demographic, and the longer that the AIDS problem continues, the less sustainable ARV treatment becomes.

Potential Treatment with Harmala Extracts

Harmala extracts provide a new possibility for treatment, and an entirely new method of blocking the viral life cycle. In assays of HIV inhibition, they induce a marked decrease in HIV activation [30] and show promising therapeutic value. They are also relatively non-toxic in normal doses and side effects at high doses are not severe, mainly nausea and vomiting coupled with nervous agitation [16, 39]. To date, no long term side effects have been reported as a result of ingesting plants containing these compounds [39]. Admittedly the degree of HIV inhibition is a fraction of that of ARV therapy [30], but there are other benefits to be considered. Acquiring harmala compounds does not require lengthy and expensive synthesis; they are readily available from a variety of plants. Extraction from the whole plant requires only hot water or alcohol as a solvent [39]. The simplicity of this process and low risk of contamination implies that this could be done on an industrial scale at relatively low cost. To simplify matters further, *P. harmala* is native to warm, dry climates, and could possibly be grown locally in areas most afflicted by HIV [39]. This would render the extraction process redundant, as seeds containing the active compounds could simply be ingested. The variety of compounds in these seeds all inhibit HIV replication by varying degrees, so preparation would not require extraction of a specific molecule [30]. Growing plants containing harmala compounds would carry only a marginal cost, and would make HIV treatment available to low income individuals who can not afford expensive ARV treatment. The low risk of side effects of harmala treatment also makes it a safer option. *In vitro* assays of carcinoma cytotoxicity indicate that harmala compounds may also be useful in treating cancers often associated with AIDS [16, 31, 33], especially Kaposi's sarcoma. Structural variants show different potency, though some offer up to 50% growth inhibition of various types of induced carcinomas [16]. This phenomenon is seemingly tissue independent, with no lasting side effects [16].

Treatment issues

The problem with HIV treatments, including ARVs, is that the possibility of transmission remains. Treatments are geared only towards lengthening the asymptomatic phase, improving longevity and quality of life. Neither ARV treatment nor the proposed harmala treatment eliminates the risk of transmission, but they do decrease the probability of passing on infection. By decreasing serum levels of the HIV virus, drugs inhibit the progression of the disease within the body as well as the ability of the virus to transfer to a new host. Implementing widespread harmala treatment in areas heavily afflicted with HIV would be geared towards increasing the longevity of infected individuals, but it may also begin to decrease the prevalence of the disease if it lowers the probability of transmission. An issue surrounding treatment is that once a patient begins to regain their health, they may begin to engage in high-risk sexual behaviours, infecting others. As a result it is essential to increase awareness and emphasize that treated patients are still infectious. Any treatment campaign would need to be accompanied by a drive to educate treated individuals about the hazards of transmission.

Drug Resistance

RNA viruses tend to be highly recombinant, and develop resistance to drug treatments relatively easily. HAART is geared at preventing this by attacking the viral life cycle from multiple angles, diluting the effect of viral recombination. This greatly decreases the probability that HIV will proliferate: HIV would need to evolve resistant variants of both the reverse transcriptase and the HIV protease proteins. Given time, however, HIV mutants will prevail and render current drugs ineffective. Harmala treatment on the other hand largely circumvents the issue of drug resistance. These compounds target a host mechanism at a stage when the virus exists only as DNA and is unable to change its expression. Its location in the host genome means that HIV is only activated by NF-κB [20, 47], a transcription factor specific to immune function. In order to evolve resistance to harmala treatment, the HIV virus would need to evolve a new promoter sequence, one with no affinity for NF-κB. This would entail losing its ability to utilize the TLR signaling pathway: HIV would no longer be able to detect co-infection, and would lose much of its virulence.

Future Considerations

Directions for Vaccine Research

The high mutation rate of the HIV virus has afforded researchers an interesting opportunity. The HIV Tat protein, which normally helps to transactivate viral replication [8], has several geographical variants. One particular variant isolated from long term non-progressor patients in Gabon, Tat Oyi, has lost its ability to transactivate replication through chance mutation [44, 57]. The structure of Tat Oyi is different enough that a substantial humoral response can be raised against it, effectively halting the progression of HIV without the help of drug therapy [57]. This provides patients with the Tat Oyi variant with innate immunity against HIV. Anecdotes report that HIV loses ability to transmit sexually or congenitally in individuals with Tat Oyi [43]. However the benefits of this innate immunity do not stop with this geographical subset of people: When antibodies cultured against the Tat Oyi variant are introduced into heterologous sera, they bind other Tat variants as well

[43]. Normally the body's humoral response is ineffective at neutralizing Tat proteins; it seems that the structure of the Tat Oyi variant is similar enough to other Tat variants to draw a humoral response towards them. Tat Oyi acts as a compromise between HIV and our immune system: It is similar enough to other variants to be recognized as a viral protein, and yet different enough to be recognized by the immune system [43]. Though in the past HIV's high rate of recombination has frustrated vaccine research, now is has afforded us a chance to use it against the virus. Theoretically antibodies fabricated against Tat Oyi would stop HIV replication, slow the progression to AIDS, and immunize a person against further infection. Preliminary testing of these antibodies *in vivo* has shown no toxicity, and allows a gradual increase in CD4 reservoir cells [57]. This seems to indicate that a Tat Oyi vaccine would in time even cure AIDS patients, ridding them of the virus. However due to the nature of introducing potentially transactivating viral proteins into AIDS patients, the Tat Oyi vaccine will require careful and lengthy testing before introduction into human subjects. In the meantime, treatment options remain the best method of combating transmission.

HIV/TB Co-infectivity

An estimated 2 billion people have infectious tuberculosis (TB), or have been exposed to the parasite [27]. With such a high prevalence it is not only one of the most successful human parasites, but a viable part of the human phenotype. Not surprisingly, *M. tuberculosum/avium* account for over 30% of deaths in people with AIDS [15]. Approximately 70% of HIV/TB co-infected individuals live in sub-Saharan Africa [27]. Both the HIV virus and the tuberculosis bacterium compliment each other, living in an unwittingly mutualistic arrangement. Immunocompromised HIV patients succumb to the *M. tuberculosum/avium* parasite more readily, progressing to the infectious stage of tuberculosis [27]. People with the infectious disease are more contagious, helping the bacteria propagate in the host and spread throughout the human population. Though tuberculosis is most commonly a pulmonary infection, in AIDS patients it can spread to other tissues [27,59]. Meanwhile, the HIV virus is strongly activated by the host's immune response to this co-infection, infecting the increased number of circulating CD4 cells. While most CD4 cells undergo apoptosis, macrophages resist this pathway and instead act as reservoirs for HIV and tuberculosis [42]. The more advanced the stage of either infection, the more it facilitates the progression of the other. The delocalization of *M. tuberculosum/avium* infection in AIDS patients creates a greater physical area of intersection, enhancing the effect of each parasite on the other. In conjunction the HIV and TB parasites create a cascade effect, each hastening the degradation of the patient's health and increasing the probability of transmission.

Strikingly high co-infectivity rates have classically been attributed to the ubiquity of *M. tuberculum/avium* in underdeveloped parts of the world. While tuberculosis is common, it should be considered that it is more than simply an opportunistic infection. It seems as if they traverse paths along the TLR signaling pathway in the alveolar macrophages, a mutual reservoir of infection for both diseases. If this is the case, then each of these epidemics may indeed be fuelling the other, and treating one may be futile unless the other is attended as well. With the emergence of multi-drug resistant strains of tuberculosis, eliminating TB may not be possible in the near future. Current drugs are becoming less and less potent with each ineffective campaign [27]. The only responsible approach is to simultaneously attack the HIV and TB reservoirs in afflicted areas of the world by employing treatment programs for both illnesses.

Novel Co-infectivity

The NF-κB/TLR dependent activation of HIV should also serve as a predictor of potentially devastating co-infection. The looming influenza pandemic threatens to create a global state of emergency. Upon arrival to AIDS-devastated areas, the influenza virus will find a plethora of immunocompromised hosts in which to spread unchecked. The most catastrophic aspect of this relationship is that the immune response elicited by the influenza virus activates NF-κB, which transactivates HIV [46]. *In vivo* assays have shown increased viral load during influenza infection. Should the influenza virus reach sub-Saharan Africa or certain areas of Asia and Eastern Europe that suffer worse from the AIDS pandemic, HIV mortality will reach record proportions. The inability of these hosts to elicit an immune response will facilitate the process of genetic recombination of influenza. Co-infectivity with multiple strains could lead to antigenic shift and new deadly strains of influenza. If this relationship proves accurate, the HIV/TB co-epidemic could be eclipsed by an accelerated and even deadlier co-infection.

Conclusion

The activation of the HIV provirus is entirely dependent on the immune infrastructure of the host. Toll-like receptor signaling allows humans to detect a variety of pathogens in the body, and orchestrate a systemic reaction. The HIV virus has embedded itself in this signaling chain, giving it the ability to proliferate in the presence of certain opportunistic infections. Knowing this pathway affords researchers the ability to target this system and prevent viral proliferation in infected individuals. Given the prohibitive cost of antiretroviral drugs, new methods of treatment should be sought out in order to treat those who cannot afford medication. The harmala family of plants contains natural compounds that block specific aspects of the TLR pathway thereby preventing HIV from detecting co-infection and proliferating. These plant extracts are a fraction of the cost of current HAART treatment, and could be made available to those who need treatment the most but cannot afford it. Their relatively low toxicity and benign side effects also make them a candidate for addition to HAART. Using this treatment provides promise of stemming the co-epidemic of AIDS and tuberculosis in sub-Saharan Africa and throughout the world.

Endnotes

1. Abel, K., Wang, Y., Fritts, L., Sanchez, E., Chung, E., Fitzgerald-Bocarsly, P., Krieg, A.M., Miller, C.J. (2005). Deoxycytidyl-deoxyguanosine oligonucleotide classes A, B, and C induce distinct cytokine gene expression patterns in rhesus monkey peripheral blood mononuclear cells and distinct alpha interferon responses in TLR9-expressing rhesus monkey plasmacytoid dendritic cells. *Clinical and Diagnostic Laboratory Immunology*, 12(5), 606-21.

2. Alberts, Johnson, Lewis, Raff, Roberts & Walter (2002). Molecular Biology of the Cell (4th ed.). New York: Garland Science.

3. Anders, H.J., Patole, P.S. (2005). Toll-like receptors recognize uropathogenic Escherichia coli and trigger inflammation in

the urinary tract. *Nephrology, Dialysis, Transplantation*, 20(8), 1529-32

4. Bafica, A., Scanga, C.A., Schito, M., Chaussabel, D., Sher, A. (2004). Influence of coinfecting pathogens on HIV expression: evidence for a role of Toll-like receptors. *Journal of Immunology*, 172(12), 7229-34.

5. Basu, S., Fenton, M.J. (2004). Toll-like receptors: function and roles in lung disease. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 286(5), 887-92.

6. Becker, Y. (2005). A point of view: HIV-1/AIDS is an allergy but CpG ODN treatments may inhibit virus replication and reactivate the adaptive immunity--hypothesis and implications. *Virus Genes*, 30(1), 127-31.

7. Beignon, A.S., McKenna, K., Skoberne, M., Manches, O., Dasilva, I., Kavanagh, D.G., Larsson, M., Gorelick, R.J., Lifson, J.D., Bhardwaj, N. (2005). Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor- viral RNA interactions. *The Journal of Clinical Investigation*, 115(11), 3265-75.

8. Bettaccini, A.A., Baj, A., Accolla, R.S., Basolo, F., Toniolo, A.Q. (2005). Proliferative activity of extracellular HIV-1 Tat protein in human epithelial cells: expression profile of pathogenetically relevant genes. *BMC Microbiology*, 5(1), 20.

9. Bogdanova, Y., Cronin-Golomb, A.M. (2005). Numerical cognition in HIV: Deficits in number-processing mediated by the parietal lobe. *Society for Neuroscience: Abstract Viewer/Itinerary Planner*, online.

10. Boulanger, M.C., Liang, C., Russell, R.S., Lin, R., Bedford, M.T., Wainberg, M.A., Richard, S. (2005). Methylation of Tat by PRMT6 regulates human immunodeficiency virus type 1 gene expression. *Journal of Virology*, 79(1), 124-31.

11. Burns, K., Janssens, S., Brissoni, B., Olivos, N., Beyaert, R., Tschopp, J. (2003). Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *Journal of Experimental Medicine*, 197(2), 263-8.

12. Burroughs, W. (2005). *Naked Lunch*. Harper Perennial: London.

13. Burton, D.R., Stanfield, R.L., Wilson, I.A. (2005). Antibody vs. HIV in a clash of evolutionary titans. *Proceedings of the National Academy of Sciences of the United States of America*, 102(42), 14943-8.

14. Center for Disease Control. (1998, October 30). *Morbidity and Mortality Weekly Report*. Atlanta: CDC Press.

15. Center for Disease Control. (1999, November). *The Deadly Intersection Between TB and HIV*. Retrieved 23 November, 2005, from [www.cdc.gov/hiv/pubs/facts/hivtb ...]

16. Chen, Q., Chao, R., Chen, H., Hou, X., Yan, H., Zhou, S., Peng, W., Xu, A. (2005). Antitumor and neurotoxic effects of novel harmine derivatives and structure-activity relationship analysis. *International Journal of Cancer*, 114(5), 675-82.

17. Chen-Park, F.E., Huang, D.B., Noro, B., Thanos, D., Ghosh, G. (2002). The kappa B DNA sequence from the HIV long terminal repeat functions as an allosteric regulator of HIV transcription. *Journal of Biological Chemistry*, 277(27), 24701-8.

18. Equils, O., Faure, E., Thomas, L., Bulut, Y., Trushin, S., Arditi, M. (2001). Bacterial lipopolysaccharide activates HIV long terminal repeat through Toll-like receptor 4. *Journal of Immunology*, 166(4), 2342-7.

19. Equils, O., Schito, M.L., Karahashi, H., Madak, Z., Yarali, A., Michelsen, K.S., Sher, A., Arditi, M. (2003). Toll-like receptor 2 (TLR2) and TLR9 signaling results in HIV-long terminal repeat trans-activation and HIV replication in HIV-1 transgenic mouse spleen cells: implications of simultaneous activation of TLRs on HIV replication. *Journal of Immunology*, 170(10), 5159-64.

20. Equils, O., Shapiro, A., Madak, Z., Liu, C., Lu, D. (2004). Human immunodeficiency virus type 1 protease inhibitors block toll-like receptor 2 (TLR2)- and TLR4-Induced NF-kappaB activation. *Antimicrobial Agents and Chemotherapy*, 48(10), 3905-11.

21. Farzin, D., Mansouri, N. (2005). Antidepressant-like effect of harmane and other beta-carbolines in the mouse forced swim test. *European Neuropsychopharmacology*, article in press.

22. Faure, E., Equils, O., Sieling, P.A., Thomas, L., Zhang, F.X., Kirschning, C.J., Polentarutti, N., Muzio, M., Arditi, M. (2000). Bacterial lipopolysaccharide activates NF-kappaB through toll-like receptor 4 (TLR-4) in cultured human dermal endothelial cells. Differential expression of TLR-4 and TLR-2 in endothelial cells. *Journal of Biological Chemistry*, 275(15), 11058-63.

23. Gangloff, M., Weber, A.N., Gay, N.J. (2005). Conserved mechanisms of signal transduction by Toll and Toll-like receptors. *Journal of Endotoxin Research*, 11(5), 294-8.

24. Gelman, A.E., Zhang, J., Choi, Y., Turka, L.A. (2004). Toll-like receptor ligands directly promote activated CD4+ T cell survival. *Journal of Immunology*, 172(10), 6065-73.

25. Ghassemi, M., Novak, R.M., Khalili, M.F., Zhou, J. (2003). Viable Mycobacterium avium is required for the majority of human immunodeficiency virus-induced upregulation in monocytoid cells. *Journal of Medical Microbiology*, 52(Pt 10), 877-82.

26. Goff, D.J., Nilson, L.A., Morisato, D. (2001). Establishment of dorsal-ventral polarity of the Drosophila egg requires capicua action in ovarian follicle cells. *Development*, 128(22), 4553-62.

27. Harries, A.D., Maher, D., Raviguone, M.C., Chaulet, P., Nunn., P.P., van Praag, E. (1996). *TB/HIV: A Clinical Manual*. Romano Canavese: World Health Organization.

28. HIV Sequence Database. (2001). *Numbering Positions in HIV Relative to HXB2CG*. Los Alamos: Los Alamos National Laboratory Press.

29. InvivoGen. (2005). *InvivoGen 2005 Product Catalogue*. Retrieved November 12, 2005, from [www.invivogen.com/docs /Catalog20 ...]

30. Ishida, J., Wang, H.K., Oyama, M., Cosentino, M.L., Hu, C.Q., Lee, K.H. (2001). Anti-AIDS agents. 46. Anti-HIV activity of harman, an anti-HIV principle from Symplocos setchuensis, and its derivatives. *Journal of Natural Products*, 64(7), 958-60.

31. Jahaniani, F., Ebrahimi, S.A., Rahbar-Roshandel, N., Mahmoudian, M. (2005). Xanthomicrol is the main cytotoxic component of Dracocephalum kotschyii and a potential anti-cancer agent. *Phytochemistry*, 66(13), 1581-92.

32. Koch, M., Frazier, J., Sodroski, J., Wyatt, R. (2005). Characterization of antibody responses to purified HIV-1 gp120 glycoproteins fused with the molecular adjuvant C3d. *Virology*, 340(2), 277-84.

33. Lamchouri, F., Settaf, A., Cherrah, Y., Hassar, M., Zemzami, M., Atif, N., Nadori, E.B., Zaid, A., Lyoussi, B. (2000). In vitro cell-toxicity of Peganum harmala alkaloids on cancerous cell-lines. *Fitoterapia*, 71(1), 50-4.

34. Leal, M.B., Elisabetsky, E. (1996). Absence of alkaloids in Psychotria carthagenensis Jacq. (Rubiaceae). *Journal of Ethnopharmacology*, 54(1), 37-40.

35. Lefevre, E.A., Krzysiek, R., Loret, E.P., Galanaud, P., Richard, Y. (1999). Cutting edge: HIV-1 Tat protein differentially modulates the B cell response of naive, memory, and germinal center B cells. *Journal of Immunology,* 163(3), 1119-22.

36. Levy, A.R., James, D., Johnston, K.M., Hogg, R.S., Harrigan, P.R., Harrigan, B.P., Sobolev, B., Montaner, J.S. (2006). The direct costs of HIV/AIDS care. *The Lancet Infectious Diseases*, 6(3), 171-7.

37. Lipford, G.B. (2005). Small molecule toll-like receptor (TLR) antagonists. *United States Patent Application*, 20050119273.

38. Lore, K., Betts, M.R., Brenchley, J.M., Kuruppu, J., Khojasteh, S., Perfetto, S., Roederer, M., Seder, R.A., Koup, R.A. (2003). Toll-like receptor ligands modulate dendritic cells to augment cytomegalovirus- and HIV-1-specific T cell responses. *Journal of Immunology*, 171(8), 4320-8.

39. Mahmoudian, M., Jalilpour, H., Salehian, P. (2002). Toxicity of Peganum harmala: Review and a Case Report. *Iranian Journal of Pharmacology and Therapeutics*, 1, 1-4.

40. McKimmie, C.S., Johnson, N., Fooks, A.R., Fazakerley, J.K. (2005). Viruses selectively upregulate Toll-like receptors in the central nervous system. *Biochemical and Biophysical Research Communications*, 336(3), 925-33.

41. Montessori, V., Press, N., Harris, M., Akagi, L., Montaner, J.S. (2004). Adverse effects of antiretroviral therapy for HIV infection. *CMAJ: Canadian Medical Association Journal*, 170(2), 229-38.

42. Olivetta, E., Federico, M. (2006). HIV-1 Nef protects human-monocyte-derived macrophages from HIV-1-induced apoptosis. *Experimental Cell Research*, 312(6), 890-900.

43. Opi, S., Peloponese, J.M., Esquieu, D., Campbell, G., de Mareuil, J., Walburger, A., Solomiac, M., Gregoire, C., Bouveret, E., Yirrell, D.L., Loret, E.P. (2002). Tat HIV-1 primary and tertiary structures critical to immune response against non-homologous variants. *The Journal of Biological Chemistry*, 277(39), 35915-9.

44. Peloponese, J.M., Collette, Y., Gregoire, C., Bailly, C., Campese, D., Meurs, E.F., Olive, D., Loret, E.P. (1999). Full peptide synthesis, purification, and characterization of six Tat variants. Differences observed between HIV-1 isolates from Africa and other continents. *The Journal of Biological Chemistry*, 274(17), 11473-8.

45. Romanelli, A., Pedone, C., Saviano, M., Bianchi, N., Borgatti, M., Mischiati, C., Gambari, R. (2001). Molecular interactions with nuclear factor kappaB (NF-kappaB) transcription factors of a PNA-DNA chimera mimicking NF-kappaB binding sites. *European Journal of Biochemistry*, 268(23), 6066-75.

46. Sun, J., Bergeron, M., Barbeau, B., Boivin, G., Tremblay, M.J. (2005). Influenza virus activates human immunodeficiency virus type-1 gene expression in human CD4-expressing T cells through an NF-kappaB-dependent pathway. *Clinical Immunology*, 114(2), 190-8.

47. Sundstrom, J.B., Little, D.M., Villinger, F., Ellis, J.E., Ansari, A.A. (2004). Signaling through Toll-like receptors triggers HIV-1 replication in latently infected mast cells. *Journal of Immunology*, 172(7), 4391-401.

48. Tardif, M.R., Tremblay, M.J. (2005). Tetraspanin CD81 provides a costimulatory signal resulting in increased human immunodeficiency virus type 1 gene expression in primary CD4+ T lymphocytes through NF-kappaB, NFAT, and AP-1 transduction pathways. *Journal of Virology*, 79(7), 4316-28.

49. Thierry, S., Marechal, V., Rosenzwajg, M., Sabbah, M., Redeuilh, G., Nicolas, J.C., Gozlan, J. (2004). Cell cycle arrest in G2 induces human immunodeficiency virus type 1 transcriptional activation through histone acetylation and recruitment of CBP, NF-kappaB, and c-Jun to the long terminal repeat promoter. *Journal of Virology*, 78(22), 12198-206.

50. Thompson, P.M., Dutton, R.A., Hayashi, K.M., Lu, A., Lee, S.E., Lee, J.Y., Lopez, O.L., Aizenstein, H.J., Toga, A.W., Becker, J.T. (2006). 3D mapping of ventricular and corpus callosum abnormalities in HIV/AIDS. *Neuroimage*, article in press.

51. Uematsu, S., Sato, S., Yamamoto, M., Hirotani, T., Kato, H., Takeshita, F., Matsuda, M., Coban, C., Ishii, K.J., Kawai, T., Takeuchi, O., Akira, S. (2005). Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7- and TLR9-mediated interferon-{alpha} induction. *Journal of Experimental Medicine*, 201(6), 915-23.

52. UNAIDS. (2004). *Table of country-specific HIV/AIDS estimates and data, end 2003*. Retrieved 18 February, 2006, from [www.unaids.org/bangkok2004/GAR20 ...] .

53. Varin, A., Decrion, A.Z., Sabbah, E., Quivy, V., Sire, J., Van Lint, C., Roques, B.P., Aggarwal, B.B., Herbein, G. (2005) Synthetic Vpr protein activates AP-1, c-Jun N-terminal kinase and NF-kappa B, and stimulates HIV-1 transcription in promonocytic cells and primary macrophages. *Journal of Biological Chemistry*, article in press.

54. Volberding, P.A., Baker, K.R., Levine, A.M. (2003). Human immunodeficiency virus hematology. *Hematology (the EducationPprogram of the American Society of Hematology)*, 294-313.

55. Wald, D., Qin, J., Zhao, Z., Qian, Y., Naramura, M., Tian, L., Towne, J., Sims, J.E., Stark, G.R., Li, X. (2003). SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. *Nature Immunology*, 4(9), 920-7.

56. Wang, J., Tao, Y., Reim, I., Gajewski, K., Frasch, M., Schulz, R.A. (2005). Expression, regulation, and requirement of the toll transmembrane protein during dorsal vessel formation in Drosophila melanogaster. *Molecular and Cellular Biology*, 25(10), 4200-10

57. Watkins, J.D., Lancelot, S., Campbell, G.C., Esquieu, D., de Mareuil, J., Opi, S., Annappa, S., Salles, J.P., Loret, E.P. (2006). Reservoir cells no longer detectable after a heterologous SHIV challenge with the synthetic HIV-1 Tat Oyi vaccine. *Retrovirology*, article in press.

58. World Health Organization. (2004). HIV/AIDS in Asia and the Pacific region 2003. Geneva: WHO.

59. World Health Organization. (2005, July). *Clinical aspects of HIV/AIDS.* Retrieved 2 March, 2006, from [w3.whosea.org/en/Section10/Secti ...] .

60. Yusim, K., Peeters, M., Pybus, O.G., Bhattacharya, T., Delaporte, E., Mulanga, C., Muldoon, M., Theiler, J., Korber, B. (2001). Using human immunodeficiency virus type 1 sequences to infer historical features of the acquired immune deficiency syndrome epidemic and human immunodeficiency virus evolution. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 356(1410), 855-66.

61. Zambon, R.A., Nandakumar, M., Vakharia, V.N., Wu, L.P. (2005). The Toll pathway is important for an antiviral response in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, 102(20), 7257-62.

References

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