

# **Towards a Cure of Chronic HIV Infection:**

## **Can HIV Vaccines and Interleukin 2 Help Promote Immunity to HIV?**

**By**

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### ***Introduction***

As a medical student I asked the advice of one of my mentors when trying to decide on a specialty of medicine to study. He said, “*My advice young man, is to choose a branch of medicine where there is therapy but no cure. That way you will always be ensured of a livelihood*”. Of course, this is the situation today with HIV infection: there is effective antiviral drug therapy, but no cure. Even though viral replication can be suppressed for several years with the administration of antiviral drugs, upon their discontinuation, the virus returns within a few weeks. This situation must be improved upon, especially given the increasing incidence and severity of antiviral drug toxicity. Therefore, it is now important to begin to think about how it can be made possible to “cure” HIV infection.

When contemplating “curing” HIV infection, it is useful to first define the term. Webster’s Dictionary defines the verb to cure as meaning “*to rectify an unhealthy or undesirable condition, especially by some specific treatment, such as medication*”. In this regard, it is important to realize that many infections caused by microscopic organisms (microbes) are cured by the immune system alone, without the need for antimicrobial drugs. Accordingly, “*the immune system is antimicrobial*”, so that the administration of antimicrobial medications simply augments and facilitates the natural host antimicrobial defenses.

With regard to HIV, why is the host unable to cure the infection if there are intact host defenses at the time of infection? Moreover, why cannot the immune system cure the residual infection when antiviral drugs have reduced the total body viral burden to very low levels? The answers to these two questions should point the way towards a cure.

It is important to distinguish between *eradication* of the very last microbe from the host vs. “*rectifying an unhealthy condition*” as definitions of a “cure”. From studies of the natural history of HIV infection, it is known that the concentration of virus detectable in plasma is predictive of the rate of progression to AIDS. Therefore, it may not be necessary to completely eradicate all HIV from the host. Instead, it may be sufficient to promote enough host control of the level of residual virus, so that progression to AIDS does not occur, or occurs so slowly that there is no discernible impact on the length of survival after infection. As well, if the level of residual virus remains below that required for transmission to another person, then the infection could be considered “cured” from a public health viewpoint.

## *How Host Defenses Combat Viral Infections*

By definition, a virus is a submicroscopic microbe (i.e. it cannot be seen with the aid of an ordinary microscope) that is only capable of replicating within living cells. Therefore, our host defenses combat viruses by preventing their invasion into cells, and by suppressing their replication or by removing them should they be successful in entering host cells. Microbes are prevented from invading the host by the natural barriers that protect against the environment, which consist of the cells of the skin, and the cells lining the respiratory, digestive, urinary, and genital tracts.

If a virus successfully crosses these natural barriers, it is capable of rapidly binding to and entering its specific target cells. Prevention of infection of these target cells is accomplished via molecules such as antibodies, which bind to the surface of the virus, and thereby facilitate the up-take of the viral particles via phagocytes (specialized cells capable of ingesting and digesting foreign matter). There are additional molecules, called opsonins, which are also capable of binding to microbes, facilitating their uptake by phagocytes. Once phagocytes ingest viruses and other microbes, they digest and kill them, reducing their number.

Should viruses escape the antibodies, opsonins and phagocytes, it is relatively easy for the microbes to find their way to susceptible target cells, where they bind to specific cell surface receptors and then enter the cell interior, which is called the cytoplasm. Once inside the cell, the outer protective coat of the virus usually disappears, exposing the viral genes (either DNA or RNA) to cellular molecules that help them to reproduce. Once the viral genes have replicated, they are capable of directing the production of viral proteins, which are ultimately packaged with the genes into new viral particles, which can then leave the cell to infect other cells and start the process all over again.

The host defenses have evolved mechanisms to combat intracellular infections. The first task is *recognition* of infected cells. The immune system can recognize virus-infected cells among normal, non-infected cells via lymphocytes, of which there are 3 major types; T cells, B cells and Natural Killer (NK) cells. T cells are especially active in viral infections. They have special cell surface receptors that can recognize and bind to viral peptides, which are short chains of amino acids, the building blocks of proteins. Usually T cells are resting. However, once the T cell recognizes a virus peptide on the surface of a virus-infected cell, signals are passed to the interior of the T cell, and it then proceeds to the next phase, which is called the *activated* stage.

T cell activation is a complex change that occurs within the T cell that has been under investigation for over 40 years. Initially, under the microscope it was possible to observe activated T cells grow in size and expand the amount of their cytoplasm relative to the nucleus in preparation for cell division. Subsequently, the cell undergoes DNA replication followed by cell division into two new cells. In this way, the initial recognition of the presence of the viral peptides on infected cells results in the proliferation of these virus-specific T cells, such that the number of T cells capable of

recognizing the virus increases markedly very rapidly, as much as 100,000-fold within a few days.

Detailed studies performed by many investigators over the past 20 years have shown that T cell activation, although initiated by recognition of the virus, is actually brought about by the action of small molecules produced by the activated cells called interleukins or cytokines, which are hormones, or molecular messengers. One cytokine in particular is crucial. Originally called T Cell Growth Factor (TCGF) and now called interleukin 2 (IL2), this cytokine is responsible for stimulating T cell proliferation. IL2 stimulates cellular proliferation by triggering cell surface IL2 receptors (IL2R), which only appear on the virus-activated T cells.

The IL2-IL2R interaction also promotes changes within the activated cells that permit them to function in new ways. Termed *differentiation*, these changes allow the T cell to produce additional cytokines, some of which attract and activate other cells that participate in the host defenses. In addition, some T cells acquire the capacity to kill virus-infected cells by secreting toxic molecules through the membranes of the target cells. These T cells are called Cytolytic T Lymphocytes (CTL), and most of them express the CD8 molecule on their surface. In addition, these CTLs secrete additional antiviral cytokines like interferon-gamma (IFN- $\gamma$ ), and tumor necrosis factor-alpha (TNF- $\alpha$ ), which bind to receptors on virus-infected cells and trigger pathways that inhibit viral replication.

CTL can also secrete IL2 in response to activation, but it turns out that most of the IL2 is produced by “*helper*” T cells, which are recognized by their cell surface expression of the CD4 molecule. Therefore, one the major ways that CD4+ T cells “*help*” CD8+ T cells is by producing IL2. Without CD4+ T cell-derived IL2 “*help*”, CD8+ T cell proliferation and differentiation to CTL and antiviral cytokine-producing T cells is blunted. For example in experimental animal systems, if CD4+ T cells are removed or if IL2 production is prevented prior to virus injection, CD8+ T cell proliferation is markedly reduced, as much as 90%, and the host defenses are severely impaired, resulting in a chronic, persistent infection.

The other major cell type that plays a role in most viral infections is the Natural Killer (NK) cell. NK cells make up ~ 10% of circulating lymphocytes (vs. 70% for T cells), and together with phagocytes, they function to serve as a natural defense against invasion by foreign microbes. When stimulated by cytokines such as IL2, NK cells also produce antiviral cytokines such as IFN- $\gamma$ , TNF- $\alpha$ , and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). These cytokines activate phagocytes as well as T cells, so that NK cells cooperate with all of the other cells that participate in the immune reaction.

### ***How Viruses and HIV in Particular Circumvent the Host Defenses***

Viruses cannot live and replicate on their own outside of a host cell. Instead, they depend on the products of host cellular genes to supplement their own genes. Viruses range in size from just a few genes, such as HIV, which has only 9 genes, to poxviruses, which

have greater than 200 genes. It is remarkable that such few genes can do so much damage, realizing that most multicellular organisms have thousands of genes. For example, humans and mice have > 30,000 genes and the small round worm *C. elegans* has almost as many, ~19,000, while the fruit fly has ~ 14,000 genes. By comparison, the typical bacteria like *E. coli* and *M. tuberculosis*, which can live on their own as single cell organisms have ~ 4,000 genes. The small number of genes in HIV may well work to its advantage, in that the fewer the genes, the fewer HIV gene products for the immune system to recognize as foreign molecules.

Larger viruses like the herpes viruses and the poxviruses have usurped and incorporated many host cellular genes amongst their own genes. Many of these cellular genes code for immunological molecules, and often the genes incorporated by the viruses circumvent the host defenses. A good example is the viral counterpart to the cytokine IL10, which was originally discovered in the Epstein-Barr Virus (EBV), which is one of the herpes virus family members, and the virus that infects B cells thereby causing infectious mononucleosis. IL10 normally functions within the immune system as a feed back, inhibitory cytokine, suppressing T cell responses. Since T cells fight EBV, virus production of viral-IL10 serves to counteract the T cell activities. Therefore, one of the ways that viruses circumvent the host defenses is to suppress the immune system by stealing and utilizing many of the immune system's own molecules.

Smaller viruses like HIV obviously are not as complicated as the poxviruses and herpes viruses, and they have not picked up many host genes. In addition, HIV has a genome comprised of only a single strand of ribonucleic acid (RNA), instead of a genome comprised of double stranded deoxyribonucleic acid (DNA), which makes up the genomes of larger viruses and all higher organisms. However, this becomes important for the capacity of the virus to outwit the immune system, because without a complimentary second strand of nucleic acid, it is difficult for the virus to replicate its genes with exact high fidelity. Consequently, there are a lot of mistakes made in the act of replication, which causes the virus to constantly change. Viruses that have changed may escape recognition by either antibodies or T cells can be selected because the new variants will be unimpeded by the host defenses.

Replicating rapidly is another way that HIV circumvents the host defenses. A rapid replication rate allows the virus to win the race with the host defenses, such that if the rate of viral replication exceeds the rate of HIV-specific lymphocyte proliferation, the immune system is continuously at a disadvantage. A high rate of viral replication also favors the selection of variants resistant to the defenses of the immune system, in that the rate of appearance of new variants is dependent on both the replication rate of the genome and the mutation rate of the genome.

In the process of replicating its genome, HIV undergoes a step called "reverse transcription", whereby it uses its strand of RNA as a guide to make a copy of complimentary DNA (cDNA). This cDNA then makes another copy of itself to form a double-stranded DNA molecule, which can be inserted into the cellular DNA. There the viral DNA can remain entirely inactive, or latent, so that the host immune system cannot

detect its presence within an infected cell. This also helps the virus evade the host defenses and persist in the host despite the immune system.

HIV has evolved another way to circumvent the host defenses, one that cripples the system. HIV apparently has found a way to inhibit IL2 production by both CD4+ helper T cells as well as by CD8+ CTL. Consequently, when confronted by invading microbes, the immune system cannot promote the rapid expansion of T cells capable of combating the infection. This appears to affect not only T cells attempting to combat HIV, but all T cells. Thus, HIV infection not only leads to a decrease in the total number of CD4+ T cells, it also disarms the T cells so that it is difficult for them to reproduce, and thereby protect the host.

### ***How can Immune-Based Therapies Overcome HIV-induced Immunosuppression?***

HIV is a chronic infection and not an acute infection, which indicates that HIV itself is not toxic to the host. Instead, HIV causes disease by inducing the immune system to turn against itself. Immunologists call this the generation of *immunopathology*. We are quite familiar with both infectious and non-infectious diseases that result from immunopathology. For example, Systemic Lupus Erythematosus (SLE), which is thought to be an autoimmune disease, has an immunopathogenesis, in that antibodies damage the kidneys and small blood vessels by reacting with an individual's own molecules.

In HIV infection, the CTL and the NK cells recognize HIV-infected CD4+ T cells and macrophages, and respond by killing them. In this way, the host defenses are able to reduce the concentration of HIV, but usually not enough to completely suppress all replicating virus. This suppressive CTL effect was nicely demonstrated in experiments with monkeys infected chronically with the Simian Immunodeficiency Virus (SIV), which is a relative of HIV that causes an AIDS-like illness in Rhesus macaques. Depletion of CD8+ T cells results in a marked increase in the concentration of plasma SIV, while after the CD8+ cells are allowed to return, the plasma SIV concentration once again declines to lower levels.

Based upon these kinds of observations, it follows that one of the most important ways to aid the host defenses in HIV infection is to boost the number and function of HIV-specific CD8+ CTL, so that they can more effectively kill virus-infected cells, hopefully depleting most if not all of the cells that are producing virus. We now know from many studies that have accumulated over the past 20 years, that the number and function of CD8+ T cells is dependent upon activation by the specific microbe, and by the action of IL2, which serves to promote their proliferation, differentiation and survival. Since there is a deficiency of IL2 production capacity in both CD4+ and CD8+ T cells during HIV infection, it follows that the administration of IL2 therapeutically might be of benefit.

Based on our understanding that T cells must be first activated by HIV molecules, and then expanded by IL2, we are now testing a way to try to boost HIV-specific immunity by using these two stimuli. While people are taking antivirals, we are administering an HIV vaccine to supply the specific HIV molecules. Simultaneously we administer IL2 as

a daily low dose of ~ 2 million Units, to promote the proliferative expansion of HIV-selected cells. Then to test whether this approach can boost HIV immunity to control viral replication, we interrupt antiviral drugs for at least 3 months, while we monitor plasma HIV concentration weekly. Therefore, this approach is designed to combine antiviral drug therapy with immunotherapies to work toward a “cure” for chronic HIV infection. It is based upon all we know of how the virus circumvents the immune system, and upon all that we know as to how the immune system successfully combats infections.