

THERAPEUTIC IMMUNIZATION: PRINCIPLES & PRACTICE

By

Kendall A. Smith

Brief History of Vaccination

More than 200 years ago Sir Edward Jenner first demonstrated that it is possible to generate immunity to a contagious disease by exposure to a similar, but different virus. Approximately 100 years later, Louis Pasteur popularized the concept of vaccination and introduced the idea that it might be possible to “attenuate” (i.e. decrease) the virulence (i.e the capacity to cause disease) of a microbe by exposing it repetitively to a different species, for example a rabbit. However, Pasteur did not have enough information available to him to actually achieve attenuation of virulence, and therefore could not have been successful. Consequently, vaccination remained a dream rather than a reality.

Pasteur’s dream of immunization with attenuated microbes finally became realized almost 100 years later, only when in 1959 Albert Sabin successfully attenuated poliovirus by multiple repeated passages in tissue culture cells. Even so, it was still impossible to know how repetitive passage in tissue culture changed the virus to make it lose its virulence. It is now known that the Sabin poliovirus vaccine has undergone multiple mutations. However, it still is not known which of the gene mutations are responsible for attenuating the poliovirus virulence. Consequently, the creation of attenuated live viral vaccines has remained a “hit-or-miss” endeavor.

Even so, the attenuated live poliovirus vaccine was a major breakthrough in vaccine research and paved the way for the development of live attenuated vaccines for many of the devastating childhood microbes, including mumps virus, measles virus, and rubella virus.

Mechanisms Responsible for Effective Immunity to Attenuated Viral Vaccines

In the 1950s and 1960s our understanding of the immune system was rudimentary, and it was impossible to understand why live attenuated vaccines were often more effective than killed vaccines. However, we now know that an optimal T cell-mediated immune response requires an intracellular infection by a live virus, so that the viral gene products are processed and placed on the cell surface in such a way to preferentially activate killer (CD8+) T cells. As well, we now realize that the CD8+ killer T cell response is crucial for an effective cellular immune response.

CD8+ T cells multiply rapidly upon recognition of virus-infected cells, and this proliferative response is dependent upon “help” derived from helper (CD4+) T cells. Recent data have shown that this “help” is delivered predominantly in the form of interleukin 2 (IL2), which is the chief T cell growth factor molecule that is produced by

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activated CD4+ T cells. Thus, the T cell-mediated immune response to viral infections occurs as a cooperative process between CD4+ T cells and CD8+ T cells. In addition to the proliferative response, IL2-stimulates CD8+ T cells to differentiate to become killer T cells, and as well, to produce large amounts of antiviral cytokines, in particular interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α).

Prophylactic Vaccination: Prevention of Infection vs. Protection from Disease

The word **immunity** is derived from the Latin *immunis*, which was defined as “free from public service and/or taxation”. The definition has become extended to mean “exempt from or protected against something disagreeable or harmful. For example, president Clinton was just granted immunity from prosecution from lying under oath when testifying in the Paula Jones case.

Accordingly, immunity to a virulent microbe can be gained either by the prevention of *infection* by the microbe, which is defined as the actual entrance of a microbe *into* the body and into the cells of the body, or alternatively, immunity can be produced by prevention of the *disease* caused by the microbe.

Traditionally, prevention of infection has been attributed to the formation of antibodies by B lymphocytes (B cells) that have been activated by a previous exposure to the microbe. These antibodies combine with microbes that gain entrance to the body through the skin or mucosal barriers (the mucosa consists of a single layer of cells that line the nose/mouth, throat, respiratory tract, digestive tract, genital tract, and the urinary tract).

If a microbe successfully makes it through the mucosae, antibodies bind to the microbe, which facilitates recognition by the phagocytic (i.e. a cell that *eats* foreign material) white blood cells (WBCs), i.e. macrophages and polymorphonuclears (PMNs), which ingest the microbe-antibody complexes and digest them before they can infect their target cells.

If a particular microbe has never infected a person there are no circulating antibodies, so that upon infection there is nothing to stop the microbe from invading the cells and tissues and establishing itself. Therefore, by vaccination with either killed or attenuated microbes before exposure to the microbe, it has been possible to stimulate the production of protective antibodies, rendering the state of immunity.

Accordingly, within the past 50 years, we now have prophylactic vaccination against most of the childhood diseases, including diphtheria, whooping cough, tetanus, polio, mumps, measles, rubella, chicken pox, hepatitis A & B, and *Streptococcus pneumoniae*, among others.

Immune Surveillance after Infection: Prevention of Disease

Despite this very effective defense system, some microbes have evolved ways to get around the protective antibodies, and HIV is one of them. Once HIV passes through or between the genital mucosal cells, it is rapidly taken up by scavenger antigen-presenting cells, of which there are 2 principle varieties, dendritic cells and macrophages. These cells then travel through special lymphatic vessels to lymph nodes where they come into close contact with T cells and B cells. Because CD4⁺ T cells and macrophages express one of the receptors for HIV (i.e. CD4 itself), these cells become infected early-on by HIV.

In addition, HIV is recognized by the CD4⁺ and CD8⁺ T cells a foreign invader, which results in their “activation”. Activated T cells change dramatically compared with their unactivated resting counterparts, in that they enlarge and become very biochemically active. This activation process prepares them for cellular division and entails the expression of a large number of new genes.

However, the fact that the cells are activated makes them much more susceptible to infection by HIV and facilitates the integration of the HIV DNA amongst the cellular genes of the cell. Accordingly, the HIV has evolved and survived in a hostile immunologic environment by learning how to infect the very cells that should eliminate the virus from the body.

Once HIV has become integrated into the genes of the cells, it can either remain dormant, or latent, or it can replicate its DNA, leading to the production of additional copies of itself. However, in the process of replicating, the HIV proteins produced inside the cell are expressed on the cell surface, where they can be recognized as foreign by T cells, both of the CD4⁺ subset, as well as the CD8⁺ subset. Subsequent to this recognition the T cells become activated, leading to their IL2-mediated proliferation and differentiation to become efficient antiviral T cells.

The result of this process in many viral infections is a constant immune surveillance, which prevents viral replication, and maintains the residual virus in a latent or dormant state. A good example of this surveillance accounts for the natural history of infection by the viruses of the Herpes Virus family; i.e. herpes simplex, cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and varicella zoster virus.

In each case, once infection occurs, the immune system reacts and eliminates the replicating viruses, but leaves a residual virus population in a latent state. The immune system keeps these residual viruses latent by a cooperative interaction between T cells and NK cells. Experimentally, it can be shown that depletion of T cells and NK cells results in the reappearance of replicating virus.

Evidence indicates that a few individuals can exert sufficient control of HIV replication, so that plasma HIV remains undetectable, and over decades, there is no decrease in

circulating CD4+ T cells. These “long-term non-progressors” have been infected, in that integrated HIV DNA can be detected in their cells and there are circulating HIV antibodies in their blood. However, their immune surveillance mechanisms are sufficient to prevent detectable viral replication, which prevents the development of immunodeficiency that eventually is identifiable as AIDS.

Therapeutic Immunization: Boosting HIV Immune Surveillance

Given the understanding that eradication of the very last HIV infected cell is impractical, and will be very difficult if not impossible, the most promising immunotherapeutic strategy is to augment the immune surveillance capabilities of the host while viral replication is maximally suppressed with HAART. The idea is to improve both the quality and the quantity of the cells that are responsible for immune surveillance.

The innate host defenses, mediated by NK cells and macrophages can be augmented by daily low dose IL2 therapy. We know now that IL2 therapy results in a gradual increase in the concentration of circulating NK cells, and also improves their function: they become more efficient killer cells and they can produce greater amounts of antiviral cytokines when stimulated by IL2.

The augmentation of HIV-specific acquired immune reactivity is dependent upon activation of T cells by HIV peptides, which are short segments of HIV protein molecules. During infection, these peptides become available to the immune system when HIV replicates. However, a vaccine, in a form that is not infectious, can also supply these same proteins and peptides.

The idea is to employ an attenuated or killed HIV vaccine during HAART, to activate HIV-specific T cells, so that they could be maximally activated *before* the antiviral drugs are withdrawn. In this way, it should be possible to manipulate the host defenses to control viral replication so that plasma HIV remains undetectable, and progression to AIDS is prevented.

Presently, there are several HIV vaccines in clinical trials, including killed HIV (Remune), “naked” DNA vaccines, and HIV vaccines constructed from other vertebrate viruses, such as vaccinia, the smallpox vaccine, and canarypox virus, a close relative of vaccinia. Remune is made by “fixing” HIV with formaldehyde. It was introduced by Jonas Salk, and was developed like the polio vaccine developed by Salk. The naked DNA vaccines are constructed from bacterial viruses that cannot replicate in human cells, and into which several HIV genes are inserted. Merck and Wyeth-Ayerst have DNA vaccines in development.

ALVAC, an HIV vaccine Constructed from Canarypox

ALVAC (Albany Vaccine) was developed as a vaccine for HIV because it is a large DNA virus, and therefore, it can tolerate the insertion of large amounts of HIV genes. Also, because it is a virus that infects birds but not mammals, it cannot replicate in human cells. This feature is attractive from a safety standpoint, in that spread of the virus cannot occur, so that there is no danger that the vaccine itself can cause disease.

Canarypox is a member of the poxvirus family, which includes small pox and cowpox, the virus first used by Jenner as a vaccine for small pox. Accordingly, this family of viruses has been known for > 200 years, and a great deal of information about the poxviruses has accumulated.

vCP1452 is a canarypox HIV vaccine that has been developed after several other generations of ALVAC vaccines. vCP1452 contains the HIV-1 envelope (*env*) and major structural (*gag*) genes, the protease (*p15*), and a synthetic gene that encodes the known peptides recognized by CD8+ T cells from the “negative effect” gene (*nef*), and RNA polymerase (*pol*) genes.

Therefore, this vaccine has been constructed to promote the presentation of viral envelope antigens, and thus improve the quality of the antibodies produced against the surface envelope. As well, it will stimulate killer T cells to recognize and react with the major structural and functional core proteins of the virus.

ALVAC vaccines against rabies, measles, CMV, Japanese encephalitis virus, as well as HIV have established a very safe profile for canarypox viruses when administered to humans. No severe (Grade III) or intolerable (Grade IV) adverse reactions have been attributed to canarypox vaccines in over 1,500 HIV seronegative volunteers immunized with an ALVAC vaccine.

The most common side effects from the vaccine are mild (Grade I) to moderate (Grade II) pain and tenderness at the injection site, which is usually the shoulder muscle. These symptoms can be accompanied by mild to moderate redness and swelling at the injection site, and mild fever (< 101°F) that usually subsides within 24 hours.

The Immune Response is Antiviral: The *in vivo* Assessment of Host Antiviral Reactivity

The desired outcome from antiviral therapy and the basis that the FDA has used for drug approval has been a sustained decrease in the concentration of plasma HIV RNA. Thus, the FDA has accepted the concentration of circulating HIV RNA as a surrogate marker that correlates with an improved clinical outcome. This policy is supported by published studies that show a correlation between the plasma HIV RNA concentration and rate of progression to AIDS.

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The net effect of the immune response to HIV is to prevent viral replication and lower the amount of detectable virus, thereby promoting viral latency. Therefore, as first demonstrated by Jenner more than 200 years ago, *the immune response is antiviral*. It follows that the most logical method of assessing the efficacy of the immune reactivity to HIV is to quantify the virus, just as is done when assessing the effectiveness of antiviral agents.

However, measurement of the concentration of plasma HIV is impossible when HAART is administered, because these drugs are very effective in suppressing viral replication. Furthermore, experimental studies in SIV infection of Rhesus macaques have shown that immune-based therapies, whether therapeutic vaccines or cytokines, are more effective when administered while HAART maximally suppresses viral replication.

Accordingly, the best way to test for the effectiveness of immune based therapies is to administer them together with HAART, and to then interrupt HAART, monitoring for the incidence and character of viral relapse.

This procedure has become accepted practice in the evaluation of therapies for Hepatitis C Virus (HCV) infection. In this infection, the therapies are administered for one year and then discontinued, and the plasma HCV concentration is quantified 6 months later. Those individuals with undetectable HCV at this time are termed “sustained viral responders”, and data indicate that these individuals will not progress to a viral relapse or to clinical liver disease.

Accordingly, The FDA accepts the treatment interruption approach to assess the efficacy of therapies for HCV, and they do so because the relapse of detectable plasma HCV correlates with HCV-induced liver disease.

We have recently reported our results of the “In vivo assessment of antiviral reactivity in chronic HIV infection”, which entails monitoring the plasma HIV and circulating lymphocyte concentrations after discontinuation of HAART (see full report in “HIV Clinical Trials” posted in this section of the web site).

This method promises to allow the determination of the efficacy of immune based therapies in phase II trials that require relatively small numbers of volunteers (i.e. ~ 100), and only a short time commitment on the part of the volunteer (i.e. ~ 6 months). In this regard, it is important to point out that many of the antiviral drugs that have been approved by the FDA have received approval in studies with similar numbers of subjects and over a similar time interval.

Consequently, the Supervised Treatment Interruption (STI) approach to the assessment of immune-based therapies promises to shorten the time to the evaluation and identification of the most effective therapeutic vaccines and cytokines.