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**RESEARCH**

**A Unified Concept of HIV Latency**

The introduction of highly active antiretroviral therapy (HAART) combining potent drugs that can inhibit reverse transcriptase, integrase and protease activities has changed the natural history of the human immunodeficiency virus (HIV) type 1 disease. Unfortunately, poor penetrability into different anatomic compartments, toxicity and drug resistance are some of the problems related to their prolonged use. The ability of HIV to mutate and become resistant, along with the ongoing viral replication during HAART, can lead to the emergence of independently evolving viral strains in different anatomic compartments (i.e., brain, testes, lymph nodes, etc.). In addition, HAART predominantly effects the viral replication in the activated or differentiating CD(+) T lymphocytes, but appears to have a very limited effect on HIV-1 preintegration complexes in the latently infected cells. Existing drug therapies do not eliminate these viral reservoirs, nor do they prevent their formation. New strategies are needed for eliminating protected areas of HIV-1 in vivo. Therefore, the persistence of latent HIV-1 reservoirs is the principal barrier in the complete eradication of HIV-1 infection in patients by antiretroviral therapy at present. African non-human primates (NHPs) naturally infected with various simian immunodeficiency viruses (SIVs) appear not to develop immunodeficiency or AIDS, whereas Asian NHPs, which are unnatural hosts, infected with SIVs, as well humans infected with HIV-1, will nearly always develop progressive loss of CD(+) T lymphocytes and a gradual destruction of immune functions. Understanding the difference in the host responses between natural and unnatural hosts, and deciphering which host factors are responsible for the non-pathogenic course of natural SIV infections, would be valuable in developing more-effective treatment or prevention strategies for HIV/AIDS. A number of factors encoded by host cells have been identified that appear to play critical roles in the SIV infection process. Two of these factors, TRIM5alpha (a member of a large family of proteins known as the TRIM proteins) and cellular apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like-3G (APOBEC3G) have been recently identified. APOBEC3G genes belong to a family of primate genes that produce enzymes (in this case, APOBEC3G) that 'edit' RNA by replacing cytosine with guanine into viral particles as the virus undergoes reverse transcription in the cytoplasm of the host cell. HIV-1, in turn, counters with a protein called viral infectivity factor (Vif), which binds to the APOBEC3G enzyme that degrades it. Several other blocking factors have been described, including lentiviral blocking factor (Lv)1 and 2. These factors appear to block the infection at a postentry step; after reverse transcription has occurred, but before proviral integration. Thus, it is crucial to understand the molecular mechanisms involved in the establishment, maintenance and reactivation of lentiviral latency. This review presents various models of HIV-1 latency and forward a new unified model of lentiviral latency.

**An Edible Vaccine for Malaria Using Transgenic Tomatoes**

Malaria, a disease caused by protozoan parasites of genus Plasmodium, is one of the world's biggest scourges. Over two billion individuals reside in the malaria endemic areas and the disease affects 300-500 million people annually. As a result of malarial-

infection, an estimated three million lives are lost annually, among them over one million children (majority under 5 years of age). The mortality due to malaria has increased because of the spread of drug-resistant strains of the parasite, the breakdown of health services in many affected areas, the interaction of the disease with human immunodeficiency virus (HIV) infection, and possibly the effects of climate change. Infants and young children with malaria often die from severe anemia, cerebral involvement, or prostration caused by overwhelming infection; many newborns die from complications of low birth weight caused by maternal malaria during pregnancy. The scarce economic resources and lack of communication, infrastructure and adequate means of travel in the endemic areas make it extremely difficult to implement traditional infection control measures (i.e., mosquito control, preventive anti-malarial drugs and nets). To make the matter worse, both malarial parasites and its insect vectors are increasingly becoming resistant to anti-malarial agents (chloroquine) and insecticides (both DDT and melathione and related chemicals), respectively. By conventional wisdom, the immune mechanisms responsible for protection against malaria will require a multiple of 10-15 antigen targets for proper protection against various stages of malarial infection. By standard vaccination protocols, such a large number of targets would not be appropriate to be used for vaccination as a single dose due to antigenic competition. It would be almost impossible to immunize over two billion individuals who live in malaria susceptible areas with several carefully crafted immunization schedules delivered 4-6 weeks apart in the form of two different antigens as a single dose. Besides, if immunization schedules could be arranged, the stability of vaccines carrying different malarial antigens, their transport, and the logistics of vaccination would be an almost impossible task to achieve under the current fiscal constraints. We are proposing a unique way to circumvent these logistical difficulties to deliver the malaria vaccines to every susceptible home at a small fraction of a cost. We hypothesize that the anti-malaria edible vaccines in transgenic tomato plants where different transgenic plants expressing different antigenic type(s). Immunizing individuals against 2-3 antigens and against each stage of the life cycle of the multistage parasites would be an efficient, inexpensive and safe way of vaccination. Tomatoes with varying sizes, shapes and colors carrying different antigens would make the vaccines easily identifiable by lay individuals.

### **Role of Micro-RNAs in Regulation of Lentiviral Latency and Persistence.**

Small interfering RNAs have been demonstrated to serve as a molecular defence against numerous retroviruses in plants and insects and, more recently, in primates. With the recent findings of micro-RNAs (miRNAs) that seem to play a pivotal role in the survival of the host, we have explored the role of miRNAs in lentiviral (LV) replication. We have previously hypothesized that, at least in the case of lentivirus infection, small interfering RNAs are involved in the inhibition of these types of viruses by the formation of intramolecular triplex formation (triplexes) between the polypurine tracks sequences of LV provirus and miRNAs and blocking the viral replication at the preintegration complex levels, placing these viruses into a suspended latency. Using several latently and chronically infected LV cell lines and human PBMCs from HIV-1-infected individuals, we show that perinuclear triplexes are formed in LV-infected cells. The number of triplexes decreased in cells with productive replication of LVs. Therefore, the degree of replication of HIV-1 and other LVs, both in the HIV-1 or other LV-infected cell lines and the HIV-1 infected PBMCs, inversely correlate with the number of cytoplasmic triplexes present in a particular cell. This correlation was further confirmed by the stimulation of PBMCs and LV-infected cell lines with appropriate mitogens. Treatment with Tagetin, a RNA polymerase III inhibitor, resulted in a significant decrease in triplexes and a dramatic

increase in the LV replication. Our data suggest that triplex formation may be an important mechanism of LV latency mediated by endogenous miRNAs.

### **Latest Developments in *In Situ* PCR**

Since the first publication on the method of in situ polymerase chain reaction (PCR), several thousand research papers have appeared in peer-reviewed journals describing various findings based solely on the application of this method or combined with other more robust methods, including solution-based PCR, immunohistochemistry, Southern blot, etc. A few years after the advent of PCR, several investigators developed in situ PCR methods that differed considerably from each other with regard to tissue preparations, fixation, mounting of slides, reverse transcription technique, primer design, target selection, size, and amplicon size, and thermocycler designs and the use, among many other fine details. This chapter describes the detail procedures that are used in the author's laboratory. It also discusses the variations and modification that can be used for the specific needs of an investigator. This protocol should serve as a primer for the investigators, and each researcher must use his or her variation according to their needs.

### **Locatization of human herpesvirus type 8 in human sperms by *in situ* PCR.**

**OBJECTIVES:** Defining the mechanism of infection with human herpesvirus-8 (HHV-8) or Kaposi's sarcoma associated herpesvirus (KSHV) is an important clinical issue. HHV-8 has been linked to Kaposi's sarcoma (KS) development in HIV-1-infected individuals, and KS develops in 40% of those infected with both viruses. A series of epidemiological data suggest that sexual transmission is one of the routes of transmission for HHV-8. In our studies, we sought to assess the cellular reservoirs of HHV-8 DNA in the semen of HIV-1-infected men and the potential role of HHV-8 infected spermatozoa in horizontal transmission. **DESIGN AND METHODS:** A nested polymerase chain reaction (PCR), in situ PCR (ISPCR) and a sodium iodide (NaI) DNA isolation technique that extracts both nuclear and episomal DNA were utilized to amplify specific genes in vitro and within intact cells to evaluate the types of seminal cells infected with HHV-8 in HIV-1-infected and uninfected men. **RESULTS :** HHV-8 was present in the spermatozoa and mononuclear cells of the semen in 64 of 73 (88%) HIV-1 infected individuals. Both the sperms as well as the mononuclear cells of the semen specimens of HIV-1 infected men were found to be infected with HHV-8. Multiplex ISPCR revealed that a significantly higher percentage of semen cells were infected with HHV-8 than HIV-1 ( $p>0.001$ ). Rare (less than one in a 100,000) sperm cells were co-infected with both viruses. A co-culture of HHV-8 infected sperm with uninfected 293 or Sup-T1 cell lines resulted in an abortive infection of these cells with HHV-8. DNA isolation by NaI yielded 73% of the positive sperm, whereas the standard phenol/chloroform method resulted in significantly lower positives (45%) from the same specimens. **CONCLUSIONS:** Design and methods: Our data strongly suggest a potential sexual/horizontal route of transmission of HHV-8, via the HHV-8 infected sperm and other semen cells, where a large percentage of HIV-1 infected men's sperm and other semen cells are infected with HHV-8. Co-culture studies have further supported the observations that HHV-8 in the sperm cells is infectious and capable of transmission of the virus to uninfected cells.

### **RNAi as Antiviral Therapy**

There are a dozen or so viruses that will continue to be a serious global health threat for many years to come, mainly due to their chronic nature. These include hepatitis C virus (HCV), human papillomavirus viruses (HPVs), West Nile virus and human herpes viruses (i.e., HSV, CMV, EBV, HHV-8, etc.). However, HIV-1 infections will remain at the

top of the list due to its high prevalence and the significant mortality and morbidity from AIDS. The development of a suitable vaccine against HIV-1 remains an important area of public interest. The initial hope of identifying the specific anti-HIV-1 antigenic epitopes that can protect HIV-1-infected individuals and serve as a potential vaccine has been replaced by the realisation that we have yet to identify a clear correlation of protective immunity against HIV-1 infection. Understanding the anti-HIV-1 protective factors and their potential role in the development of a vaccine or inexpensive therapy remains one of the major obstacles in HIV-1 research. In the last quarter century--since the realisation of AIDS--previous studies have established that the role of humoral or cellular immune responses in protecting human hosts against HIV-1 have been inconclusive. Moreover, most of the publicized and awaited clinical trials on vaccines have failed. The recent discovery of RNA interference (RNAi) has raised the possibility of developing a new generation of vaccines that can stymie human viruses, particularly HIV-1 replication at various stages of its life cycle at the intracellular level. Various transcripts in the HIV-1 life cycle can be targeted, and specific small double-stranded RNAs (small interfering RNAs) can be developed against these HIV-1-specific targets. However, some recent data suggests that RNAi-based therapeutics against this virus should be viewed with strong caution. Specifically, there are multiple factors that make HIV-1 a difficult infection to 'cure' because of HIV-1 latency. The changing nature of HIV-1 genomes and the possible presence of microRNAs within the HIV-1 genes can suppress RNAi directed against HIV-1 gene targets. Thus, HIV-1 would be a difficult epidemic to overcome by RNAi-based therapeutics.

### **Zinc and prostate cancer.**

The role of zinc in the development and progression of prostate malignancy and its potential application in the prevention and treatment of prostate cancer (PCa) are contemporary critical issues for the medical/scientific community and the public-at-large. The overwhelming clinical and experimental evidence provides a compelling rational basis for the expectation and concept that prostate zinc accumulation is an important factor in the development and progression of prostate malignancy; and that zinc could be efficacious in the prevention and treatment of PCa. In contrast, various epidemiologic studies have produced divergent and conflicting results regarding the efficacy of dietary and supplemental zinc against PCa. Before reaching any definitive conclusions regarding this complex issue, one should have a complete understanding of the clinical and experimental evidence associated with the involvement of zinc in the normal and malignant prostate. Also, an understanding of interacting effects of confounding factors on the absorption, assimilation, and bioavailability of supplemental dietary zinc is important. The purpose of this review is to present the current state of the clinical and experimental information regarding zinc relationships in the normal prostate and in the pathogenesis PCa. The evidence in support of a potential beneficial effect of zinc supplement versus potential harmful effects on PCa is assessed. A discussion of the divergent results of the epidemiologic studies is presented along with a description of important factors and conditions that impact or mask the effects of dietary zinc on PCa development and progression. We also hope to bring more attention to the medical and research community of the critical need for concerted clinical and basic research regarding zinc and PCa, for the development of appropriate human prostate models to investigate these relationships, for further appropriately designed epidemiologic studies, and for future well-controlled clinical trials.

### **Role of zinc and zinc transporters in the molecular pathogenesis of diabetes mellitus.**

Diabetes is one of the most common chronic diseases in the United States. An estimated 18.2 million people in the US (6.3%) have diabetes; among them 2.8 million are African Americans (AAs). On average, AAs are twice as likely to have diabetes as European Americans (EAs) of similar age. AAs disproportionately suffer from various diseases in the US. Many of these diseases include hypertension, cardiovascular disease (CVD), diabetes mellitus (DM-beta predominantly Type II), and cancers of the prostate and pancreas. A number of risk factors such as smoking, a high fat diet, little physical activity, stress, and meager access to health care have been the subject of numerous investigations. However, the factor of the interaction between genetics and the environment has received very little attention in the scientific community. Of note, the content of zinc in pancreatic beta cells is among the highest in the body; however, very little is known about the uptake and storage of zinc inside these cells. We hypothesize that one of the major reasons AAs disproportionately suffer from DM (as well as some other illnesses like prostate cancer, CVD and hypertension) is due to their inherent inability to transport appropriate amount of zinc in the crucial cell types that require relatively higher amount of zinc than the other cell types. In this article, we will explore in detail the possible genetic and environmental link between human zinc transporters (hZIPs) and their differential expressions in the islet beta cells from AAs as compared to other racial groups, particularly EAs, in both normal healthy individuals and diabetic patients. We hypothesize that the hZIPs play an important role in the development of diabetes, and the main reason AAs disproportionately suffer from DM (as well as other illnesses like prostate and pancreatic cancers, hypertension, and CVD) as compared to EAs may be due to the low degree of expressions of the critical zinc transporters in the beta cells. Understanding the molecular events in the pathogenesis of DM with regards to regulation of zinc uptake would be critical to the evaluation of the natural history of diabetes in humans and especially in various racial groups. If a direct link between zinc transport and diabetes can be established, then a special nutritional formula, medication or other intervention might be especially designed to test the ability to decrease the incidence of this disease in DM susceptible groups, particularly in AAs.

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