VIEWPOINT

A New Perspective on HIV Vaccine Design: A ViewPoint

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Abstract
The unsuccessful outcome of a number of vaccine trials in the quest to conquer HIV-1 demonstrates the difficulties inherent in fighting diseases that afflict our world, and the poor disproportionately, especially the developing nations. A quarter century has elapsed since AIDS became a recognized major threat to human health, yet the ever-growing volume of scientific data has failed to meet its prime objective, a cure for AIDS or an HIV prevention vaccine. This brief article suggests several intriguing possibilities for researchers to consider, including small RNA-based immunity, as they seek to find a vaccine for the HIV-1 infection that threatens not only individuals and families, but in some cases entire nations.

Keywords: African non-human primates (ANHPs), Classical immunity (CI), Cell-mediated immunity (CMI), elite suppressor (ES), Humoral immunity (HI), innate immunity, long-term nonprogressor (LTNP), microRNAs (miRNAs), molecular immunity (MI), Vaccine

1. Introduction

The unsuccessful outcome of a number of vaccine trials in the quest to conquer HIV-1 demonstrates the difficulties inherent in fighting diseases that afflict our world, and disproportionately the developing nations (1-3). A quarter century has elapsed since AIDS became recognized as a major threat to human health, yet the ever-growing volume of scientific data has failed to meet its prime objective, a cure for AIDS or a HIV prevention vaccine. This brief article suggests several intriguing possibilities for researchers to consider, including double-stranded (ds) small RNA-based immunity, as they seek to find a vaccine for the HIV-1 infection which threatens not only individuals and families, but in some cases entire nations.

Our logic that the current efforts to find a vaccine that travels down classical immunity pathways will not arrive at its desired destination is based on scientific data in several areas, including the following:

i. In the course of scientific investigation, data sometimes emerge that cannot be readily reconciled with existing theory. Yet scientists are, by nature, reluctant to reach for a new theory when old concepts are still seemingly viable, albeit in need of revision or update. Usually, the consequences of this scientific reticence are simply enlivened verbal debates at academic conferences or written contests in scholarly journals. However, in the field of human immunodeficiency virus type 1 (HIV-1) pathogenesis, the effects of a decade-long lack of progress in understanding the fundamental biology of retroviral infection can be measured in human terms—literally twenty-three million people may be infected with HIV-1 by now, and many will likely die of AIDS and its complications if rapid progress is not made. Effective treatments must be developed which work at the molecular level—for that is the scale at which retroviruses work—and an effective vaccine for HIV-1 is desperately needed as the epidemic continues to expand worldwide in an unthrottled fashion (1-3).

ii. Clues to effective treatment and vaccine have long been before us, but conventional wisdom has repeatedly misguided us in the course of scientific investigation. Just three years ago, for example, most scientists thought HIV-1 infection included a long latency period where there was little or no viral activity but we now know this hypothesis may be completely wrong and that associated lines of inquiry were but blind allies (reviewed in 4-5). Worse still, inconsistencies between observed facts and prevailing theory have led to the expenditure of considerable capital in a counterproductive argument over the etiologic agent of AIDS—long after overwhelming data have incontrovertibly shown HIV-1 to be the cause of the disease (4). Still, the dissenters against the "HIV-AIDS hypothesis" have made many valid observations, particularly involving the various "cofactors" that clearly influence the course of HIV-1 pathogenesis (reviewed in 4). Yet mainstream scientists seem to have focused more energy upon being appalled at the dissenters rather than trying to examine and accommodate the kernels of truth in their arguments. Existing immunologic theory makes the accommodation of these dissenters' concepts—as well as, considerable quantities of other enigmatic epidemiological and laboratory data—exceedingly difficult.

iii. The fact is that retroviruses are a unique form of infectious agent and one that has direct access to the genome of host species (6-7). The genetic nature of retroviruses is fundamentally different from all other infectious agents, a characteristic that may allow the virus to cause substantial genetic damage to the host (7), even permanent change to the germ line of the host species (in fact, some molecular biologists argue that the action of retroviruses has been a critical factor in the course of vertebrate evolution). Yet conventional humoral immunity (antibody formation) and cell-mediated immunity seem to be ineffective against most retroviruses (reviewed in 1-5). It is almost inconceivable that higher organisms have evolved without some special means to control this special sort of pathogen, or else retroviruses would long since have caused massive genetic damage to myriad host species, which fortunately they have not done (8-12). As a matter of fact, abundant data, derived both in vitro and in vivo, show that in mammals the majority of the eurkaryotic intracellular defenses have arrived from transposons and retroelements (9-16), and that all life forms appear to be quite capable of controlling the actions of retroviruses, but the observed characteristics of the immunologic response do not seem to fit any existing theory of immunology.

iv. The vast majority of humans with high risk behaviors, when exposed to significant doses of HIV-1, become infected (in a traditional definition of infectious diseases) and develop antibodies to HIV-1 antigens. However, many individuals remain uninfected with the virus, despite histories of multiple high-risk sexual exposures to the virus (see below for details). For example, it has been shown that the CD4+ cells of some individuals have resisted very high doses of the virus (about 1,000-fold more virus than that required to establish infection). Also, in these individuals, the majority of cells failed to support viral replication (reviewed in 4-5, see below for details).

v. While the HIV-1-pandemic has been the central focus for health-care providers, and has captured most of the public's attention, many species of African nonhuman primates infected with various strains of simian immunodeficiency viruses (SIVs) are providing valuable perspectives that can help us to better understand host-retrovirus interaction (4, 17-18).

vi. For example, over 50% of African green monkeys are infected with a sub-strain of simian immunodeficiency virus subtype SIV_{agm} in the wild, yet no clinical pathology has been associated with this infection to date (4, 17). Similarly, sooty mangabeys have been shown—both in the wild and in breeding colonies—to

be infected with another sub-strain of SIV_{sm} (4, 17). Like the African-green-monkey infection, the sooty-mangabey infection appears to cause no disease in its native host. These and many African nonhuman primates are the natural hosts of SIVs, yet they harbor the virus their entire lives without developing the disease (4, 17).

vii. There is a striking homology between this SIV of sooty mangabeys and human immunodeficiency virus type II (HIV-2) (reviewed in 4, 17), but there is a marked difference in the clinical course, with the HIV-2 infection significantly prolonged (reviewed in 4, 17).

viii. There is significant sequence homology between HIV-1 and SIV_{cpz}, a simian immunodeficiency virus isolated from chimpanzees, which causes no apparent illness in the naturally infected chimpanzees (1-6). Of particular note, chimpanzees experimentally infected with HIV-1 fail to develop overt disease despite establishment of infection as evidenced by transient viremia and development of HIV-1 specific antibodies and HIV-1 specific cytotoxic T-cells (1-6).

ix. The vaccine efforts utilizing nonhuman primates have shown quite clearly that if the monkeys are first infected with a non-pathogenic lentivirus, then challenged with a genetically-closely-related pathogenic variety, they do not develop disease (reviewed in 4, 17-18). However, if they are first infected with high doses of a pathogenic variety that is genetically unrelated to any prior lentivirus infection, then the monkeys do develop AIDS-like disease.

x. This complex pattern of clinical expression among lentiviruses is shared by other species of retroviruses that infect humans. For example, the human foamy or spumaviruses have yet to be clearly associated with any disease in humans despite certain populations with a high prevalence of infection, and infectious virus can be readily cultured in explanted tissues from these individuals (reviewed in 9).

xi. Human T-cell leukemia/lymphoma virus type II (HTLV-II) has been shown to be endemic in certain American Indian populations, but without clinical disease (reviewed in 9).

xii. Human T-cell leukemia/lymphoma virus type I (HTLV-I) causes disease in a small minority of patients, leading to either adult T-cell leukemia if acquired in infancy or a chronic neuropathic disease if acquired later in life (note the ages of immunoincompetence) (reviewed in 9).

xiii. Similarly, although less dramatic, substantial disease variability has been observed with the clinical course HIV-1 infection. There are reports of long-term survivors for as long as 15 years (reviewed in 4, 8, 17-18), and recent estimates show that at least 1% of HIV-1-exposed individuals may never develop disease (4). In contrast, other reports document patients who rapidly progress to immunodeficiency in a matter of a few years (4).

xiv. Pediatric HIV-1 infection is typified by a bimodal pattern of disease progression (reviewed in 4- 5). Therefore about 20% of perinatally infected infants exhibit rapid progression towards AIDS, with immunological deterioration, low CD4+ T-cell count, high viral burden, failure to thrive, delay in development or regression in intellectual capacities, and very high mortality rates. About 80% of children with perinatal HIV-1 infection show a relatively slower development of disease, long-term survival, low viral burden, and limited morbidity with HIV-1 infection (reviewed in 4).

xv. The documented exposure of over 2,400 health care workers is most curious, as only four have seroconverted and none has developed AIDS (reviewed in 4 and 8). And then there are the cases of spontaneous clearance of HIV-1 (49-50), or the low frequency of successful transmission of HIV-1 resulting from a single intercourse with an infected partner, even though HIV-1 is present in almost 80-100% of human semen specimens. Two other anomalous observations are the reported isolation of HIV-1 from individuals who remained HIV-1-seronegative, and the observation that some men with many different partners with whom they practiced receptive anal sex still remain seronegative (reviewed in 4 and 8).

xvi. Retrovirus-based vectors have predominated in gene therapy trails and successful ex vivo transfer of genes have been demonstrated (reviewed in 4). However, no human disease has been cured by utilizing the retroviral vectors yet, even though several studies have demonstrated that therapeutic gene transfer to humans via retroviral vectors can be detected in vivo for several years; no long term biological responses could be documented. The most publicized therapy, utilizing retroviral vectors containing an adenosine deaminase enzyme expression system for the treatment of severe combined immunodeficiency, resulted in failure. In every case, the retroviral vectors appeared to have shut down after few days to few months after the infusion of rector containing cells. It is hypothesized that this observed phenomenon is the result of natural intracellular defenses against retroviruses and until

these intracellular mechanisms have been well defined and better understood, gene therapy protocols that use retroviral vectors will prove useless.

xvii. In mice, the presence of two different endogenous proviruses have been identified as protective against infection with certain exogenous retroviruses (reviewed in 8). Similarly, such a phenomenon had been noted in chickens, where the presence of certain endogenous retroviruses seems to protect against exogenous viruses, most probably through intracellular molecular immunity (reviewed in 8).

xviii. Fv1, an endogenous Gag-related gene, has been described recently (reviewed in 4 and 8) in certain strains of mice, which makes them resistance to murine leukemia virus (MuLV). Fv1 gene product is able to block the virus in the early phase of the viral life cycle. The course of infection is blocked after RT, but before the establishment of the integrated provirus in the host genome.

xix. Some identical, monochorionic, monozygotic twins, born to HIV-1 infected mothers, show discordant results. This means that one is infected with HIV-1 and other one stays uninfected. This is because in monozygotic, monochorionic fetuses, the blood circulation is such that the blood first goes through one of the twins and then proceeds to the second. In this situation, the first twin is exposed to high doses of HIV-1, and the second gets lower doses of the retrovirus... In this case, the first one gets infected while the second one is "vaccinated" against HIV-1 (reviewed in 4).

xx. Most of the research efforts on retroviruses over the past 25 years have focused on the mechanisms of disease production by these pathogens. Now it is time to explore the mechanism by which infected hosts defend themselves. This article maintains that evolution has created intracellular protective mechanisms to specifically battle retroviruses. Many of the previously anomalous phenomena reported by various investigators would be explained on the basis of this hypothesis. For example, it could explain why SIV_{agm}, which has the same overall genomic organization as the other lentiviruses, causes no known disease in its native host, the African green monkey. Similarly, it can explain why the SIV_{sm} causes no significant disease in its natural host, the sooty mangabey and yet causes an AIDS-like illness in experimentally infected, naive, rhesus macaques, and in cynomolgus monkeys, experimentally exposed to SIVagm (reviewed in 1-6). Extensive analyses of the immune responses of African green monkeys and sooty mangabeys against their respective SIVs show no unusual activity against the virus. They exhibit weak, if any, neutralizing antibodies, no cell-mediated immune response and viral loads in their systems are completely independent from their immune responses to the viruses (reviewed in 4, 8, and17).

xxi. It is hypothesized, on the basis of substantial experimental data and the experiments of nature, that the final disease potential of retroviruses depends on host-retroviral interaction that is primarily governed by the initial viral dose, the replication capacity of the virus, and the immunocompetance of the host. The survival of the host primarily depends on the rapid development of intracellular "molecular immunity" and is altogether independent of humoral or cell-mediated immune responses. This "molecular immunity" is able to prime the majority of target cells with the appropriate defenses, outracing the pathogenic effects of the retroviruses. There is enough documentation to show that miRNAs play an important role in protecting the host against lentiviral and retroviral invasion (8-16). In addition, in both primates and humans, retroviral specific "molecular immunity" could be raised by the following modes:

a) If the host is exposed to very low doses of the pathogenic virus. The examples of low seroconversion in health care workers, clearance of HIV-1 virus from certain individuals, and the long-term nonprogressors with the nef-defective HIV-1 strains are examples (reviewed in 4).

b) If the host is exposed to a nonpathogenic strain of the retrovirus first before any exposure to a genetically-related pathogenic strain of the virus. Reports of natural immunity in various monkey species against relatively pathogenic SIVs could be explained on the basis of this hypothesis. Since the African primates are exposed to various types of lentiviruses in the wild, they may be protected against a wide range of lentiviruses (19-20). On the other hand, the Asian primates that are evolutionarily naive for certain lentivirus strains would be susceptible to even relatively mild types of lentiviral infection. Similarly, neonates and young humans or primates exposed to even relatively mild pathogenic strains of lentiviruses would develop immunodeficiency due to late maturation of this molecular immunity system. For example, a case has been reported where a woman delivered a baby infected with HIV-1; 12 years later she is without symptoms but her child has died of AIDS (reviewed in 4 and 8). Similarly, it is reported that an attenuated SIV, designated SIVD3 (a mutant of

SIV deleted in the nef and Vpr genes), induced a lethal AIDS-like disease in two of the four macaque neonates infected orally, but the infection remained attenuated in the adult after intravenous infection (19-20).

xxii. Since 1991, there have been over 4,000 vaccine development trials utilizing various nonhuman primate models of AIDS and involving hundreds of thousands of humans (1-3). Numerous different vaccine strategies were utilized, but none of these strategies in humans that utilized Classical immunity models has been successful (1-3). In the non-human primate models all the vaccination strategies have been unsuccessful or remain unresolved except that attenuated live virus or genetically related non-pathogenic viruses have resulted in consistently high levels of protection following challenge with pathogenic genetically-related virus (reviewed in 1-3, 8, 17-18).

Intracellular Molecular Defense

Many different kinds of pathogens render the human body vulnerable to infection. These invaders differ greatly in their life cycles, the structure of their surfaces, and their mode of entry into the host, so each type of invader must be countered by a correspondingly diverse set of defensive mechanisms within host immune systems.

Primitive forms of life are believed to be RNA in nature; therefore, it follows that the invaders of host RNA genomes were also RNA-based. These were self-replicating nucleic acids; thus, the intruders evolved in a manner that allowed them to integrate into nucleic acids. During early evolutionary stages, it was challenging to separate host from parasite. Therefore, the mixing of genomes presumably caused enormous radiation of these early life forms (4, 6).

This developmental phase subsequently evolved into unicellular and later multicellular life forms, and the related radiation of speciation. As this process of speciation matured further, the invading microlife forms did likewise and came to be organized into genomes. Even as they evolved, novel invasion strategies came to life that could threaten ever larger and complex genetic structures. This Darwinian evolution of host-parasite enmity, this perennial defense and counterdefence chess game, resulted in the proliferation of new species of both hosts and parasites (6). As organisms developed the means to oppose the integration capacities of invading transposons and retroelements through expression of endogenized pieces of nucleic acids as well as specialized proteins, the opposing parasites also devised methods to fight host defenses.

We maintain that this primeval evolutionary phase gave birth to molecular immunity. What was initially a simple immune defense system based on homology recognition eventually gave birth to exceptionally specialized defense systems that we now see in the form of miRNA- and RNAi-based immunity. This initial system was founded on evolution of a singular molecular-based recognition system capable of distinguishing the similarities between self (non-coding nucleic acid sequences) and the genetic fragments of invading agents (4, 8). Consequently, ds-hairpin small RNAs, expressed from previously integrated retroelements, developed the capacity to disable the reintegration and the replication of numerous intracellular genetic parasites, including DNA and RNA viruses, retroviruses, transposons, and retrotransposons. Because the genetic mutations and recombinations within and between intracellular agents were consistently creating potentially newer iterations of genetic invaders, consistent with Darwinian evolutionary theories, molecular immunity came to be a constantly evolving phenomenon, which periodically hit bumps on the road to self-preservation. One recent bump that Homo sapiens has hit is the arrival of HIV-1/AIDS (21). However, throughout the evolution of life forms, comparable bumps have threatened, and even eliminated, many life forms, among them large chimpanzee colonies in the recent past (21-22).

At this point, it is helpful to note that the first immunity to develop was molecular immunity based on small dsRNAs, and that this is still the most prevalent immunity, and constitutes the primary defense against intracellular invaders (4-5, 8-9, 23-25). Molecular immunity is found in every life form, no matter how primitive or how advanced (4, 12-16, 23-27). This immunity, primarily derived from retroelements, has profoundly influenced the evolution of prokaryotic and eukaryotic life forms (13-15). Researchers have observed the existence of genome sequences at the ~50% level for most modern life forms that display genetic resemblance to either retroelements or transposable elements (9, 13-15). Out of this miniature drama in which miRNA defensive systems did battle against retroelements intruders, numberless flora and fauna emerged, as did persistence capacity (4, 13, 15, 25).

As time passed, many life forms chose accommodation with retroelements rather than biological combat (9-14), a pattern that played an intermediary role for "molecular immunity" (4), with its defensive system made up of expression of small fragments of non-coding genetic fragments drawn from the previously incorporated double-stranded RNA of retroelements. Molecular immunity appears to have origins in both Prokaryotes and Archea (13-15, 25).

Endogenous Retroviruses: Protective Lessons

Endogenous retroviruses heavily colonize vertebrate genomes, which share approximately 50% of their genomic DNA. These endogenous retroviruses have emerged from host cell retroviral infections via evolutionary progression, which permits the permanent integration of viral genomes into host DNAs, and facilitates multigenerational transmission (25-26). Endogenous retroviruses obstruct replication cycles of exogenous pathogenic retroviruses that are transmitted horizontally. We hypothesize that endogenous retroviruses have afforded protection to hosts against pathogenic retroviruses that share genetic sequences similar to those of the integrated viruses. Researchers Arnaud et al. (27) have recently characterized the molecular virology and evolutionary history of the Jaagsiekte sheep retrovirus (endogenous beta-retroviruses, enJSRVs), and pointed out the crucial function of integrated retroviral genes in the struggle to oppose exogenous retroviruses. These scholars found that (i) two loci from enJSRV, which had invaded the host genome prior to speciation within sheep (i.e., genus Ovis) approximately three million years ago, had acquired, following integration, a defective and mutated viral protein that was able to of block exogenous retroviruses; (ii) both transdominant enJSRV loci had became permanently established in the host genome by at least the time of sheep domestication (i.e., 10,000 years ago); (iii) the intrusion of endogenous JSRV/enJSRV retroviruses persists to this day; and (iv) there are new (< 200 years ago) viruses that elude the transdominant enJSRV loci. Hence, momentous virus-host combat goes on; hosts counter retroviral infections via endogenization, and the judicious selection of endogenous retroviruses affords molecular defense.

Evolution of Cytoplasmic Replication Strategy

As life forms grew increasingly complex, increasingly sophisticated parasites also emerged; some evaded integration into the host DNA; rather, they replicated beyond the confines of the nuclear system while cannibalizing raw matter from the host and from associated synthetic machinery (28). To find viable protection against these novel RNA and DNA viruses became paramount. Consequently, small interfering RNAs, as well as triplex-forming microRNAs (tfmiRNAs) countered by challenging, and effectively interfering with, the cycles of viral replication (8, 29-32). Hosts attempted genetic editing (i.e., APOBEG3G enzymes: 22). For their part, viruses responded with miRNAs in order to disable host miRNAs (33). A balance resulted, and host genomes continued to accommodate more and more retroelements until in many life forms almost half of their genomes had REs; these seem to have provided the requisite number of small dsRNA permutations to block retroelements invasions (4, 8). Increasing retroelement numbers called for the control, without damaging host replication, of numerous potentially active endogenous retroelements as well as coding genes. In fact, the present mechanism for effecting mammalian gene regulation resembles a huge orchestra, one that promulgates life's musical scores through complex patterns of synchronic balance. Early in their progression, hosts evolved sizeable numbers of retroelements, and also began to co-opt endogenized RE-miRNAs for both internal and external regulation (26-28,30-36). Moreover, as life forms accumulated multiple organs and layers, cellular differentiation and specialization led to selected gene expression in varied cell types on a differentiated basis. This, in turn, prompted the expression of non-coding genes at differential stages (37). Meanwhile, evolution led to resource conservation, and life forms developed cell surface receptors for purposes of expression for gathering resources and for differentiation. Accompanying these developments was the invitation of specific retroviruses, and other viruses as well, to invade cells in specific ways and at specific levels of development. As for miRNAs, they were differentially expressed in a manner that allowed them to check invasion through miRNA arrays (34-37).

Each host cell developed the means to block retroelements and to curtail other viral entry (4,8-9). However, to operate properly required that surface receptors routinely regulate nutrition and systematically communicate signals (34-35). Retroelements and viruses targeted the most vulnerable parts of host cells. The contest still continues, as observed in both the negative and positive utilization of entry routes for such menaces to life on the planet as SIVs, HIV-1, and human herpesvirus 6 and 7. CD4 molecules are important players in the contest; they are critical for the proper functioning of CD4+ T cells, as well as other immune cells (38-43). There are cases (e.g., Caenorhabditis elegans) in which miRNA deterrence has gained such ascendancy as to prevent invasion by any natural virus (44). When it comes to unnatural viruses, however, C. elegans are vulnerable to unnatural viruses, including human varieties that prove deadly in vitro (44). These worms have not developed the necessary miRNAs to quell unnatural infections (45).

Innate Immunity and Development of Immunity Based on Pattern Recognition

As multicellular life forms developed more sophisticated

means of communications and signaling pathways, pathogens also evolved to exploit the surface receptors. Therefore, organisms developed another layer of defense system that was based on pattern recognition. This so called innate immune system lacked the fine specificity that is so precise in case of nucleic acid homology dependent immune defense that has been so well documented in the form of RNAi or miRNAs, but it has an uncanny ability to recognize self versus non-self based on recognition of repeating patterns of molecules on the surface of invading agents.

The surface of microorganisms is generally covered with repeating patterns of molecular structures; their nucleic acids likewise display predictable patterns. Bacterial DNA, for example, contains unmethylated repeats of dinucleotides CpG. Viruses nearly always require dsRNA in their life cycles (7). A singular immune system developed that could recognize so-called foreign pattern recognition in the invading agents through pattern-recognition receptors (PRR). One such receptor capable of pattern recognition is mannose-binding lectin or MBL. MBL and a number of other protein receptors discern particular sugar patterns on pathogenic surfaces while simultaneously recognizing that these particular patterns are not evident in the host. This protein exists in human blood plasma as a free protein and participates in the activation of complement, which constitutes yet another portion of the innate immune system, and forms a crucial link between various immune defense layers and levels. Through pattern-recognition receptors (PRR), mannose-binding lectin (MBL) can sense invading pathogens and accurately distinguish them from self (24).

For multicellular, multi-organs life forms the initial line of systemic defense is, naturally, dependent on those protective gears that check invader entry. Examples include the anti-microbial enzymes of lacrimal glands, keratinized skin, respiratory epithelia, etc (46-47). Once the invading life forms reaches the host target cells, the first immune systems to counter the invading microorganisms are those that are perpetually ready to resist an invader. The PRR system systematically discriminates between self and non-self through a scanning process that analyzes the differences in patterns on the surface of newcomers, and judges their similarity or difference vis-à-vis the patterns found on self. When necessary, they then attack and destroy the harmful intruders. From a practical perspective, the surfaces of many microorganisms bear repeating three-dimensional patterns of mannose, a sugar that is present on the surface of many microorganisms in a repeated fashion and with a specific orientation. This 3D pattern is sensed by MBL, which performs a binding action, and activates a complement cascade that pokes holes in membranes of the invaders, which causes them first to leak and then to perish (24, 47).

Extracellular hijacking of nutrients and development of "Classical Immunity"

As intracellular invasion became increasingly problematic, pathogens evolved new niches and approached ways to invade hosts. Subsequently, in larger forms of life, pathogens evolved that could dwell in the blood, and in body fluids and cavities, in order to seize raw materials (46-48). Significantly immune to miRNAs, and having never previously entered cells, these parasites remained relatively invisible to miRNA. New defenses, however, evolved to deal with these unseen insurgents; natural killer cells (phagocytosis), innate immunity, and later classical immunity developed to quell this new type of intruders. Then, maybe as long ago as 400 million years, jaw-fish commenced the development of antibodies that could offset invading antigens, which gave rise to classical immunity's humoral arm (46-47). Interestingly, this newly acquired immune defense also owes a debt of gratitude to transposons, the mother of molecular-based immune- and miRNA-based immune systems (48) .

As extracellular fungi, parasites, and bacteria sought to intercept resources in the large hosts upon which they preyed, classical immunity was not necessarily needed to defend against intracellular DNA/RNA viruses and retroelements (4, 8, 12-15). With relative ease, molecular immunity quelled them (4,8,25). Meanwhile, retroelements did not cease their evolutionary adaptation; in fact, they seem to have produced countermeasures and genetic codes that utilized molecular immunity to bypass miRNAs and other small RNAs (4, 8-9, 12-14). Hosts developed in a way that allowed them to check invader entrance through the use of viral receptors, to modify the genetic codes of intruders upon entry by editing those codes, and to stymie the replication cycles of retroelements (33). Still, a great deal is left for us to discover. Homologous sequence recognition, the primary means of host defense, has served over a vast period of time (8-9,12-15,25). It is important to note that host defenses developed in a series of layers, and classical immunity appears to be the newest layer that has evolved to counter extracellular invaders (47). It exerts no significant influence on intracellular retroelements' replication, including HIV-1 replication (1-4). The response of classical immunity (CI) to HIV-1 antigens is typical of its response for other antigens; however, it has very little effect on reproduction of retroviruses, lentiviruses, or retroelements (1-4). Perhaps this helps us

understand why so many studies note CI's "effectiveness" in dealing with HIV-1 replication. Such studies report only part of the overall picture. CI has developed the capacity to sense and respond to "foreignness" when confronted with any invading epitope or antigen (47). This does not mean, however, that it has achieved "immunity" from pathogens that deliver the foreign entity, something we are already painfully well aware of following thousands of clinical trials utilizing hundreds of thousands of human volunteers who received antigens or vaccines based on CI, trials that failed to impart any immunity against HIV-1 (1-3). Consequently, these pathogens constitute living proof of CI's ineffectiveness in combating HSV, HCV, HIV-1 and other menaces to human health.

Classical Immunity Is Not Very Useful Against Retroelements

Historically, both humoral immunity (HI), which is antibody-mediated, and cell-mediated immunity (CMI), which is CD8+ T cell mediated have been useful in vaccine development (47). However, to date they have not prevailed against HIV-1. These two pillars of classical immunity allow customary immunological responses whenever an invading substance is judged to be "foreign" (47). Briefly put, molecular immunity scrutinizes sequences with shared homologies vis-à-vis non-coding miRNA sequences, and effectively incapacitates them (4, 25). Reliant on a lymphocyte recognition system, classical immunity serves humans by recognizing alien substances and directing a protective response by either CMI or antibody-mediated immunity (HI) (46-48). Classic immunity has afforded the classic means of protection when bacteria or other foreign substances (including viruses and HIV-1 retroviruses) threaten the body (47). However, classical immunity has an unfortunate limit: it recognizes only extracellular antigens (47). Its health- and life-preserving heroics against extracellular pathogens fail to block intracellular pathogens; it provides very limited preventative checks once pathogens have invaded the cell (as with such genetic parasites as retroviruses, lentiviruses, transposable elements, and retroelements) (4, 25). The beneficial but non-universal protection that HI and CMI offer humans ultimately results in considerable numbers of pathogenic casualties. Vast numbers of people consequently succumb to mycobacterium tuberculosis (with its CI-defying specialized sheath) and malarial parasites (whose intracellular replication in red blood cell hosts threatens one fifth of the world's population and annually snuffs out the lives of more than a million children) (49). Retroviruses remain beyond the grasp of classical immunity as they infect vital immune cells (e.g., CD4+), invade the very genomes of infected cells, and multiply their threat as they divide, but only after a deceptively dormant state (7). The classical cell- and antibody-mediated mechanisms routinely fail to effectively hinder HIV-1 invasions or the threats posed by SIVs (17-18). Even if such mechanisms do not fail to identify their dangerous intruders, an antibody response may actually be more harmful than helpful (4). Within cells, classical immunity lacks utility (1-4).

microRNAs

The creation of anti-HIV-1 therapeutic agents may be hastened by utilizing recent insights into miRNAs and the manner in which they regulate retroelements. Retroelements, which constitute a significant proportion of all eukaryotic genomes, can alter genes, and thereby threaten host genomes (4-5,50). Moreover, they may be blocked through molecular methods that check mutagenic activity (26-27). Recent research has suggested that complex molecular means can silence mutagenic ability, and systematically express such components of integrated retroelements as introns, miRNAs, and other elements (e.g., LINES, SINE, LINES, and Alu) (4, 26). Numerous non-coding small RNA (as well as introns) derive from retroelements, and through molecular means express the building blocks of incorporated retroelements that may be used to silence endogenous retroviruses, and exogenous retroelements intruders. (4,8-9,12-15,25).

New Approaches for New Dilemmas

As recently as ten years ago, classical immunity appeared sensible as the main model for an HIV-1 vaccine, but by the end of the past century, immunity theory emerged that focused on small dsRNA (4,25,50). Based on research into worms and plants, this particular form of immunity, which deals with RNA interference (RNAi) and miRNAs, checks viruses in plants via gene silencing, and functions abundantly in eukaryotic life (4-5, 12, 25, 50). In 2006, two United States scientists – Andrew Fire and Craig Mello – earned a Noble Prize for their groundbreaking work in the discovery of RNAi (50). Meanwhile, the AIDS pandemic evaded the traditional tools that had seemed so promising (1-3). Building on research about humans with HIV-1, as well as macaques infected with SIV, researchers touted CMI's promise in countering immunodeficiency (1-4). However, no study could show nonexistent viability, in either strong cell-mediated immune response or broadly-based neutralization of antibodies (1-3). VaxGen's unsuccessful human trial in 2003 brought major distressing

news (2-3, 51-53). Great hopes had been placed on its Env (envelope)-specific methodology, which narrowed in on gp (glycoprotein) 120 but ultimately was unable to "neutralize primary HIV-1 isolates in vitro," and prevent HIV-1 infection. Moreover, it did not affect the viral load of participants in the trial that became infected with HIV-1 (51-53). To date, no HIV-1 vaccine trial has brought forth effective broadly reactive antibodies (1-3). Mainstream researchers should now be open to additional methodological approaches as we pursue our joint quest to produce a vaccine for HIV-1 (1-2, 52).

Lessons from Other Primates

Genetic similarities between humans and chimpanzees help explain scientific fascination with these primates. Following infection with HIV-1, chimpanzees experience viremia early on but subsequently do not develop disease. Obviously, an inherent potential for HIV-1 inhibition replication deserves ongoing research efforts (4, 17-18). There have been trials in which immunized chimpanzees suffered from viremia initially, even when HIV-1 neutralizing antibodies could be observed and when high response levels with regard to cell-mediated immunity occured (17-18). In other cases, chimpanzees that had not been vaccinated coped successfully with the viremia challenge, and then stayed free from infection (17), which implies that some form of natural immunity, probably intracellular immunity, is functioning (8, 17-18).

Vaccine Research and Strain Diversification

One leading explanation for the unsuccessful anti-HIV-1 vaccines is the vast diversity of HIV-1 viral strains and quasi-species (1-3). In fact, genetic variability is extensive, and typifies viral isolates. HIV-1, by definition, hampers normal immune response; extensive mutation disrupts classical immune reactions. Simian immunodeficiency viruses (SIVs) - like HIVs complex - endemically spread infection among more than 40 African non-human primates (ANHPs, reviewed in 4,17-18). Among those ANHPs infected with lentiviruses naturally, numerous "quasispecies," often termed "SIV swarms," emerge within just a few days from the time of incipient infection, yet the infection remains controlled (17-18). This suggests that strain diversity is likely not the principal culprit behind immunity failures in human beings (17, 54). Researchers have been intrigued that there are HIV-exposed seronegative female sex workers who remain seronegative in spite of their exposure to HIV-1 (i.e, EU), and have found them resistant to many HIV-1 types and clades (53-54). If strain diversity were actually the primary reason for vaccine failure, then EUs, as naturally resistant individuals, would typically lose protection not long after exposure to a new viral strain (54-58). We recommend the abandonment of current paradigmatic patterns, and renewed focus on ANHPs that, as natural hosts, avoid immunodeficiency to different SIV strains, in spite of having displayed initial viremia. Animals may, in fact, hold important keys to unlock the answers to AIDS (8,53-57).

Potential Solutions from long-term nonprogressors (LTNPs), and Elite Suppressors (ES)

It is now considered a truism that persons exposed to HIV-1 routinely become infected; generally, therefore, the number of exposures relates directly to infection risk. However, this truism is not always true. For example, unique humans possess the capacity to stay infection free in spite of repeated sexually risky exposures (54). About one percent of the human family possesses a homozygous defect in the human chemokine receptor 5 (CCKR5), an HIV-1 coreceptor that facilitates resistance to HIV-1 when monocyte-tropic (58). Why do some healthcare workers evade infection when exposed directly to HIV-1? More than 2,084 healthcare workers in the United States have suffered reported accidental HIV-1 exposure, and were carefully monitored by the Centers for Disease Control (CDC). The number of unreported incidents is believed to be far higher, perhaps 10-20 times as much. Most of these workers were inadvertently exposed to the infected body fluids of persons known to be seropositive for HIV-1, yet they opted not to disclose their situation to the CDC. Still, although there was no additional source of HIV-1 exposure, only four developed seropositivity (59-60). This is even more remarkable because many of these health professions suffered deep percutaneous exposures in which there was bleeding seen from the site of the needle injury. Studies of the risk of HIV-1 following percutaneous infected blood exposure found that a high percentage have some type of natural immunity (59-60).

Human Resistance to HIV Infection

Numerous scholars have advanced explanations as to why some individuals somehow cope with HIV-1 without medical intervention, and how an identified group, Kenyan sex workers, avoid seropositivity even following repeated HIV-1 exposures (54). Plumber et al. have written about "a clustering of resistance" that involves family groupings: daughters, mothers, nieces, and aunts showed a common resistance to the usually deadly retrovirus (54, 61-62). Nevertheless, explanations have not yet been

conclusive. HIV-1-infected patients who maintain viral loads of <50 copies/ml when untreated are known as elite suppressors (ES). Researchers have suggested that these elite suppressors, along with long-term nonprogressors (LTNPs), have been infected with HIV-1 variants that could be considered defective (61). Other studies have indicated that these individuals have unusually high levels of the HLA (human leukocyte antigen)-B*27 and -B*57 alleles, which indicates that viral replication is decisively influenced by host factors. Bailey et al. (63) have analyzed variations in the immune responses and in the viral isolates of an HIV-1 transmission pair. Both patients tested positive for HLA-B*57, but the transmitter acquired AIDS, while the recipient (an ES and seropositive for HLA-B*27) failed to do so. Examination of isolates from each individual demonstrated that they were replication competent. Escape mutations, found in the HLA-B*57-restricted epitopes, were found in each patient, which indicates that these mutations, in and of themselves, fail to adequately explain the divergent outcomes found in these two patients.

The cases mentioned above raise basic questions that, if conclusively answered, could shift the focus of the global quest to conquer AIDS. What is the relationship, if any, between primate resistance to SIV and human resistance to HIV? Traditional research approaches into classical immunity may help, but we maintain that a paradigm shift that focuses on intracellular miRNA immunity is warranted. (4,8,25). The extensive data gathering efforts of earlier researchers are to be praised, as should their insightful interpretations, but we maintain that contemporary researchers should use the factual and interpretive contributions of the past as building blocks of a new research edifice as opposed to mere scaffolding for ineffective theoretical structures.

Potential Paths Forward:

Although miRNAs comprise but 19-21 nucleotide double-stranded molecules, these miRNAs affect the regulation of more than one-third of the entire human genome (25). Augmented miRNA comprehension in recent years is paralleled by hopeful aspirations for milestones in dealing not only with HIV/AIDS but also with cancer, and with infectious and noninfectious diseases generally (64-65). miRNAs influence gene regulation and expression in cancerous cells as well as their normal counterparts (25) and miRNAs have been implicated in severe hematological malignancies, including primary effusion and B-cell lymphoma; and lymphoblastic, chronic myelogenous, and acute myeloid leukemia (25). Many authors have written of the hopeful future roles of miRNAs in the diagnostic and therapeutic arenas for cancer patients. The investigation of miRNA research has risen to new heights in recent years. For instance, in cancer pathogenesis, they are believed to act, in different settings, as either tumor suppressors or as oncogenes, and to exert regulatory effects on genes that are fundamental to cancer progression. Differential expression in tumor cells is a promising area of miRNA research. In diverse tumor subtypes, researchers surmise that differential expression could facilitate advances in cancer treatment by indicating new predictive markers (25, 37).

What we have seen about miRNA's potential invites us to allocate both hope and resources to the study of yet more exact tools to assess miRNA's regulatory role in specific settings. One miRNA can target more than 200 genes, which renders miRNA both a potent tool and a multifaceted weapon to employ against varying health challenges (4,25).

Conclusion

Will it be a paradigm shift that finally solves the HIV vaccine riddle? Thomas S. Kuhn's Structure of Scientific Revolutions suggest that past discoveries have required such a shift. Hopes for a classical immunity solution to finding a vaccine for the HIV have proven illusory (1-3). We maintain that scientists might profitably seek undiscovered solutions in the realm of intracellular immunity to discover how effective resistance can be sustained against the HIV-1 retrovirus. Lentiviruses (LVs), particularly SIVs, cause endemic infection in forty or more African non-human primates (reviewed in 4-5,17-18,55), and consequently provide potential models for HIV-1 molecular analysis. Since they are natural hosts to SIVs (17), why do African non-human primates not progress to acquired immune deficiency syndrome (AIDS), even in cases of high levels of viremia (18,55)? Clearly all primates are not alike, as evidenced by the susceptibility of some Asian macaques to suffer from viremia and also from critical losses of CD+ T lymphocytes (17-18), a key consideration in the progression to AIDS by both non-human Asian primates and human primates. To date, no traditional immunological methodology has pieced together the tough ANHP-immunodeficiency puzzle. We hypothesize that protection for ANHPs is rooted in discriminating and differential SIV homologous miRNA expression that establishes stable complexes with the threatening virus (8). We further assert that a tiny percentage of humans exhibit an analogous protective mechanism that renders them resistant to HIV-1 or provides long-term latency (8). Retroelements, ancient intracellular invaders, may be blocked by microRNAs and/or non-coding RNAs (ncRNAs), which are defense mechanisms based on small double-stranded nucleic acid.

(9, 25, 66-68).

In spite of the disappointment accompanying unsuccessful HIV-1 vaccine trials based on classical immunity, (1-3,69-70), we can take comfort in knowing that foundational research brings us ever closer to possible solutions. Simian-based models to fight AIDS have, likewise, proven disappointing, yet focus is often easier following earlier failures, and so it must be with HIV research. With all due respect for the contributions of classical immunity to human life and longevity, so useful in earlier vaccine successes, we argue that it is not the principal defensive mechanism against retroelements and retroviruses. We argue that enhanced investigation is warranted into defenses based on the protection afforded by miRNAs and dsRNAs (ncRNAs). We advocate the concentration of future efforts on the comprehension of "molecular defense" as a means of checking the global HIV-1 pandemic (4, 69-70).

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