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NATURAL IMMUNITY AGAINST HUMAN IMMUNODEFICIENCY VIRUSES: PROSPECTS FOR AIDS VACCINES

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1. ABSTRACT

We have hypothesized and will present evidence that there may be another form of immunity, other than humoral and cellular immunities, which operates against retroviruses. In order to distinguish it from the traditional immune responses, we have named this form of immunity **"molecular immunity"**. The major goal of this hypothesis is to better define the "messenger molecules" that are critical in forming the **molecular immunity** against retroviruses, and to further determine the activation pathways of this relatively unexplored form of immunity. We will provide evidence that this natural immunity against retroviruses and specifically against HIV-1, can be activated and optimized, and have made some interesting observations. We believe that resistance to HIV-1 and to other retroviruses can be induced by various means, including low dose exposure, infection with replication defective viruses and exposure to non-pathogenic but genetically related viruses.

2. INTRODUCTION

Even though a vast majority of human with high risk behaviors, exposed to HIV-1 become infected, some individuals remain uninfected with the virus, despite histories of multiple high-risk sexual exposure to the virus (1-3). For example, it had been shown that the $CD4^+$ T-cells of some individuals resisted very high doses of virus (about 1000-fold more virus to than what was required to establish infection). Also, in these individuals, the majority of cells failed to support viral replication (4). Recently, it was shown that three chemokines, MIP-1 alpha, MIP-1 beta and RANTES suppress HIV-1 ability to infect $CD4^+$ lymphocytes (5) and, more recently, that the cellular receptors through which these chemicals exert their effect-the coreceptors CCR5 and CXCR4 (6-7). Intense genetic analyses of the CCR5 coreceptors have revealed that certain individuals (about 1%) have a 32-base pair deletion allele, $CCR5\Delta^{32}$. The individuals who posses homozygous defect in CCR5 are resistant to monocyte-tropic strains of HIV-1 (8-9). It have also been documented that about 11-17% of Caucasians and 0-1.7% of African Americans are heterozygous for this $CCR5\Delta^{32}$. But it appears that presence of only 1 deleted CCR5 does not protect the individuals from HIV-1 infection (4, 8-9), but there is an indication that it may slow down the progression of AIDS (8-9). However, these observation do not explain the fact that why the majority of health care workers who got exposed to HIV-1 did not become infected with the virus? There are over 2084 health care workers in the U.S. who were accidentally exposed to HIV-1 and were monitored by the Center for disease control CDC, only 4 individuals who

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have no other source of exposure did become seropositive (10-12). Furthermore, several investigators have reported isolation of HIV-1 from individuals who remained HIV-1-seronegative and free of disease (13-16).

The central role of $CD8^+$ T-cells and their anti-retroviral-factor(s) (CAF) has been well documented (17-24). CAF from healthy HIV-1-infected or uninfected individuals can suppress HIV-1 replication without killing the infected cells. These are non-CTL, noncytolytic, non-MHC restricted $CD8^+$ T-cells, characterized by their ability to reduce HIV-1-replication. We wish to present an abundance of previously-published data, including our own, to support the hypothesis that CAF belongs to an unique form of natural immunity, distinct from cell-mediated (CMI) and humoral arms of immunity (HI). We also wish to show how several substances of abuse may adversely affect the anti-retrovirus effects of $CD8^+$ T-cells. The mechanisms by which $CD8^+$ T-cells inhibit retroviral replication are poorly defined.

3. THE RETROVIRUSES AND THEIR ABILITY TO INTEGRATE INTO THE HOST GENOME

The fact is that retroviruses are a wholly unique form of infectious agent and one that has direct access to the genome of the host species. The genetic nature of retroviruses is fundamentally different from all other pathogens known—a characteristic which allows the virus to cause substantial genetic damage to the host, even permanent change to the germline of the host species (in fact, some molecular biologists argue that the action of retroviruses has been a critical factor in vertebrate evolution—it is estimated that about 5 -10% of the mammalian genome has been manipulated and moved around by reverse transcriptase of retroviral origin during evolution). Yet, the two well-characterized forms of immunologic response—humoral immunity (HI) and cell-mediated immunity (CMI)—do not seem to be effective against many retroviruses (reviewed in 25).

It is almost inconceivable that higher organisms have evolved, to the present degree, without developing some special means to control this unusual sort of pathogens, or else retroviruses would long since have caused irreparable genetic damage to a myriad of host species. Furthermore, there is abundance of data, derived both *in vitro* and *in vivo*, that shows mammals are indeed quite capable of controlling the actions of retroviruses (see below).

Thus, we propose a new interpretation of previously-derived data which we feel is far better at accommodating these unusual data as well as the observed pathogenesis of HIV-1, Simian immunodeficiency virus (SIV) and other Lentiviral infections. We postulate that there is a third form of immunity (besides humoral and cell-mediated immunities) at work with retroviruses, which we call "molecular immunity". We hypothesize this molecular response is akin to both humoral and cell-mediated responses, but it involves a particular virus-specific messenger molecule that delivers to individual cells crucial information about the pathogen in question. This molecule serves as a critical score and cue to a whole orchestra of proteinaceous instruments playing at the molecular level, which must perform in symphony in order to overpower the discordant genes of the infecting retrovirus in question. Furthermore, the critical messenger molecule seems to be associated with a specific $CD8^+$ subset of T-cell lymphocytes, and various stimuli, cytokines/chemokines, and other "cofactors" that modulate the response of these $CD8^+$ cells also modulate the course of retroviral infection, particularly during the initial stages of the infection (see below). Most importantly, the body can be primed with genetically-related non-pathogenic strain, such that the body can develop a persistent genetic "molecular immunity" that specifies a particular retrovirus. The body, then, manifests protective natural defenses against this retrovirus and genetically-closely-related strains of virus, with or without the concomitant presence of classical humoral or cell-mediated responses (see below).

The mechanisms by which $CD8^+$ T-cells inhibit retroviral replication are poorly defined. The problem is that interpreting HIV-1 data through conventional immunologic theory could be compared to sailing a rocky coastline with a map of continental features found on another planet. Meanwhile, the HIV-1 epidemic continues to expand unchecked worldwide, and initially-promising intervention protocols result in ultimate failure in nearly every laboratory where they are tried (26-28). Instead of languishing in this intellectual abyss, let us explore in earnest new realms in our quest for fundamental understanding of infection by retroviruses.

4. WHAT IMMUNE MECHANISMS ARE RESPONSIBLE FOR INHIBITING RETROVIRAL REPLICATION: A NEW HYPOTHESIS:

Rapid viral replication during the course of HIV-1 infections is now considered to be an important factor in the *in vivo* pathogenesis of this human retroviral disease (29-31). Quantitation of viral burden in plasma, peripheral blood mononuclear cells (PBMC) and lymphoid organs have been closely correlated with HIV-1 stage, clinical status and CD4-positive T-lymphocyte counts (29-36). It appears that, from the time of HIV-1-infection until the development of the AIDS, the major sites of viral replication are the secondary lymphoid organs (35-36). CAF from HIV-1 individuals, SIV-infected monkeys, Feline immunodeficiency virus (FIV) infected cats, or from uninfected healthy individuals can suppress corresponding retroviral replications (17-24, 37). This observed suppressive activity is non restricted by MHC, does not require cell-to-cell contact between effector and target cells and can be mediated through soluble factors (17-24). Therefore, this $CD8^+$ cell- mediated HIV-1 resistance is unique and does not fit into the known concepts of humoral or cell-mediated immune response, which requires cell-to-cell contacts between targets (T) and the effector (E) cells, requires certain E-

to-T ratio to be effective and is MHC restricted. Therefore, we believe that CD8+ cell-mediated anti-retroviral immunity belongs to a unique form of natural immunity, distinct from CMI and HI. We wish to present existing data to support this hypothesis.

Based upon the observations presented below, we postulate that there may be a third form of immunity (besides humoral and cell-mediated immunities) at work with retroviruses, which we call "molecular immunity". We hypothesize this molecular response is akin to both humoral and cell-mediated responses, but it involves a particular virus-specific messenger molecule that delivers to individual cells crucial genetic information about the pathogen in question. Furthermore, the critical messenger molecule seems to be associated with a specific CD8+ subset of T-cell lymphocytes, and various stimuli, cytokines/chemokines, and other "cofactors" that modulate the response of these CD8+ T-cells also modulate the course of retroviral infection, particularly during the initial stages of the infection (see below). Most importantly, the host can be primed with genetically-related non-pathogenic strains, such that the host can develop a persistent genetic "molecular immunity" that specifies a particular retrovirus. The body, then, manifests protective "molecular immunity" against this particular retrovirus and genetically-closely-related strains of virus, with or without the concomitant presence of classical humoral or cell-mediated responses (38-43: see below).

Many others have recently published on the important role of CD8+ lymphocytes and associated cytokines/chemokines and their receptors in HIV-1 infection, but we believe that these data are manifestations of only part of the picture (4-9, 17-23, 44-47). In the following pages we have examined various aspects of our hypothesis, particularly those linked to what we have termed "molecular immunity" and which appears to be responsible for controlling HIV-1-replication in human and other retroviruses in their animals.

5. SCIENTIFIC BASIS FOR CURRENT VACCINES

Almost all the current designs for an HIV-1 vaccine are based on one of the following: inactivated whole virus; live-vector-driven subunit or recombinant subunit virus vaccines; chemically-synthesized peptides; viral pseudo types; or DNA vaccines (reviewed in 53-54). Yet none of the experiments to date in either humans or primates have produced satisfactory results (reviewed in 26-28, 48). If partial protection is achieved, it is produced with either low doses of the pathogenic virus itself, or with a strain of genetically-closely-related simian immunodeficiency virus (SIVs) with a low pathogenicity (41-43). In addition, inactivated and recombinant approaches to vaccine design leave us with other concerns—can these vaccines prevent the cell-to-cell transmission of HIV-1 or entry of virions through the mucosal route, since these vaccines do not generally provoke an IgA-type of response? (49-56).

All of these designs of vaccine are directed towards arming the hosts with HIV-1-specific humoral immunity (HI) or cell-mediated immunity (CMI). This focus is due largely to history and momentum—HI and CMI are two well-studied and well-understood immunologic phenomena, and agents stimulating these responses have led to many successful vaccines in the past. However, new data suggest that there are problems in relying upon these approaches for a vaccine against retrovirus like HIV-1.

5.1. Development of vaccine based on humoral immunity

Most common attempts to develop a vaccine against HIV-1 or SIV (the best existing primate model for AIDS) focus on inactivated virus or various forms of recombinant vaccines (48-51). Despite extensive research, these attempts have yet to yield successful results. If some sort of protection is reported, it is only under very specific conditions (i.e. with small infectious doses or with secondary challenge with a less pathogenic virus, etc.) (38-43, 54). With SIV, the numerous attempts to develop protection with viral subunits, whole inactivated SIV, recombinant vectors expressing viral proteins and various other combinations have met with almost total failure. Even when various vaccines induced high levels of SIV-neutralizing antibodies or CMI, no protection was observed against the pathogenic strain of simian immunodeficiency virus (SIV) (SIVmac or SIVsm) (56-59).

More surprisingly, infection of a chimpanzee—our closest primate relative—with HIV-1 results in an initial viremia, but without the manifestation of any subsequent disease. Somehow this primate possesses a natural ability to prevent replication of HIV-1 and adverse consequences of the infection (56-59). However, in various trials, vaccines have failed to prevent this initial viremia in immunized chimpanzees—even in the presence of high HIV-1-specific neutralizing antibodies and CMI responses (55-59). Yet other non-vaccinated chimpanzees (such as the control animals in the above trials) are *able to contain the initial viremia of HIV-1 equally well in the absence of either of these immune responses* and neither of these animals develop any evidence of the HIV-1 infection (60-64). The correct interpretation of these observations may hold the key to a successful vaccine against HIV-1.

On the contrary, the development of so called "enhancing antibodies" which actually increase viral replication have been well documented in both human and animal studies. Furthermore, in certain animal trials, specific vaccines have resulted in the development of antibodies which markedly enhanced viral replication and disease progression (reviewed in 65). Currently, there are numerous suggestions to target beta-chemokines or their receptors (by synthetic or pharmacological agents) to block HIV-1 entry. We believe that such suggestions should be viewed with great care, since previously soluble CD4+ antibodies have suffered total failure (26-27, 50-52). Also,

one should keep this fact in mind that the affinity for gp120 to CD4 receptors in their native form is very strong—the Ka association constant gp120-CD4 binding is about $2x10^{9-11}$. However, the affinity of gp120 to soluble CD4 molecules produced by hybridomas or recombinant antibodies are usually much lower (2-3 log lower). In addition, receptor-ligand interactions are dynamic and they are always in a state of flux between association and dissociation, providing the high affinity HIV-1 to latch on to the target cells and enter the cells if co-receptors are present.

5.2. Development of vaccine based on cell-mediated immunity

The "CMI hypothesis" was strongly forwarded by late Jonas Salk and his colleagues (66), who believed that a protective vaccine against HIV-1 would induce cellular rather than humoral immunity. Salk also hypothesized that humoral immunity would not only be non-protective, but it would also increase susceptibility to HIV-1 infection.

This "CMI hypothesis" was based upon several observations which supported the protective role of CMI. For example, at an earlier stage of the AIDS epidemic, Clerici *et al* (1) had shown that a majority of individuals exposed to HIV-1 were still seronegative for HIV-1, and a small percentage of unexposed or low-risk subjects showed evidence of pre-existing HIV-1-specific CMI. Lymphocytes (PBMCs) from these individuals released IL-2 and exhibited evidence of proliferation when exposed to the gp120 envelope antigen of HIV-1. Furthermore, macaque monkeys (which develop an AIDS-like immunodeficiency upon infection with SIVmac) that are exposed to low-doses of live SIVmac, usually exhibit CMI responses to SIVmac, without producing antibodies specific to SIVmac or developing any evidence of infection (reviewed in 67). On the other hand, all but one of the macaques in this study became infected when injected with high doses of SIVmac, and these animals developed SIVmac antibodies but showed no evidence of CMI. From these observations, Salk *et al* deduced that cell-mediated immunity (TH1) can protect against pathogenic retroviral infections (i.e. HIV-1 and SIVmac) whereas humoral responses (TH2) makes the host susceptible to infection with these types of viruses (66,68).

In a nutshell, the CMI-hypothesis proposed by Salk *et al* (66) holds that a patient's fate is determined by which of two types of immune effector cells-TH1 or TH2 is predominant in responding to HIV-1. According to this hypothesis, HIV-1-infected subjects switch from TH1 protective to a TH2- disease enhancing response. Since the above-referenced study was published, there have been many studies which do not support the CMI-hypothesis. For example, Romagnani *et al* (68) tested this hypothesis and failed to confirm the hypothesis of TH1/TH2 switch during the progression of HIV-1 infection. They did not observe any increase of IL-4, IL-5 and IL-10 production during progression of disease. Since then, it has been documented with HIV-1-infected humans that rapid progressors as well as long-term nonprogressor (LTNP) both exhibit good HIV-1-specific humoral and cell-mediated immunity, suggesting that neither humoral nor CMI response provides protection against HIV-1 (68). Furthermore, in chimpanzees, the natural protection against HIV-1 can occur in the absence of either CMI or HI responses (69).

In the simian models of AIDS, the role of humoral vs. CMI response against retroviruses remains unclear. In the African green monkeys, neither whole inactivated virus (designed to produce strong CMI nor HI have managed to protect these animals against SIVagm infection. In addition, several excellent studies of humans have reported that the decline in the initial HIV-1 viremia occurs before either neutralizing antibodies or HIV-1-specific CMI responses appear in the infected individual (69-70).

6. ANOMALOUS OBSERVATIONS WITH RETOVIRAL INFECTIONS: IS THERE "MOLECULAR IMMUNITY" ?

Viral vaccines work by imitating natural immune responses to pathogens and subsequently clearing the infectious agents from the host's system. However, retroviruses possesses a much more complex life cycle than any other infectious agent for which we have previously developed vaccines. We must come to better understand the nature of any unique immune mechanism that may exist against retroviruses if we wish to develop an effective vaccine.

First of all, on evolutionary viewpoint, it is difficult to believe that during the course of evolution, higher organisms did not develop some sort of defense mechanism in order to protect themselves from the onslaught of retroviral infections. For example, regions between maize plant genes are packed with retroelements which make up more than 50% of the two billion base pairs that constitute this plant's nuclear DNA (71). Obviously, a mammalian cell can not possibly accommodate so many retroviral genes and must have developed intracellular defenses to counteract insertion of such genes. Retroviruses have the ability to acquire and alter the structure of host-derived sequences, leading to altered genes, pseudogenes, and oncogenes in the host species; they have the ability to insert their own genome into the host's germline, potentially making subsequent generations "transgenic hosts" for this now endogenous virus (72-73); and finally, the seemingly-random insertion of provirus can cause genetic damage to the host, leading to disruptions in the activation or control of specific genes near the site of proviral integration. Therefore, we have hypothesized that higher eukaryotes must possess some sort of intracellular immunity (which we have named molecular immunity**)** specifically evolved to combat such clear and present genetic dangers. In fact, already there are substantial published data to indicate such immunologic responses exist (74-77).

6.1. Epidemiological studies

Even though a majority of individuals exposed to HIV-1 become infected, rare individuals remain uninfected

with the virus, despite histories of multiple high-risk sexual exposure to HIV-1 (1, 13-16). In some cases this may simply be the result of defective viruses resulting in abortive or quiescent infection. In other cases, there appears to be a clear evidence of resistance to infection. For example, it has been shown that the $CD4^+$ T-cells of some individuals resist infection with high doses of virus (about 1000-fold higher concentration of virus than what is require to establish infection). While, a small fraction of cells become infected with such a high viral dose, the viral-replication does not take place (13, 78-79). Recently, it has been shown that certain individuals (about 1%) have homozygous defect in one of the HIV-1 coreceptors, CCR5, which makes them resistant to monocyte-tropic strains of HIV-1 (8-9). However, this observation does not explain the fact that why so many of health care workers who got exposed to low doses of HIV-1 did not become infected with the virus? For example, there are over 2084 health care workers in the U.S. who were accidentally exposed to HIV-1 and were monitored by the CDC, (the actual figure may be 10-20 times higher, since most individuals who are accidentally exposed to bodily fluids of HIV-1-seropositive individuals do not inform the CDC). Yet only 4 individuals who have no other source of exposure did became seropositive (18-20) and personal communication: Dr. Denise Cadre, CDC). Considering that many of these individuals had deep percutaneous exposures resulting in visible bleeding from the sites of needle injuries. Recent, more extensive studies indicate that the estimated risk for HIV-1 infection after percutaneous exposure to HIV-1 infected blood is about 0.3% (10-13). This is a very small percentage, considering these sorts of exposure generally involve needle pricks with infected blood. Also, this does not quite fit into the beta-chemokine receptor hypothesis described by Cocchi and others (5-9).

Epidemiological studies indicate that various classifiable subgroups infected with HIV-1 vary considerably in their median incubation period and their susceptibility to HIV-1 infection (80). For example, the frequency of successful transmission of HIV-1 resulting from a single intercourse with an infected partner is relatively low (0.2 to 1%), even though HIV-1 is present in 80-100% of human semen specimens from HIV-1-infected individuals. Some individuals also lack any evidence for infection with HIV-1, despite multiple sexual contacts with HIV-1-infected partners (81-82). Other epidemiological studies suggest that some individuals are truly resistant to HIV-1-infection (13, 78-79). It has been shown that the rate of infection in individuals exposed to a whole unit of infected blood is about 30% (83)—a very high percentage and one that is associated with high-dose exposure

Furthermore, several investigators have reported isolation of HIV-1 from individuals who remained HIV-1-seronegative and free of disease. Detels *et al* (13) have observed that some men with many different partners with whom they practiced receptive anal intercourse have remained seronegative, despite repeated exposure. Surprisingly, Bryson *et al* (84) have documented the clearance of HIV-1 infection in perinatally-infected infants, who subsequently remained without a detectable HIV-1-infection for five years. More recently, Roques *et al* have also documented 12 cases of perinatally-infected children who cleared the virus (85). Besides these well-documented cases, several other investigators have also reported evidence of individuals who seroreverted, and the prior infection of these individuals with HIV-1 were documented by HIV-positive blood cultures, positive serum HIV-1 p24 antigenemia, and in some cases, positive PCR assays (86).

But perhaps most intriguing of all are the reports of the so called molecular immunity in humans infected with HIV-1 who are long-term nonprogressors (LTNP). The majority of individuals infected with HIV-1 progress to AIDS. The average time from first infection with HIV-1 to death in the progressors is less than 10 years. However, the clinical manifestations of HIV-1-associated illnesses appear much earlier, 4 to 6 years after the infection. In about 5% of individuals infected with HIV-1, the so called "long-term nonprogressors", the natural history of HIV-1-infection is altered. These individuals remain healthy and many of the clinical manifestations of HIV-1-infection are either absent or not as prominent as in the progressors (i.e., low $CD4^+$ cell count, HIV-1 p24 antigenemia, generalized lymphadenopathy and other AIDS-associated infections, lymphomas and Kaposi's sarcoma). In recent years, several reports have emerged, some of which indicate that an attenuated *Nef*-defective HIV-1 variant may be one of the causes of the LTNP status of individuals, from which these defective HIV-1 variants were isolated (87). Deacon, *et al*. (83) have described a single index case, an HIV-1-infected blood donor, whose blood or blood products were transfused into six individuals. All of these recipients have remained free of HIV-1-related diseases after 10 to 14 years. The analysis of HIV-1 isolates have shown a *Nef*-defective gene. In addition, Kestler, *et al*. (87) have reported that, rhesus monkeys, experimentally infected with *nef*-negative simian immunodeficiency virus (SIV_{mac}) manifest no signs of immunosuppression. The actual contribution of *nef*-defective HIV-1 in the LTNP of this retroviral infection is still controversial and several LTNP do not show evidence of *nef*-defective HIV-1 in their PBMCs (but potential defects at other genetic loci have not been done yet) (88). In addition, the functional analyses of the *nef*-defective HIV-1 viruses, isolated from ten LTNPs, indicated no significant differences in the replication properties of these isolates (88). Many factors, including multiple HIV-1 variants with different degrees of virulence and replication capabilities, numerous host factors, and environmental influences may play important roles in the ultimate outcome of infection with HIV-1 (89-90). It appears that there is race between the rapid replication of the virus and development of anti-retroviral immunity. If an individual is allowed to develop the proper "molecular immunity" against the virus, then the odds may move in favor of the host. We hypothesize that infection with these relatively-attenuated viruses or low doses of HIV-1 (like in health care workers: 10-12), results in molecular immunity to respective viruses in the infected hosts (38-43, 91-95). A particular point that bolsters this interpretation is the fact that many of the individuals infected with *nef*-defective HIV-1 almost certainly later came in contact with fully

pathogenic strains of HIV-1 but remained nonprogressors due to the induction of molecular immunity by the attenuated strains. For example, one of the documented nonprogressors was a hemophiliac and probably had multiple exposures to virulent strains of HIV-1 (through frequent injection of unscreened Factor VIII). Similarly, several other nonprogressors were homosexual men and also had likely been exposed to various quasispecies of HIV-1, including the fully virulent strains of HIV-1(87-88, 93). Similarly, experimental infection of monkeys with *nef*-deleted SIVmac has resulted in protection against subsequent infection with the high-dose, full-length, wild-type, virulent strain of the homologous virus (93).

7. OTHER EVIDENCE FOR "MOLECULAR IMMUNITY": NO MANIFESTATION OF DISEASE WITH OTHER LENTIVIRAL INFECTIONS

Although most of our attention is focused on HIV-1, there are several interesting aspects of molecular immunity which could be learned from the related SIV lentivirus. The various SIVs are the most-closely-related viruses, some of these strains cause AIDS-like diseases in various species of primate. But there are a number of seemingly anomalous host-virus interactions, and these each give clues to how primates deal with lentiviral infection.

For example, nearly 50% of African green monkeys are infected with a sub-strain of SlVagm in the wild, yet no clinical pathology has been associated to date (reviewed in 96). Similarly, sooty mangabeys have been shown—both in the wild and in breeding colonies—to be infected with a sub-strain of SIVsm (96-99). Like the African-green-monkey infection, the sooty-mangabey infection appears to cause no disease in its native host, even though SlVagm and SIVsm are both known to cause AIDS-like disease in other species of monkey. Somehow, these monkeys have developed a means of controlling these retroviruses. In fact, in the breeding colony of sooty mangabeys where the original SlVsm isolate was discovered, as many as 80% of the animals were infected—some for over a decade—without manifesting any evidence of disease (100). Also, there is a striking homology between SIVsm of sooty mangabeys and HIV-2 (100). and there is correspondingly significant sequence homology between HIV-1 and a simian immunodeficiency virus, SIVcpz, which was originally isolated from chimpanzees, who were without evidence of disease (101-102). Of particular note, chimpanzees experimentally infected with HIV-1, fail to develop overt disease despite establishment of infection as evidenced by transient viremia, (103-104), and development of HIV-1 specific antibodies (105) and HIV-1 specific cytotoxic T-cells (106-107). These observations strongly suggest that these primates already have a molecular immunity to these lentiviruses. *The question is that why exposure of these SIV-strains , which are non-pathogenic in one species of primates, becomes pathogenic when other species of primates are exposed to them.*

In humans, this complex pattern of varying pathogenecity among lentiviruses is also manifested in the clinical expression of other retroviruses. For example, the "human foamy virus (HFV)" or spumaviruses infects humans but has not been associated with any known disease. This lack of clinical expression persists despite a high prevalence of infection among certain human populations, and infectious virus can be readily cultured from the infected tissue specimens from these individuals (108-109).

Human T -cell lukemia virus (HTLV)-II has been shown to be endemic in certain native American Indians without any manifestation of clinical disease (110). In contrast, infection with the similar HTLV-I in a minority of individuals leads to either adult T-cell leukemia *if acquired in infancy, or a chronic neuropathic disease if acquired late in life* (110). Despite many similarities among these retroviruses, there are marked differences in the level of clinical expression. Yet, it is apparent by the maintenance of infectious disease processes within these populations that low level of virions are being produced from the integrated proviral sequences.

Similarly, although less dramatic, substantial disease variability has been observed with the clinical course of HIV-1 infection. For example, about 5% of HIV-1-infected individuals are long term non-progressor (LTNP) for as long as 15 years (1-4, 80-82, 88). In contrast, other reports document patients who rapidly progress to immunodeficiency in a matter of a few years (30). Furthermore, when one compares HIV-1 with HIV-2, there is a marked difference in the clinical course, with the HIV-2 infection being significantly prolonged (30). The prior example regarding documented exposure of >2,000 health care workers is most curious, as only 4 have seroconverted and none has developed AIDS (10-12). And then there are the cases of spontaneous clearance of HIV-1 (84-86), or lack of transmission of HIV-1 in individuals who have multiple unprotected sexual contacts with HIV-1-infected partner, even though HIV-1 is present in almost 80-100% of human semen specimens (80-82). 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 (1-2).

8. 'MOLECULAR IMMUNITY" NEEDED TO BE EXPLAINED:

Most of the research efforts on retroviruses over the past 10-15 years have focused on the mechanisms of disease production by these pathogens. Now it is time to explore the mechanisms by which infected hosts protect themselves and the potential factors which adversely effect the anti-retroviral 'molecular immunity'. If one assumes that evolution has created some sort of intracellular protective mechanisms

to specifically battle retroviruses—different from humoral or cell-mediated immunity—then many of the previously anomalous phenomena reported by various investigators could be explained on the basis of this alternative hypothesis. For example, it can 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, but does cause AIDS-like disease in other species. 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 cynomolgus monkeys. While there are genomic differences between the rapidly-fatal variant of SIV_{sm} and other SIV_{sm} subtypes, the differences fail to clearly define the pathogenic moiety of this virus (107). Similarly, in the oncoviral family of complex retroviruses, there is HTLV-I, for example, which if acquired in infancy in southeast Japan, leads to leukemia (ATLL) in a small minority of the population, while in the much larger majority, the virus remains clinically latent throughout life (108). In certain Caribbean populations, however, infection with this retrovirus tends to occur later in life, and among this population, HTLV-I leads primarily to a neuropathic disease (109). HTLV-II, in contrast, which is endemic in certain isolated populations throughout the world causes no known disease (110). HIV-1, which has devastated certain human populations, is most closely related to SIV_{cpz} which appears to cause no disease in either naturally-infected or experimentally-infected chimpanzees. What is becoming increasingly clear is that the final disease potential of retroviruses lies in the complex interaction of the retrovirus with the infected host. *Perhaps the survival of the host depends on the rapid development of intracellular defenses that are able to "prime" the majority of target cells with the appropriate defenses, outracing the pathogenic effects of the retroviruses. In the primates and humans, we hypothesize that the defenses are already in place against retroviruses and it may be the environmental factors which can tilt the balance in favor of the pathogens (see below).*

9. ROLE OF $CD8^+$ CELL IN THE DEVELOPMENT OF ANTI-RETROVIRAL IMMUNITY

The pivotal role of $CD8^+$ T-cells in the development of the anti-retroviral-specific-natural defenses have been well documented (17-24). Briefly, $CD8^+$ T-cells or factors from $CD8^+$ T-cells (CAF) from healthy HIV-1-infected or uninfected individuals can suppress HIV-1 replication without killing the infected cells (10-12, 17-24, 111-115). These are non-CTL, noncytolytic $CD8^+$ cells, characterized by their ability to reduce HIV-1 p24 antigen levels and RT levels in the culture fluids of PBMCs infected with all strains of HIV-1 & 2, SIVs and FIV (10-12, 111-115). This anti-retroviral activity does not appear to be restricted by MHC, or require contact between target and effector cells, occurs at low $CD8^+$ /$CD4^+$ ratios and is oligoclonal in nature (111). The nature of its anti-retroviral activity is via soluble messenger agents, which are unrelated to any known cytokines or chemokines, though currently, this conclusion is controversial (17-24, 116) The exact mechanisms of these antiviral effects are unclear. However, recently, several experimental evidence have been reported which show CAF exert their anti-HIV-1 effects by specifically interrupting HIV-1 transcription (19, 116).

10. ARE BETA-CHEMOKINES THE ANSWER FOR $CD8^+$ CELL FACTORS?

Recently, there are remarkable series of articles demonstrating the coreceptors for HIV-1 in various cell lines (4-9, 44-47). It has long been thought that HIV-1 may bind to its primary CD4 coreceptors, but coreceptors were certainly necessary for the viral entry (44-47). This was determined from data in murine and other animal cell-types, in which CD4-expression would not permit productive infection. Feng *et al* (46) demonstrated that a transmembrane chemokine receptor, CXCR4, serves as a cofactor for T-cell tropic, but not monocyte tropic, strains of HIV-1. Another coreceptor, CCR5 appears to be major co-receptor for macrophage /monocyte tropic strains of HIV-1 (45). These coreceptors bind variety of beta-chemokines i.e. RANTES, MIP-1 alpha and MIP-1beta and are secreted by variety of cell types. What these beta-chemokines do? Are they the suppressive factors scientists are looking for? It appears that they may not be the "elusive" $CD8^+$ cell factors or CAF! Their effect is on pre-entry level (4-9, 44-46), whereas, CAF appears to works on pre-RT, RT-levels and/or transcription levels (17-24, 111-116). It is hypothesized that beta-chemokines may serve as blocking agents for their respective receptors and hence as anti-HIV-1 suppressive factors. Recently, it has been reported that, analyses of the two exposed-uninfected (EU) people (who have exhibited a significant degree of resistance to high doses of macrophage tropic strains of HIV-1 infection *in vitro*), have shown that such a resistance is due to the presence of a homozygous genetic defect in the CCR5 receptor (4, 8-9). We believe that $CD8^+$ cell factors are different than the beta-chemokines described by Cocchi *et al* (5), since it has been noted that : 1) the concentration of $CD8^+$ cell factors and chemokines are independent variables in HIV-1-suppressing culture media; 2) that chemokine-specific neutralizing antibodies do not block the $CD8^+$ cell factor activities; 3) that $CD8^+$ cell factor-susceptible HIV-1 strains are variably sensitive to chemokines; 4) both T-cell tropic and monocyte/macrophage-tropic strains are sensitive to $CD8^+$ cell factors; 5) the levels of chemokines and $CD8^+$ cell factors peak at different times, *in vitro*; and 6) $CD8^+$ cell factors have very broad range against several lentiviruses (unpublished data). The homozygous defect of CCR5 receptors reported to be present in about 1% of the U.S. population, does not explain the fact that why a vast majority of health care workers (>97.7%), who got

exposed to HIV-1, did not become infected with the virus (10-12).

From some of the statements regarding the nature of CAF, it could be interpreted that molecular immunity may be similar to so called transfer factor (TF), described in the old immunology literature. TF was described as less than 10 kd, dialyzable, cell-free extract of lymphocytes that was able to transfer CMI from antigen-responsive to antigen-nonresponsive hosts. The activity of TF was antigen-specific but generalized immunopotentiation was also achieved. The TF was reported to be DNAse resistant, and heat-sensitive. Many clinical studies to date employed TF in the therapy of neoplasm, immunodeficiency states and infectious diseases. It would be obvious from the data that CAF effects described by many investigators (17-24, 111-115) do not fit into the described effects shown by TF.

11. SOME COFACTORS CAN COMPROMISE IMMUNITY

We hypothesize that the protective natural defenses against a specific group of retrovirus and genetically-closely-related viruses would be life long unless untoward events or cofactors adversely affected the anti-retroviral "molecular immunity" by inducing alterations in the subsets of lymphocytes that are involved in molecular immunity (i.e $CD8^+$ cells). These cofactors are especially important during the initial stages of infection, at which time they can determine the future course of infection. "Untoward events or cofactors" are of several types, including exposure to low-dose radiation, UV-light, cyclophosphamide and protein-synthesis inhibitors (118). Another type of cofactor is co-infection with another pathogenic virus or exposure to specific viral products—these other viruses need not necessarily be retroviruses (reviewed in 118). A third type of cofactor is temporary immunoincompetence due to abuse of certain substances, such as alcohol (119-123), cocaine (124-127) or even certain anti-HIV-1 drugs (118)—these latter cofactors may be of particular significance during the initial exposure (118). No doubt there are also, yet unknown cofactors, which may interfere or inactivate the "molecular immunity" pathways, which will render the host susceptible to acute infection of retroviruses.

12. FACTORS WHICH INTERFERE IN THE ANTI-RETROVIRAL "MOLECULAR IMMUNITY" PATHWAYS

If we have natural defenses against HIV-1 infection, why does infection with HIV-1 result in such devastating consequences for humans when several primates, including our closest evolutionary relative, the chimpanzee, which carries genetically similar retrovirus, SIVcpz, is able to resist clinically significant infection with HIV-1 and SIVcpz ? There are two main possibilities. We believe that infection with HIV-1 in human (and other lentiviruses in primates) follows two possible outcomes:

i) Initial single exposure to *a very low dose* of virus, resulting in "priming" of the hosts' "molecular immunity" and, subsequently arming the host defenses against that specific type of lentivirus. If exposed to high doses of virulent HIV-1, after a reasonable time (i.e. 1 week), host will be resistant to a subsequent infection.

ii) Initial exposure with *a high dose of HIV-1*, or multiple exposure to smaller doses of HIV-1, before the completion of "priming phase", resulting in overwhelming of the host's 'molecular immunity mechanisms" resulting in "unprimed" host who will be susceptible to HIV-1 infection.

13. TWO OUTCOME SCENARIOS

a) Why primates, experimentally exposed to low doses of SIV or preexposed to genetically related non-pathogenic SIV, develop resistance to high doses of the pathogenic SIVs (38-43, 91-95); b) why thousands of health care workers exposed to HIV-1-infected body fluids are still seronegative (10-12); c) why there is clear-cut separation of pediatric patients, one group that progresses rapidly to AIDS within a year after birth and the other group which apparently clear HIV-1 from their system (30); d) why patients or primates infected with *nef*--negative or *nef*-defective virus have done well (88, 93, 128); e) why there is so much variability in the susceptibility of PBMCs infected with HIV-1, *in vitro* (122-124), f) why some men with many different partners with whom they practiced receptive anal intercourse have remained seronegative, despite repeated exposure (1-3, 80-82, 117); g) why HTLV-II has been shown to be endemic in certain native American Indians without manifestation of clinical disease (110) and why infection with HTLV-I in the majority of individuals leads to no adverse consequences but causes diseases, (either adult T-cell leukemia if acquired in infancy, or a chronic neuropathic disease if acquired late in life), in very small minority and in individuals whose "molecular immunity" is not fully active (110); h) why all the primates infected with various strains of SIV_{agm} in the wild exhibit no clinical signs of AIDS or other related illnesses (96-100), but if exposed to different strains (to which they were previously unexposed) get sick (100-107). The explanation of this manifestation, if we accept the two dose hypothesis, is that primates get exposed to doses of SIVs *in utero* or during nursing period, making them immune to that particular strain or related strain of SIVs, but when experimentally exposed to genetically different strain of SIVs (which is almost always with high doses of SIVs) they get sick.

We would also suggest that any agent(s) which induces certain dysfunctions in $CD8^+$ lymphocytes may lead to non-specific activation of the immune system. Also, any agent(s) which non-specifically stimulates T-lymphocytes (i.e. T-cell mitogens PHA, ConA, anti CD3/CD28, IL-2 etc.) could induce alterations in the anti-

retroviral immune mechanisms. Several experiments by various investigators have provided information regarding these events. Zack *et al.* (129) evaluated the molecular events after HIV-1 entry into $CD4^+$ lymphocytes and after entry into unstimulated human PBMC. They showed that the HIV-1 genome is blocked from completing reverse transcription by some unknown host factor(s). Even though viral RNA and incomplete reverse transcripts of proviral DNA may persist for a short period of time, they are labile and are degraded by host factor(s). Stimulation of PBMC with a T-lymphocyte mitogen results in the breakdown of this natural defense system against HIV-1. More recently, Best *et al* (130) have identified a gene, *Fv1*, an endogenous Gag-related gene found in certain strains of mice, which makes them resistant to murine leukemia virus. The *Fv1* gene product, is able to block 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. The location of anti-retroviral action of *Fv1* (cytoplasm or nucleus) is still undetermined (130). In a different series of experiments, Bukrinsky *et al.* (131) have examined the PBMC from HIV-1-seropositive individuals, for the presence of HIV-1 provirus. In their studies, they observed that in the resting (quiescent) T cells from asymptomatic individuals, HIV-1 existed as unintegrated full-length HIV-1 but not as an integrated form. This observation suggests that certain naturally occurring host factors may be preventing the integration of HIV-1 provirus. However, when the PBMC were activated with mitogen *in vitro*, HIV-1 provirus integrated into the host genome. The same authors further noticed that in AIDS patients, the percentage of integrated HIV-1 was higher and was associated with HLA-DR-positive T cells (activated subsets of T cells). Recently, Sonza et ai (116) have shown that in fresh PBMC-monocytes also, HIV-1 replication is blocked prior to RT and integration steps. Therefore, there is evidence of natural defense against HIV-1 at pre-reverse transcription stages, as well as pre-integration stages.

In summary, there is growing evidence that neither the humoral immune responses nor the traditionally understood viral-specific CTL appear to play any important role in the inhibition of HIV-1 replication *in vivo*. The majority of the HIV-1 vaccine strategies that have received the greatest attention to date, including the use of recombinant HIV-1-envelop glycoprotein immunogens, live vaccinia virus subunits and many vaccine approaches to elicit virus-specific CTL have given disappointing results. We believe that the real natural defenses against HIV-1 lies inside the $CD8^+$ T-cells and understanding the mechanisms by which these intracellular "molecular immunity" pathways operate would provide us the tools we need to fight against HIV-1.

14. THE TWELVE STEPS OF MOLECULAR IMMUNITY

We now wish to present twelve steps on how we think molecular immunity against retroviruses actually works, then we will describe data which we feel support this hypothesis:

i) Initial exposure: Upon initial exposure to a retrovirus, the host organism requires a brief lag period to marshal its molecular responses.

ii) Initial viral activity: During the lag period, the retrovirus actively replicates or infects some cells then becomes dormant, depending on the nature of the retrovirus.

iii) Lag period of host: During the lag period, myriad intracellular defense mechanisms become activated, and these responses have evolved over time to block the replication of the retrovirus at every important step of its life cycle.

iv) Virus-specific messenger molecule: Following the lag-period, which seems to last 2-5 days in healthy individuals, retrovirus-specific messenger molecules are produced by the host. These molecules relay pathologic information to uninfected cells, probably in some sort of genetic fashion, and seem to be persistent for long-term immunity.

v) Cascade of molecular responses: The messenger molecules are highly specific to the particular retrovirus in question, and they activate a cascade of events in the uninfected cells, which allow these cells to arm themselves against infection by that particular retrovirus.

vi) Immunity against genetically-closely-related viruses: Although this immunity is highly specific to the retrovirus which initially infects the host, molecular immunity also inhibits the infection by retroviruses that are closely related genetically.

vii) Late evolutionary, and ontogenic development of molecular immunity: The development of molecular immunity seems to have occurred relatively late in evolution, and consequently, it matures relatively late in the ontogenic development of the organism. For humans, molecular immunity seems to develop between the ages of 0.5-3 years. Prior to this maturation, infection of a young host with a retrovirus of relatively low pathogenicity or a low-dose infection with a pathogenic retrovirus may prove uncontrollable or fatal.

viii) $CD8^+$ lymphocytes are basis of response: A subset of $CD8^+$ lymphocytes initiate the molecular response to retroviruses, and these cells also dispatch the messenger molecules to various cells in the organism, including $CD4^+$ T-cells, monocyte/macrophage cells, and cells of the CNS (i.e. neurons, astrocytes, oligodendrocytes). Upon exposure to the messenger signals, the recipient cells synthesize their own anti-retroviral agents, which are designed to inhibit the early steps in the life cycle of retrovirus, such as RT, RNase H and integrase activities. Interruption or dysfunction of the primary responder cells—namely specific $CD8^+$ lymphocytes—can result in unchecked replication of the retrovirus.

ix) Cofactors can compromise immunity: Protective molecular immunity against a specific strain of retrovirus and genetically-closely-related viruses would be life long unless untoward events or cofactors adversely affect the intracellular mechanisms of protection or the subsets of lymphocytes that are involved in molecular immunity (particularly $CD8^+$ T-cells). These cofactors are especially important during the initial infection and lag period, at which time they can determine the future course of infection. "Untoward events or cofactors" are of several types, including exposure to low-dose radiation, UV-light, cyclosporins, cyclophosphamide, or protein-synthesis inhibitors. Another type of cofactor is co-infection with another pathogenic virus or exposure to specific viral products—these other viruses need not necessarily be retroviruses. A third type of cofactor is temporary immunoincompetence due to abuse of certain substances, such as alcohol, cocaine or even certain antiviral drugs—these latter cofactors may be of particular significance during the initial exposure and lag periods. No doubt, there are also yet unknown cofactors, which interfere or inactivate the molecular immunity pathways, and which will render the host susceptible to acute infection of retroviruses. (118-125).

x) Low-dose inoculation can provide molecular immunity: A low-dose inoculation of a pathogenic retrovirus will result in the development of protective molecular immunity against the specific retrovirus or against genetically-closely-related retroviruses. Provided cofactors do interfere excessively in the initial immunologic response. This is akin to a subclinical infection, where low-dose exposure to a pathogen results in the development of protective immunity without overt signs or symptoms of the disease. Similarly, exposure to a non-pathogenic live retrovirus provides an immunity against genetically-closely-related pathogenic retroviruses (54-58). The degree of success in developing molecular protection is directly proportional to the degree of genetic relatedness of challenging retroviruses, and inversely proportional to the degree of pathogenicity of the inoculating retrovirus.

xi) Active replication necessary for effective vaccine: The activation of molecular immunity requires exposure of primary target cells to live retrovirus. A heat-inactivated, formalin-fixed whole virus, or virion subunits produced by recombinant vectors, or peptides and subunits produced by bacterial or baculovirus expression systems, or any other form of a vaccine where the virus is unable to complete a single cycle of growth, would not be able to bring molecular immunity to its full potential and thus would not protect the host. In other words, the very mechanisms of retroviral infection—not the antigenic nature of the virus itself—elicit the molecular response that leads to protective immunity (54-58, 66).

xii) Possible HIV-1 vaccines: The hypothetical vaccines that should produce protective immunity against HIV-1 are the following live-virus vaccines (in descending order of desirability): 1) an engineered replication defective HIV-1 virus (i.e *env*-defective virus), 2) very low doses of a genetically-closely-related, non-pathogenic lentivirus, such as SIVcpz, 3) low doses of a non-pathogenic HIV-1 virus, such as a particular *nef*-defective strain, or 4) a controlled, very low-dose inoculation with fully pathogenic HIV-1 (obviously this method would require very stringent conditions).

In summary, a new hypothesis has been forwarded which provides numerous examples, both from experimental data and experiments of nature that actual protection against retroviruses is from a third, yet unexplored, form of immunity- "molecular immunity". Also, there are ample evidence to show that traditional form of immunities- cell-mediated and humoral- do not play any significant role in protection against retroviruses.

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