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Covalent vaccination and catalytic antibodies: A new way of looking at an HIV vaccine

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Introduction

There is global concern regarding HIV drug-resistance, drug toxicity, and increasing drug costs. Many health care professionals believe that eradicating HIV will require development of a vaccine that prevents infection by the virus. Yet, one by one, classical vaccine approaches used for combating other infections have proved ineffective for HIV in clinical trials.

Nearly three decades of research has been invested in understanding how HIV overcomes immune defenses and why candidate HIV vaccines have been ineffective. Immune defense is provided by an ensemble of molecules and cells with innate and adaptive capability to counter infectious microbes. The innate immune capabilities have evolved over millions of years of evolution. Their functional importance resides in the immediate blockade of infection, for example, killing of microbes by macrophages that migrate to the infection site due to an inflammatory response. Vaccines generally work by inducing adaptive immunity developed over days to weeks. The functional mediators of adaptive immunity are antibodies and T lymphocytes that specifically recognize microbial antigens and help remove the microbe. HIV has developed various mechanisms to overcome natural immune defenses of humans. These mechanisms are also the reason for the failure of classical immunological approaches to yield an effective HIV vaccine.

Challenges posed by HIV immune properties

Thousands of different HIV-1 strains have emerged. Most infections are initiated by strains that utilize chemokine coreceptor CCR5 for entry into host cells. Coreceptor CXCR4-dependent strains emerge with time. Both types of strains use CD4 as the primary host receptor to infect T cells and macrophages. Different parts of the world are dominated by strains belonging to different HIV-1 subtypes.[1] Subtype C strains are found primarily in the developing world and account for a majority of infections globally.

A central problem is that most exposed components of HIV mutate rapidly, generating structural variations of the viral coat proteins. The mutable coat regions are also its dominant antigenic regions, also known as epitopes, against which the immune system produces antibodies and T lymphocytes.[2] The original infecting strain induces a robust immune response, but new quasi-strains develop over the course of infection, and protection against the virus is transient at best. Similarly, the antigenic constituents incorporated in previously-tested candidate vaccines were drawn from a single HIV strain or at most a few strains. The candidate vaccines induced antibody responses and T cell responses mostly directed to the mutable coat protein regions, compromising their efficacy against structurally divergent virus strains in different individuals and in different parts of the world.

Over the course of the humoral immune response, antibody complementarity determining regions (CDRs) undergo rapid mutations under the selective pressure of antigen binding. This process generally generates neutralizing antibodies capable of high affinity antigen binding. One of the few immune vulnerabilities of HIV is the maintenance of its exposed CD4 binding site (CD4BS) on the surface of the coat protein gp120 in mostly constant form. The CD4BS is essential for virus-host cell binding and infection. Despite minimal chemical variability of the CD4BS, the immune system fails to mount a sufficiently protective antibody response to the CD4BS.

The reasons are complex. First, individual epitopes within the CD4BS are conformationally plastic, that is, the three-dimensional epitope structure can change during the process of infection. Initial CD4 binding at the CD4BS region located in the outer gp120 domain ($CD4BS^{od}$) may induce a conformational change of the CD4BS core region composed of amino acids 421-433 ($CD4BS^{core}$) that is essential for stable HIV-host cell binding. Consequently, the $CD4BS^{core}$ might exist in a conformation vulnerable to immune attack only transiently during the process of CD4BS-CD4 binding. Second, HIV utilizes an unusual evolutionary trick to preclude production of a protective antibody response by B lymphocytes. The $CD4BS^{core}$ expresses superantigen character.[3,4] Superantigens bind specifically to innately-produced antibodies expressed on the surface of B lymphocytes, the B cell receptors. Unlike the stimulatory binding of traditional antigens to the B cell receptor, superantigen binding occurs at the antibody framework

regions, and the functional consequence is down-regulation of B cell differentiation, premature cell death and failure to mount an adaptive antibody response. We suggested that the innate superantigen recognition capability of antibodies was originally developed by Darwinian evolution processes over millions of years as a defense against primordial microbes.[5] HIV appears to have evolved a CD4BS with superantigenic character as the means to preclude an adaptive antibody response.

Novel vaccine approaches

Induction of neutralizing antibodies is the cornerstone of effective vaccination. Following failure of candidate protein and polypeptide vaccines to induce sufficient neutralizing antibodies to the free virus,[6] the focus shifted to developing candidate DNA vaccines that induce cytotoxic T cells directed to HIV infected cells.[7] This approach was also ineffective. The RV144 vaccine composed of full-length gp120 protein and a canary pox vector expressing the *gp120/gag/protease* genes reduced the risk of infection by 31%.[8] It is unclear whether this is a statistically or clinically meaningful effect. Many in the field of HIV vaccine development believe that combined induction of neutralizing antibody and cytotoxic T cells is the favored approach. As the individual antibody and cytotoxic T cell responses to the mutable HIV regions are ineffective, it is not clear how combining these responses can be the basis for effective vaccination. Our view is that HIV vaccination will be feasible once an immunogen is identified that induces a sufficient immune response to a structurally constant region of HIV essential for virus infection and propagation.

The coat protein gp41 expresses certain structurally conserved regions. The vaccine approach of Barton Haynes at Duke University entails an epitope of the HIV gp41 coat protein located in the proximity of the lipid membrane.[9] Polyspecific antibodies that recognize this epitope in conjunction with membrane lipids neutralize genetically divergent HIV strains. Membranes of uninfected cells also contain the lipids as self-antigens. The immune system is generally tolerant to the self-antigens, and anti-HIV antibodies that react with self-antigens can exert deleterious effects on the host. Nonetheless, there is strong interest in the notion that breaking tolerance to self-antigens may guide development of an immunogen capable of inducing HIV neutralizing antibodies.

Concerning the epitopes of the CD4BS, there is no evidence for insufficient physical exposure as the cause of insufficient antibody production. Similarly, an intrinsic defect in the CDR adaptive mutational process is theoretically possible, but there is no evidence that this is the reason for insufficient anti-CD4BS antibody production following HIV infection or administration of the previously-tested vaccine candidates. Burton and coworkers have identified rare antibodies that recognize a segment of the CD4BS (the CD4BS^{od}) and neutralize genetically divergent HIV strains comparatively broadly.[10] Reverse-engineering of peptides with structure complementary to the neutralizing antibody binding site can be conceived as a route to a vaccine that induces the synthesis of similar neutralizing antibodies upon administration to humans. A peptide immunogen designed using as template a neutralizing antibody to a segment of the CD4BS did not induce broadly neutralizing antibodies.[11] Targeting a larger CD4BS surface area by a reverse-engineered immunogen could be more fruitful.

Our studies have identified the CD4BS^{core} as the proverbial Achilles heel of the virus. In the rare circumstances that anti-CD4BS^{core} antibodies are produced, they neutralize HIV strains from across the world with exceptional potency.[12,13] Such antibodies were found in non-infected patients with lupus, an autoimmune disease that is rarely associated with concurrent HIV infection, and in long-term survivors of HIV infection. It appears that HIV is highly vulnerable to neutralization by specific antibodies to the CD4BS^{core} region, but the adaptive immune response to the region is insufficient to control infection under normal circumstances. A clear path to an HIV vaccine that induces broadly neutralizing antibodies can be foreseen if the following milestones can be reached: a) Reproduction of the correct CD4BS^{core} conformation in the vaccine candidate, and b) Rapid adaptive production of neutralizing anti-CD4BS^{core} antibodies upon administration of the vaccine candidate.

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Our preclinical studies in experimental animals based on the covalent vaccination strategy suggest the feasibility of attaining the foregoing milestones.[14,15] Central points in the strategy are:

- The vaccine candidate, an electrophilic polypeptide containing the CD4BS^{core}, which binds covalently to B cells, resulting in production of broadly neutralizing antibodies. The polypeptide is activated chemically by linking lysine side chain to the strongly electrophilic phosphonate diester group. Naturally-occurring nucleophilic sites are found ubiquitously in B cell receptors.[16,17] Noncovalent binding of the CD4BS^{core} peptide epitope to the B cell receptors positions the electrophilic group within covalent binding distance of nucleophilic groups. The ensuing covalent bonding between the electrophile and nucleophile liberates a very large amount of energy that initiates productive signal transduction, IgM→IgG/IgA antibody class switching and differentiation of the cells into antibody-secreting plasma cells.
- Recruitment and clonal expansion of the small subset of B cells capable of producing antibodies with innate, pre-existing specificity directed to the CD4BS^{core}. The

CD4BS^{core} binds at a site located mainly in the framework regions of B cell receptors. Neutralizing antibody production occurs without dependence on typical adaptive mutational processes occurring in the CDRs. However, adaptive improvement of the antibodies due to mutations of the framework regions is feasible, as suggested by evidence from immunization of animals with electrophilic gp120 and an electrophilic CD4BS^{core} peptide mimetic.[18,19] Robust neutralization of diverse HIV strains by the antibodies in tissue culture was evident. The antibodies displayed specific recognition of the CD4BS^{core}, confirming mimicry of the native CD4BS^{core} by the vaccine candidates.

• Immunization with full-length electrophilic gp120, which overcomes the physiological hurdle in producing anti-CD4BS^{core} antibodies. Neutralizing antibodies to electrophilic gp120 displayed binary epitope reactivity, that is, the simultaneous ability to bind the CD4BS^{core} at the antibody framework region site and a second spatially distant epitope at the traditional antigen binding cavity formed by the CDRs. The binary specificity suggests that simultaneous stimulatory binding of the second immunogen epitope at the CDRs compensates for the down-regulatory CD4BS^{core} binding at the framework regions.

Taken together, our studies indicate the feasibility of developing an HIV vaccine capable of directing the innate CD4BS recognition capability of B cells towards a favorable maturational pathway, eventually resulting in synthesis of broadly neutralizing antibodies.

Catalytic antibodies (Abzymes)

Reversible CD4BS binding by antibodies alone is sufficient to neutralize HIV. A subset of antibodies produced by B cells express the ability to catalyze the breakdown of peptide bonds, destroying gp120 permanently.[20] A single catalytic antibody molecule is reused to cleave thousands of gp120 molecules over its biological half-life in blood (1–3 weeks). The neutralization potency of catalytic antibodies, therefore, is superior to traditional antibodies that bind the antigen reversibly on a 1:1 basis. Antibody catalytic sites belong to the serine protease enzyme family, consisting of nucleophilic sites similar to the archetypical Serine-Histidine-Aspartate catalytic triad of trypsin. Catalysis occurs by formation of a covalent intermediate and water attack on the intermediate, regenerating an antibody molecule that is reused for additional catalytic cycles.

Catalytic cleavage of gp120 occurs by noncovalent CD4BS^{core} binding followed by cleavage of peptide bonds. The catalytic sites are present in antibodies produced without exposure to HIV.[21,22] Sexual transmission of HIV generally occurs through the rectal and vaginal mucosal surfaces. Only a minority of sexual intercourse events with an infected individual results in transmission of the virus. Secretory IgA class antibodies found at mucosal surfaces of non-infected humans catalyze rapid gp120 cleavage and neutralize HIV in tissue culture.[23] It may be hypothesized that the catalytic IgAs constitute a natural defense against mucosal HIV transmission.

In addition to inducing reversibly-binding antibodies, the covalent vaccination approach described in the preceding section stimulates adaptive improvement of the nucleophilic function of antibodies. This is feasible because covalent binding of the electrophilic vaccine candidate selects B cell receptors with the greatest nucleophilic reactivity.[24,25] In turn, the improved nucleophilic reactivity enhances antibody inactivation of HIV as follows. First, specific pairing of the antibody nucleophile with the weakly electrophilic carbonyls of gp120 forms stable immune complexes with covalent character. Covalently binding antibodies were induced by immunization with the electrophilic analogs of full-length gp120 and a synthetic gp120 peptide. Reversibly bound antibodies dissociate from HIV readily. As the covalent bond is very strong, the covalent antibody-HIV complexes do not dissociate, increasing the HIV neutralization potency. Second, if the antibody combining site supports water attack on the covalent gp120-antibody complex, catalytic gp120 cleavage occurs. A subset of antibodies obtained by immunization with the electrophilic CD4BS^{core} peptide catalyzed the cleavage of gp120 rapidly.

The neutralization potency of catalytic antibodies is superior to traditional antibodies that bind the antigen reversibly on a 1:1 basis.

Treatment of HIV using reverse transcriptase and protease inhibitors requires vigilant management because of the potential for toxicity and emergence of drug-resistant strains. This has generated interest in passive immunotherapy using monoclonal antibodies. Control of viremia upon infusion of reversibly binding anti-HIV antibodies in humans was transient, suggesting emergence of antibody-resistant viral mutants. Very large quantities of the antibodies were necessary to reduce viral load, a reflection of modest antibody neutralizing potency. Can catalytic antibodies be used for passive immunotherapy of HIV infection? The answer depends on the epitope specificity and neutralizing potency of the catalysts. Targeting the CD4BS^{core} minimizes the opportunity for development of antibody resistant strains, as CD4 binding and mutations in the CD4BS^{core} are predicted to result in loss of CD4 binding activity. Indeed, anti-CD4BS^{core} antibodies from long-term survivors of HIV infection neutralized the autologous HIV strain potently. There is no evidence, therefore, for emergence of resistant strains despite the selective pressure imposed by the anti-CD4BS^{core} antibodies over prolonged durations. Anti-CD4BS^{core} antibodies neutralize HIV in tissue culture with nanogram/ml potency, supporting their potential therapeutic application.

In addition to gp120, two additional HIV proteins essential for virus infection are cleaved by catalytic antibodies, reverse transcriptase and integrase.[26,27]

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Intracellular expression of catalytic antibodies to these proteins holds potential for early blockade of viral propagation via interference with copying viral RNA into proviral DNA and DNA integration into the host genome. Gene therapy protocols for intracellular antibody expression[28] can be conceived for persistent delivery of catalytic anti-HIV antibodies. Reactivation of HIV infection can occur due to integration of the viral genome into host DNA. Drugs that deplete proviral DNA reservoirs are under investigation to address the problem of HIV latency.[29] Catalytic antibodies combined with a proviral DNA-depleting drug may be suitable for consideration as an alternative therapy for the infection.

Prioritization and funding of new technology development

A prophylactic vaccine and a cure for patients infected with HIV are needed urgently. However, there is considerable pessimism because of repeated clinical failure of candidate vaccines. The seemingly insoluble nature of HIV has even inspired an argument for use of the limited available funding for improved delivery of available anti-retroviral drugs to infected patients rather than further research investment. This argument is misguided. Innovative preclinical approaches are essential if the objective of eradicating HIV infection is to be met. Our positive preclinical studies using the covalent vaccination and catalytic antibody approaches are an example. These approaches were developed under basic immunology grants funded by the National Institute of Health over the past two decades. Additional developmental efforts will be necessary to obtain a standardized covalent vaccine and catalytic antibody candidates for human trials, but there is hope for translation of the preclinical immunological advances into clinical success.

In the U.S., elaborate governmental arrangements are in place to prioritize the competing developmental approaches for funding, including excellent scientific peer-review arrangements. However, programmatic allocation of funds is inspired at least in part by non-scientific reasons. The literature is replete with claims of potential clinical advances. On the other hand, most HIV vaccine development projects are likely to yield incremental advances at best. An example is the continued testing of vaccine formulations that induce immune responses primarily to mutable regions of HIV. Likewise, intensive efforts have been undertaken to identify immune markers correlating with the marginal risk reduction observed in the RV144 vaccine trial. As there is doubt whether the vaccine candidate really reduced the risk of infection, it is hard to accept that meaningful correlates of risk reduction will emerge. A policy change that forthrightly admits the limited utility of classical vaccine approaches and explicitly encourages credible, novel approaches would be a welcome event.

Scientific approaches that diverge radically from established paradigms are invariably subject to rigorous peer evaluation. Independent reproduction of the evidence is usually necessary prior to widespread acceptance of the new scientific approach. These are essential safeguards against mistaken conclusions and spurious claims. Antibodies obtained by the covalent vaccine approach have been independently verified to neutralize diverse HIV strains in tissue culture. Factors that might result in artifactual neutralization have been carefully eliminated.[30,31] Similarly, the chemical and immunological principles underlying antibody catalysis have been amply validated by researchers across the world. Occam's razor is yet another safeguard against unproductive science—when confronted with alternative explanations that are equal in other respects, the hypothesis that makes the fewest novel assumptions should be selected for further study. It is necessary to invoke B cell superantigenicity as the cause of poor CD4BS immunogenicity, as no competing hypothesis explains the empirical findings adequately. Similarly, the innovation of covalent bonding of the vaccine candidate to B cells is necessary, as no alternative strategy is available to induce a robust anti-CD4BS antibody response. In summary, the preclinical scientific findings support translation research aimed at realizing the clinical utility of the technology.

Conclusion

Recent immunogenicity and virus neutralization data encourages the belief that it may be possible to develop a covalent HIV vaccine that induces broadly neutralizing antibodies directed at the CD4 binding site of the virus. Catalytic antibodies to HIV appear to be a natural defense mechanism against HIV, and it may be possible to apply broadly neutralizing catalytic antibodies as an alternative therapy for HIV infection.

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