How Basic Immunological Principles May Instruct the Design of a Successful HIV-Type 1 Vaccine

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Abstract

This article is dedicated to Dr. Peter Doherty. While Peter continues to make groundbreaking discoveries in the field of immunology, he also provides outstanding scientific mentorship to his trainees. Here we contemplate our past training with Peter, Peter's teachings of basic immunological principles, and how basic principles may instruct the design of a successful human immunodeficiency virus-type 1 vaccine.

Keywords: mentor, infectious diseases, immune response, vaccine development, superinfection

Training with Peter Doherty

WE WERE TRAINED as fellows in the laboratory of Dr. Peter Doherty where we were taught the basic concepts of immunology. Peter described how lymphocytes recognize and then eradicate invading viruses. While teaching and engaging trainees in his ongoing projects, Peter also encouraged scientific independence. Unlike many mentors who focus primarily on their own ideas, Peter encouraged young scientists to formulate new hypotheses. He supported scientific freedom both within his laboratory and for trainees who graduated from his laboratory to advance their independent careers.

In the mid-1990s, after we had graduated from Peter's laboratory and had taken new positions in Peter's Immunology Department at St. Jude Children's Research Hospital (St. Jude), we initiated development of a clinical-grade vaccine. We were soon meeting with Food and Drug Administration (FDA) officials and learning Good Manufacturing Practices (GMPs) required for the development of clinical biologicals. These steps paved the way for the preparation of clinical vaccine material and the conduct of a first phase I clinical vaccine study at St. Jude. Today, the *Children's GMP LLC* on the St. Jude campus produces dozens of products for clinical applications.

It was while we were advancing our first vaccine candidates in 1996 that Peter and Dr. Rolf Zinkernagel received the Nobel Prize in Physiology or Medicine for their groundbreaking discovery of major histocompatibility complex (MHC) restriction. In the 1970s, Peter and Rolf found that immune T cells only recognized infected target cells when the T cell and target cell shared MHC (14,54–57). This discovery drove further research into T cell receptor, viral peptide, and MHC interactions (20) and has since served as the foundation for decades of basic and clinical advances in vaccine development and T cell immunotherapies. Peter accepted the Nobel Prize with humility. Even today, when young scientists meet Peter, they are impressed with his humility and willingness to stop and discuss science. Peter is never too busy to listen and provide advice. In sum, Peter taught us and teaches us how to perform research, how to enjoy research, how to share research, and how basic immunological principles can translate to extraordinary improvements in human health.

Basic Immunology Concepts and Vaccine Development

Evolution has armed mammals with an impressive means of immune protection. The sophisticated joining of immunoglobulin or T cell receptor variable, diversity, and joining (V-D-J) gene segments in developing B cells and T cells provides humans with as many as 10^{20} different receptors [one model predicts that the receptor number is $>10^{60}$] (13,28,32). Each lymphocyte bears a different receptor and each receptor has a different antigenic specificity. Immune receptors bind their targets (free antigen for B cells and peptide-MHC complexes for T cells) using highly specific

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"lock-and-key" (target-to-receptor) interactions. Accordingly, the enormous diversity of unique immune cells/receptors has the potential to protect humans against virtually any pathogen in nature.

Vaccine developers may take advantage of diverse B and T cell receptor repertoires by designing look-a-like vaccines. When a vaccine "looks like" its target pathogen (i.e., carries antigens that are structurally matched), the vaccine will safely activate (by "lock-and-key" interactions with lymphocyte receptors) the B cells and T cells that can cross-react with the pathogen. These lymphocytes then amplify and serve as an army, ready to tackle pathogen when an exposure occurs at a later date. Lymphocyte activation before pathogen exposure is essential, particularly for persistent viruses such as human immunodeficiency virus-type 1 (HIV-1), which in the absence of primed defenses can establish permanent residence in immune-privileged sites.

HIV-1 Vaccine Development

HIV-1 is a formidable human pathogen. In part, this is because HIV-1's attachment envelope protein (Env) can vary [although Env diversity is limited by its requirement to bind conserved CD4 (26) and co-receptor molecules]. HIV-1 is not the first diverse pathogen to pose a challenge to vaccine development; in other fields, vaccines against diverse pathogens have already been designed and licensed. In the 1950s, Jonas Salk produced a successful polio vaccine by combining representatives of three circulating poliovirus serotypes into a cocktail (4–6,22,24). *Streptococcus pneumoniae* vaccines have similarly proven effective, because they are cocktails that represent diverse serotypes. Cocktail vaccines for *S. pneumoniae* were being formulated as early as 1945 and resulted in a vaccine, still used today, comprising 23 purified capsular polysaccharides for representation of 23 different serotypes (2,50). When pneumococcus conjugate vaccines were first developed, only seven serotypes of pneumococcus were included (Prevnar), but breakthrough infections occurred and vaccine valency was accordingly increased (19,21,47). The current conjugate vaccine formulation (Prevnar 13) includes 13 serotypes (1), and new vaccine candidates comprising even more distinct serotypes are being developed. It should be noted that the public health benefits conferred even by the smallest vaccine cocktail formulations have been immeasurable.

Adding to lessons from other vaccine fields, insight into successful HIV-1 vaccine development can be gained by analyses of natural virus infections. Studies have shown that animals previously exposed to HIV-1 (or simian immunodeficiency virus [SIV] or chimeric HIV-SIV [SHIV] in nonhuman primate models) are often protected from superinfection (9,10,12,15,33,37,43,45). Furthermore, the passive transfer of sera from an infected animal to a naive animal can be protective (27,34,46).

The immunity against exogenous virus that is conferred by infection is a consequence of a complex interplay between endogenous virus and the immune system. When a naive individual is naturally infected with HIV-1, the founder virus is limited in diversity and the consequent immune response is similarly limited (49). Virus is not cleared, but it is instead sequestered in privileged sites. Virus then mutates, generating new Env structures that can support HIV-1 infection and can escape the contemporaneous systemic immune response (31,49). When new virus variants circulate, they activate new lymphocytes, increasing the breadth of the immune response. Activated B cells also experience somatic mutation, after which cells that bear receptors with improved affinity and avidity toward viral antigens are amplified (17). After several rounds of virus escape and lymphocyte activation, immune breadth is sufficient to recognize diverse HIV-1 and thereby protect against virus infections from an exogenous source (29,35,49). Vaccines designed to recapitulate the Env diversity that is introduced by a natural virus infection are likely to prove successful (29).

Yet another lesson informing HIV-1 vaccine development can be gleaned from the RV144 HIV-1 vaccine study (30). Although this clinical trial suggested a vaccine efficacy of only ∼30% (in a modified intention-to-treat analysis), it is noteworthy that the vaccine included only three different Env. One genetically engineered Env was expressed with other HIV-1 proteins by recombinant canarypox and two Env were included in a protein boost (51). Trial results were disappointing, but they pointed to the potential efficacy that could be afforded by a larger vaccine cocktail (as was observed in the pneumococcal vaccine field).

Creating an HIV-1 Cocktail Vaccine

Successful multivalent or "cocktail" vaccines (such as those tested in the 1940s and 1950s) were designed to represent antigenically distinct target pathogens by mapping (cartography) studies that tested antigen–antibody interactions (4–6,24,41). Similar antigen–antibody mapping studies have been initiated in the HIV-1 vaccine field by using virus isolates/proteins and sera/antibodies (3,23,25,38,58). These have defined antigenic clusters, but they have not yet been used to advance a vaccine to licensure.

Results from antigen–antibody mapping studies emphasize that the virus's clade (sequence) and country of origin do not always predict antigenicity (3,23,40,48). Rather, some viruses from two different clades and countries share antigenicity, whereas some viruses from the same clade and country do not. This result is expected, because B cell and T cell epitopes are influenced by structure (including three-dimensional and four-dimensional protein configurations), not just sequence, and because structure can be altered by one or a few amino acid changes within or distant from a target epitope (7,8,11,16,36,44).

Cartography studies in the HIV-1 field could be easily expanded and fine-tuned using high-throughput antigen–antibody assays to create vaccine cocktails representing most functional Env antigens. The immune system naturally responds to a plethora of diverse antigens in the human environment and can also respond to large multicomponent vaccines (e.g., the pneumococcus vaccines described earlier or much larger vaccine libraries) (18,39,42,52,53).

Presumably large HIV-1 cocktail vaccines will eventually be developed, shown to induce protective immunity, and licensed. Inferring from previous successful vaccines such as Prevnar, it is possible that HIV-1 vaccine development will be iterative—advancing in vaccine efficacy with each subsequent iteration.

The multi-Env cocktail vaccine approach is slowly gaining momentum in the HIV-1 vaccine field. Goals are to

recruit an array of lymphocytes with diverse receptors and to promote somatic mutations to improve receptor affinity, avidity, and breadth (17). These outcomes may together, ultimately, protect humans from a deadly disease.

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