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The value of HIV protective epitope research for informed vaccine design against diverse viral pathogens

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Abstract

The success of vaccine regimens against viral pathogens hinges on the elicitation of protective responses. Hypervariable pathogens such as HIV avoid neutralization by masking protective epitopes with more immunogenic decoys. The identification of protective, conserved epitopes is crucial for future vaccine candidate design. The strategies employed for identification of HIV protective epitopes will also aid towards rational vaccine design for other viral pathogens.

Keywords

epitope; glycan; H1N1; HIV; vaccine

An effective HIV vaccine is still elusive despite more than 20 years of concerted efforts. With the success of highly active antiretroviral therapy for treatment, as well as recent studies indicating the efficacy of pre-exposure prophylaxis [1,2], continued enthusiasm for HIV vaccine research might appear injudicious. Indeed, the most successful trial to date was RV144 that exhibited 30% protection. This modestly successful trial has provided hints about the correlates of protection against HIV-1 acquisition [3,4].

Inducing protective antibody responses against HIV is challenging due to lack of correlation between protective antibody responses and neutralizing activity. Several protective epitopes have been identified due to isolation of new broadly neutralizing monoclonal antibodies (bnAbs). HIV vaccine trial (RV144), demonstrated a reduced risk of infection associated with the presence of antibodies binding the V1/V2 region [3]. A recent study described the bnAb elicitation mechanism; the antibodies develop primarily from B cells initially selecting for a long, complementarity determining region 3 loop rather in addition to affinity maturation or somatic mutation [4]. As broadly neutralizing humoral responses occur rarely,

that prompts efforts to identify non-neutralizing epitopes for alternative effector mechanisms such as antibody-dependent cellular cytotoxicity and identification of protective epitopes beyond the ostensible envelope targets [5]. This last approach led to the identification of a novel protective epitope in HIV Tat among animals vaccinated with multimeric HIV gp160, HIV-1 tat and SIV gagpol. These responses were not observed in viremic animals [5]. Protection-linked biopanning was able to establish a link between an epitope in Tat and antibody-mediated neutralization, though it has not yet been studied whether this response would provide prevention of infection. Interestingly, there are no bnAb that bind to epitopes outside of the HIV envelope. The efforts to identify protective epitopes beyond Env may yield dividends not only in HIV vaccinology, but vaccine research in other infectious diseases such as influenza and dengue.

More recently, it has been elegantly demonstrated that natural variation in Fc glycosylation is a key determinant in controlling spontaneous HIV antiviral activity [6]. On the other hand, major changes from complex glycosylated to deglycosolated forms have been shown to play a critical role in the development of HIV-specific antibodies [6]. Therefore, it has been suggested that HIV glycans are an attractive vaccine target because structural variation of the glycan structure is considerably lower than that of the viral protein sequence. Recently, two studies have highlighted the need for more intensive studies on the role of the glycan shield: a network theory has been proposed based on highly conserved glycans in different HIV clades and demonstrate the existence of subtype-specific glycosylation patterns that provide the rationale for how certain subtypes are more susceptible to neutralization [7]. Furthermore, it has been suggested that certain HIV clades use alternate glycosylation patterns to evade neutralization with broadly cross neutralizing antibodies while maintaining the protective gp120 glycan shield; based on structural studies, it has been proposed that multiple modes of viral neutralization exist which involve glycan recognition sites. It has also been shown that bnAbs can tolerate structural diversity within a highly conserved variable region of the glycan shield [8,9]. Together, these studies reveal that there is a site of vulnerability in the viral envelope, which can be an attractive target for vaccine development and suggest that more investigation is needed to explore the existence of highly conserved structurally proximal clusters of glycosylation that will help identify protective epitopes on carbohydrate-neutralizing clusters. Although these studies are inspiring, extensive research is required to generate a comprehensive depiction of viral fusion glycoprotein-associated immunosuppression. New tools such as synthetic screening libraries of homogenous glycopeptides have aided in glycan epitope characterization [10,11]. Like HIV-1, the host targets of the immunomodulatory motif found in other species of viruses remain poorly defined and await further studies.

Elicitation of protective antibodies is considered the primary goal of the annual influenza vaccine. Although influenza does not have the same inherent hypervariability as HIV, vaccinations must be given on an annual basis to elicit protection against the predicted dominant strain. The protective antibodies are generated to the surface glycoprotein hemagglutinin (HA) of the virion. These antibodies block binding of HA to sialylated host cell receptors; the most neutralizing antibodies are those with epitopes in the globular head region. The recent emergence of H7N9 in China has directed more research towards antibodies targeting the membrane proximal stalk domain of HA, which exhibit low

neutralization titers in standard HA inhibition assays [12]. Similar to membrane proximal external region -specific antibodies against HIV, these antibodies prevent fusion of the viral and cellular membrane (reviewed in [13]). These types of neutralizing antibody responses are measurable in standard neutralization assays; however, HA inhibition assays can only detect HA neutralization activities via direct antibody binding, excluding others such as antibody-dependent cellular cytotoxicity. With the focus of the yearly vaccine on eliciting HA antibodies that target the globular head, it may be more advantageous to target conserved, although perhaps poorly immunogenic, epitopes on the stalk. This may afford benefits such as broader antiviral efficacy within and across subtypes, as well as obviate the need for yearly vaccinations as a result of point mutations in HA variable regions [14]. More recently, Medina *et al.* demonstrated that introducing glycosylation sites onto the HA head of pandemic H1N1 resulted in attenuated infection as well as protection from pre-existing immunity to wild-type strains [15]. Glycosylated H1N1 was able to elicit a broad, polyclonal, cross-neutralizing response against glycosylated and wild-type virus. When this glycosylation site was removed, antibodies were elicited that were able to prevent infection from antigenically diverse strains. These data suggest that glycosylation plays a considerable role in viral pathogenesis and immunity to the degree that glycosylation alteration may be a potential strategy to improve influenza vaccines.

The annual live-attenuated influenza vaccine induces protective T-cell responses in addition to the humoral immune responses at the mucosal and cellular level [16,17]. This additional T-cell elicitation imparts a higher level of protection than achieved with antibodies alone, as in the traditional detergent-inactivated vaccine.

The perceived importance of T-cell responses in an effective HIV vaccine has fluctuated over the last 10 years. T-cells have been shown to control HIV infection, but recent trials testing T-cell eliciting candidates such as the recombinant adenovirus serotype 5 vector did not prevent HIV acquisition [18,19]. A recent meta-analysis showed that although vaccine responses clustered into epitope ‘hotspots’, these epitopes were not evident in chronic HIV-1 infection [20]. These hotspots were found in variable regions, reinforcing the idea that an effective T-cell response will be difficult to elicit. A majority of CD8 T-cell responses were found to epitopes within variable regions during early infection in ART-naïve patients [21]. However, responses against conserved epitopes were correlated to lower setpoint viremia. These studies emphasize the importance of designing vaccines to elicit T-cell responses to conserved epitopes. The difficulty in developing next-generation HIV T-cell vaccines that potentially target conserved epitopes has renewed the focus on neutralizing antibodies, though the success seen with the live-attenuated influenza vaccine and its bimodal cellular and humoral responses should convey the importance of identifying protective epitopes for both T-cells and antibodies.

What then should be the focus of future protective epitope research? We believe there should be efforts directed to identify new protective epitopes associated with protection and improve understanding of presently known protective epitopes with the aim of leveraging this knowledge into rational vaccine candidate design, including what effective epitope presentation requires. Research into new protective epitopes beyond those associated with bnAbs may generate insight; the epitopes of less protective antibodies using conformational

epitope mapping identified epitopes present on trimeric Env that spanned the V3 and V4 loops [22]. New methods to present known protective epitopes more effectively are being developed, including gp41 membrane proximal external region epitopes persistently expressed by chimeric foamy virus [23]. A novel respiratory syncytial virus vaccine candidate was generated using computational protein design to improve scaffold proteins [24]. A scaffold protein is used to stabilize the conformation of the protective epitope. This approach elicited respiratory syncytial virus neutralizing responses in macaques; this method of generating unique scaffold proteins specifically adapted to each epitope may be beneficial for ongoing HIV and influenza vaccine efforts. The stability of established cytotoxic T lymphocytes epitopes binding resistance-associated HLA alleles was correlated to increased immunogenicity [25].

The value of continued research into protective HIV epitope mapping will not only improve the prospects of HIV vaccine design, it will improve vaccine design and monoclonal antibody-based therapy for other viruses with similar antigenic variation and complexity. Indeed, this research can be applied beyond viruses to any antigen the humoral immune system might encounter. The dividends gained from this research can potentially be applied to a wide field, including bacterial pathogens.

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Biography

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