LEVERAGING TECHNOLOGICAL ADVANCES TO ACCELERATE HIV VACCINE DEVELOPMENT

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Over the last three decades, advances in antiretroviral (ARV) drug treatment have transformed HIV-1 infection from a progressive fatal disease into a stable and manageable chronic viral infection. Yet, despite this progress, more than 1 million people died from AIDS-related illnesses in 2015, in large part due to limited access to ARV drugs.

Consequently, an effective HIV vaccine continues to offer the greatest promise to control and eventually defeat the ongoing global HIV/AIDS epidemic. The development of a protective HIV vaccine will most likely require the elicitation of broadly neutralizing antibody (nAb) responses.

This white paper discusses current methods for assessing nAb responses and defining genetic determinants of neutralization escape. It also describes the breadth and potency of nAb responses elicited during HIV infection and the primary challenges facing the development of a broadly protective vaccine. Finally, it shares recent efforts to generate new broadly neutralizing monoclonal antibodies (bnAb). The breadth, potency, origin and maturation of these bnAb are the subject of intense investigation, and the features of bnAb are informing novel vaccine designs and strategies today.

Measuring nAb Activity

Over the last 10 years, pseudovirus reporter assays have largely replaced conventional nAb assays performed using replication competent virus stocks and primary peripheral blood mononuclear cell (PBMC) culture. These pseudovirus assays reduce the time, resources and expenses associated with measuring nAb activity, and they offer higher reproducibility and reliability. Researchers can use this approach to interrogate nAb responses to envelope proteins of any HIV or SIV isolate, which significantly facilitates the characterization of nAb escape variants.

A Deeper Understanding of nAb Responses

The improved convenience and throughput of pseudovirus assays have yielded significantly more insight into the timing, breadth and potency of nAb responses mounted after HIV infection.[1] Potent nAb responses emerge rapidly, however these early responses are typically narrow. Furthermore, the ability of HIV to continually escape nAb pressure enables ongoing viral replication and diversification. Although responses vary significantly among individuals, the development of bnAb responses is not uncommon, but generally requires prolonged infection.

Potent nAb Responses Emerge Rapidly Following HIV Infection

Serial plasma samples have been tested for HIV nAb activity against pseudoviruses generated from the same plasma collections. The resulting data demonstrate that detectable nAb activity emerges by week eight post-HIV infection and possibly as early as week four post-infection.

Early HIV Infection is Defined by Potent, Yet Narrow, nAb Responses

nAb responses mounted during the first year of HIV infection are generally restricted to the autologous virus and are unable to neutralize the heterologous viruses of other HIV-infected subjects. Thus, the development of narrow nAb responses is one of the main obstacles to the natural immune control of HIV infection and the elicitation of an effective vaccine response.

HIV Perpetually Escapes nAb Pressure, Enabling Ongoing Replication

Another key barrier to the development of an effective HIV vaccine is illustrated by the fact that each plasma sample can neutralize only the viruses that precede the contemporaneous virus population. Viruses that emerge later in infection are not susceptible to existing nAb responses. The continuous selection of HIV envelope variants that escape concurrent or prior nAb activity explains why potent nAb responses to infection fail to control viral replication.

nAb Responses Among Individuals Vary Dramatically

A further challenge facing the development of an effective HIV vaccine relates to the variation in nAb responses that emerge among individuals following natural HIV infection. This variation includes:

- nAb responses that continually and incrementally adapt to recognize new variants
- The failure of nAb responses to emerge despite ongoing HIV replication
- The emergence of nAb responses directed to early variants but that fail to evolve to recognize subsequent escape variants

Understanding the virus and host immune factors that give rise to these variable responses will aid the design of broadly protective vaccine responses.

Strong Antibody Responses Are Associated With Directed Sequence Evolution

When the envelope sequences of emerging variants are interrogated, stark distinctions are observed among infections that provoke different nAb responses. HIV infections accompanied by potent nAb responses are characterized by highly directional envelope sequence "divergence." In contrast, infections that are not accompanied by potent nAb responses are characterized by non-directional sequence "diversification." These patterns are consistent with a model in which sustained selective pressure drives the continual emergence of nAb escape variants fueled by the error-prone replication of HIV.

nAb Breadth Generally Expands with Prolonged Chronic Infection

While early nAb responses elicited by HIV infection are narrow, nAb breadth can expand significantly during prolonged infection. This initial expansion includes nAb responses directed at autologous virus variants. However, as nAb responses expand to recognize emerging autologous virus variants, they eventually acquire sufficient breadth to neutralize heterologous virus variants. Several vaccine initiatives are now exploring progressive immunization strategies aimed at recapitulating the elicitation of incrementally broader nAb responses.

A Tool to Measure nAb Activity

The PhenoSense® nAb assay performed at Monogram Biosciences is a patented recombinant pseudovirus assay that was developed to interrogate nAb responses directed against an entire HIV population, as well as the individual molecular clones or variants within a population.

The PhenoSense assay begins with the assembly of plasmids expressing an envelope protein derived from any HIV or SIV isolate or a reference strain. Envelope expression vectors are generated by isolating virus RNA from plasma, performing RT-PCR, cloning envelope amplification products and propagating those envelope expression vectors in bacteria. The vectors are then used to generate pseudovirus HIV stocks containing a luciferase reporter gene. Prior to inoculation of target cells, those pseudoviruses are harvested and incubated with a source of nAb or mAb. Finally, nAb activity is measured by assessing the ability of antibody preparation to reduce luciferase activity in the target cells.

Case Study: Isolation of New Monoclonal Antibodies Possessing Broad Neutralizing Activity – Informing New Approaches in HIV-1 Vaccine Discovery

A collaboration among the International AIDS Vaccine Initiative, Monogram Biosciences, Theraclone Sciences and the Scripps Research Institute enabled a highly productive series of studies. The products of this collaboration demonstrate how novel experimental approaches are informing immunogen design and vaccine development. These novel approaches included:

- Utilization of the PhenoSense nAb assay to identify the 1 percent of subjects exhibiting "elite" (cross-clade) nAb activity among more than 1,000 donors[2]
- Development of a high-throughput microtiter assay to rapidly screen cell lines derived from about 30,000 activated memory B cells of a single donor to identify two novel broadly neutralizing mAb[3]
- Broader application of this high-throughput screen to cell lines representing activated memory B cells of four donors resulting in the identification of 17 novel broadly neutralizing mAb[4]
- Application of a most-recent common ancestor approach to design an HIV envelope sequence that elicits broad nAb activity and is a candidate for HIV vaccine immunogen design[5]

Identifying "Elite" Neutralizers

The success of this collaborative effort relied heavily on the identification of infected individuals who developed antibody responses capable of neutralizing a broad spectrum of genetically diverse strains of HIV. To identify these "elite" neutralizers, a large panel of pseudoviruses was generated that expressed HIV envelope proteins representing all well-established HIV-1 group M subtypes.

Based on these data, a much smaller multi-subtype panel was selected based on an ability to interrogate nAb breadth and potency that was representative of the larger panel. This smaller panel was then used to rank the nAb responses of individual serum samples using a scoring algorithm.

Generating New Monoclonal Antibodies

A preliminary attempt to isolate antibody gene sequences from the B cells of one individual was conducted with the intent to create cell lines that produce mAb exhibiting broad nAb activity. This effort produced two new broadly neutralizing mAbs, PG9 and PG16, which exhibit significantly broader nAb activity than existing mAb that were previously considered broadly neutralizing, such as b12, 2G12, 2F5, and 4E10.

The successful isolation of PG9 and PG16 prompted efforts to repeat this process using the B cells of additional elite neutralizers, which resulted in the generation of an expanded series of broadly neutralizing mAb (i.e., the PGT series). These mAb comprise several distinct binding specificities, enabling new insights into which determinants on the HIV envelope are capable of eliciting broadly neutralizing responses.

Subsequent studies characterizing these broadly neutralizing mAb, have demonstrated that their neutralizing activity against individual HIV strains can vary significantly, spanning several orders of magnitude. In some instances, the neutralization activity may be incomplete and unable to fully neutralize HIV infection even at the highest antibody concentrations tested.

Mapping Determinants of bnAb Activity

A novel bioanalytical approach was developed and applied to map the binding specificities of newly developed mAb.[6] Amino acid patches on the surface of the HIV envelope protein were surveyed to identify variation within patches that were strongly correlated with changes in nAb activity. The method relies on a matching dataset of HIV envelope sequences and nAb activity, derived using a pseudovirus library. The mapping results of this new method are consistent with the results obtained using more tedious and time-consuming, conventional approaches.

The Path to Successful Vaccination Strategies

Today, nAb responses are generally characterized using pseudovirus assays that rely on reporter gene readouts. Using these assays, researchers have come to appreciate that HIV infection elicits potent nAb responses that broaden during infection. The continual emergence of nAb escape variants prevents nAb responses from controlling virus replication.

The inability of vaccine candidates to elicit broadly neutralizing antibody responses remains the most significant challenge in HIV vaccine development. However, recent efforts have yielded a new generation of human mAb with broad, potent nAb activity. The characterization of these antibodies has the potential to inform and improve vaccination strategies and reinvigorate the pursuit of an HIV vaccine.

References

1. Richman DD et al. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci USA* (2003) 100(7):4144-4149.
2. Simek MD et al. Human immunodeficiency virus type 1 elite neutralizers: Individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J Virol* (2009) 83(14):7337-7348.
3. Walker LM et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* (2009) 326(5950):285-289.
4. Walker LM et al. Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* (2011) 477:466-470.
5. Hoffenberg S et al. Identification of an HIV-1 clade A envelope that exhibits broad antigenicity and neutralization sensitivity and elicits antibodies targeting three distinct epitopes. *J Virol* (2013) 87(10):5372-5383.
6. Evans MC et al. Predicting HIV-1 broadly neutralizing antibody epitope networks using neutralization titers and a novel computational method. *BMC Bioinformatics* (2014) 15:77.