OVERVIEW

A vaccine capable of eliciting antibodies neutralizing a broad range of HIV-1 strains must target a region of the HIV-1 virus that is highly conserved across strains. The Reinherz consortium is aimed at eliciting antibodies directed against a region of the viral gp41 envelope protein known as the membrane proximal external region (MPER). Structural information on this lipid-embedded region will be exploited for vaccine immunogen design and delivery to the immune system in the form of lipid-coated nanoparticles. This vehicle system has the advantage of concomitantly facilitating delivery of adjuvants including Toll-like receptor (TLR) ligands. In addition, nanodisc wafers shall be used to restrict antibody access to the MPER segment, thereby selecting desired anti-MPER specificities.

RESEARCH OBJECTIVES

1.) Structural analysis of HIV-1 MPER from clade C viruses with and without the appended transmembrane (TM) gp41 segment in lipid environments.

2.) Creation of MPER nanoparticles consisting of a polymer core particle encapsulated by an outer lipid vesicle or “skin” for MPER presentation to the immune system.

3.) Elicitation of humoral immune responses to MPER immunogens, assessment of antibody titer, specificity and HIV-1 neutralizing activity in serum IgG as well as characterization of individual bone marrow plasma cells.

PROGRESS

Although infrequently elicited during the course of natural infection and rarely, if at all, upon conventional vaccination, the membrane-proximal external region (MPER) of the HIV-1 gp41 glycoprotein is the target of several human broadly neutralizing antibodies (bNabs): 4E10, 2F5, Z13e1 and more recently, 10E8. How these bNabs bind to their lipid-embedded epitopes and mediate antiviral activity is unclear, but this information may offer important insight into a worldwide health imperative. The Reinherz research team, including Gerhard Wagner, Jim Sun, Likai Song and Mikyung Kim, has utilized EPR and NMR techniques to define the manner in which these bNabs differentially recognize viral membrane-encrypted residues configured within the L-shaped helix–hinge–helix MPER segment. Two distinct modes of antibody-mediated interference of viral infection were identified. 2F5, like 4E10, induces large conformational changes in the MPER relative to the membrane. However, although 4E10 straddles the hinge and extracts residues W672 and F673, 2F5 lifts up residues N-terminal to the hinge region, exposing L669 and W670. Detailed analysis of 2F5 extraction using the above techniques in conjunction with hydrogen exchange mass spectrometry (HX-MS) with John Engen demonstrates that 2F5 recognition is stepwise, involving a paratope more extensive than the core binding site contacts alone, and dynamically rearranging via an apparent CDRH3 scoop-like movement essential for MPER extraction from the viral membrane. Core epitope recognition on the virus requires induction of conformational changes in both the MPER and paratope. Hence, target neutralization through this lipid-embedded viral segment places stringent requirements on antibody combining-site plasticity. In contrast, Z13e1 affects little change in membrane orientation or conformation, but rather immobilizes the MPER hinge through extensive rigidifying surface contacts. Thus, bNabs disrupt HIV-1 MPER fusogenic functions critical for virus entry into human CD4 T cells and macrophages, either by preventing hinge motion or by perturbing MPER orientation. HIV-1 MPER features, important for targeted vaccine design, have been revealed, with implications extending to bNab targets on other viral fusion proteins. 10E8 also binds to W672 and F673, but with a different extraction profile to that of 4E10.

Progress was made in NMR spectroscopic studies to characterize three clade C MPER segments in a lipid environment. These clade C peptides all share the typical helix-hinge-helix motif first observed in a HxB2 peptide from clade B. We showed that the MPER consists of a structurally conserved pair of viral lipid-immersed helices separated by a hinge with tandem joints that can be locked by capping residues between helices. This design fosters efficient HIV-1 fusion via inter-converting structures while at the same time affording immune escape. Disruption of both joints by double alanine mutations at Env positions 671 and 674 (AA) results in attenuation of Env-mediated cell-cell fusion and hemifusion as well as viral infectivity mediated by both CD4-dependent and CD4-independent viruses. The potential mechanism of disruption was revealed by structural analysis of MPER conformational changes induced by AA mutation. A deeper acyl chain-buried MPER middle section and the elimination of cross-hinge rigid-body motion almost certainly impede requisite structural rearrangements during the fusion process, explaining the absence of MPER AA variants among all known naturally occurring HIV-1 viral sequences. Furthermore, those broadly neutralizing antibodies directed against the HIV-1 MPER, including 10E8, exploit the tandem joint architecture involving helix-capping, thereby disrupting hinge function.

In addition, the Irvine group’s work on phospholipid-enveloped biodegradable microparticles and nanoparticles includes multilamellar vesicles, polymer nanoparticles and stealth liposomes as vaccines displaying MPER segments on lipid. In collaborative efforts within the group, specific anti-MPER antibodies have been generated with broad specificity for clade B and C sequences. Improvement in affinity through more optimal T follicular helper cell elicitation, and other particle and immunogen tuning efforts, are ongoing to generate useful neutralizing antibodies. The impact of surface MPER density and adjuvant choice are key elements of this investigation that are now well studied. Activators of the STING pathway, such as cyclic dinucleotides, are being used to effectively enhance antibody titer ten-fold. By incorporating microengraving methods of the Love laboratory at MIT, individual bone marrow plasma cells elicited by such immunization can be interrogated and monoclonal antibodies rescued using single cell PCR cloning for repertoire analysis and characterization. In this regard, we recently demonstrated that several immunizations with MPER/liposomes induce high levels of bone marrow long-lived plasma cell (LLPC) antibody production. Single-cell immunoglobulin gene retrieval analysis shows that these plasma cells are derived from a germ line repertoire of B cells with a diverse representation of immunoglobulin genes, exhibiting antigen-driven positive selection. Characterization of LLPC recombinant monoclonal antibodies (rMAbs) indicates that antigen recognition is achieved through convergence on a common epitopic focus by utilizing various complementarity-determining region H3 (CDRH3) lengths. Importantly, the vast majority of rMAbs produced from these cells lack polyreactivity yet manifest antigen specificity in the context of lipids, shaping MPER-specific paratopes through selective pressure. Taken together, these findings demonstrate that the MPER is a vaccine target with minimal risk of generating

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Grant at a Glance

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Grantee Institution
Dana-Farber Cancer Institute, Boston, USA

Project Title
HIV-1 clade C MPER immunogens eliciting 10E8-like specificities

OPPID
1108179

Grant Award
Up to $2 Million, awarded in July, 2014

Collaborating Institutions
◊ Harvard University, USA
◊ Massachusetts Institute of Technology, USA

(Cont.)

off-target autoimmunity.

Inclusion of the TM segment of gp41 with the MPER in microparticle display and its impact on immunogenicity is a focus of intense interest for the Consortium. While structural characterization of epitope-paratope pairs has contributed to the understanding of antigenicity, by contrast, few structural studies relate to immunogenicity, the process of antigen-induced immune responses in vivo. Using a lipid-arrayed MPER as a model antigen, we investigated the influence of physicochemical properties on immunogenicity in relation to structural modifications of MPER/liposome vaccines. Anchoring the MPER to the membrane via an alkyl tail or transmembrane domain retained the MPER on liposomes in vivo, while pre-serving MPER secondary structure. However, structural modifications that affected MPER membrane orientation and antigenic residue accessibility strongly impacted induced antibody responses. The solvent exposed MPER tryptophan residue (W680) was immunodominant, focusing immune responses despite sequence variability elsewhere. Nonetheless, immunogenicity could be readily manipulated using site-directed mutagenesis or structural constraints to modulate amino acid surface display. These studies provide fundamental insights for future immunogen design aimed at targeting B cell antibody responses, including induction of bNabs.

Although we have generated antibodies with high specificity against the MPER that lack polyreactivity, no BNAbs emerged. Antibodies elicited to the MPER in liposomes do not react with gp160 trimers expressing the same MPER sequence as arrayed on the liposome vaccines, unlike 4E10, 2F5 or 10E8. This striking result emphasizes a limitation of the current immunization strategy, namely that antibody can vector onto the lipid bilayer to bind MPER in myriad orientations. However, on the virion, the MPER is largely occluded by the three outer blades of the trimer. To the contrary, 2F5, 4E10 and 10E8 all approach the MPER at a tilted/flat angle relative to the membrane We reason that there will be a much greater chance to generate BNAbs if we recapitulate this restricted approach angle. To achieve this goal, advanced nanodisc technologies developed in the Wagner lab are being exploited. We shall utilize the spacing between two opposing nanodiscs to restrict antibody accessibility. In these immunogen designs, we can leave the MPER flat or, alternatively, lift the N-helix of the MPER off the lipid nanodisc surface by attaching it to an opposing nanodisc via cysteine coupled thiol chemistry, further tunable by DNA double-stranded oligonucleotides to form an MPERTM embedded nanodisc wafer. The distance between the two opposing nanodiscs is adjusted by DNA pillar length to approximate the space between gp160 trimer blades and the viral membrane.