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Review

Immunogen design for HIV-1 and influenza ☆

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ABSTRACT

Vaccines provide the most cost effective defense against pathogens. Although vaccines have been designed for a number of viral diseases, a vaccine against HIV-1 still remains elusive. In contrast, while there are excellent influenza vaccines, these need to be changed every few years because of antigenic drift and shift. The recent discovery of a large number of broadly neutralizing antibodies (bNAbs) and structural characterization of the conserved epitopes targeted by them presents an opportunity for structure based HIV-1 and influenza A vaccine design. We discuss strategies to design immunogens either targeting a particular antigenic region or focusing on native structure stabilization. This article is part of a Special Issue entitled: Recent advances in molecular engineering of antibody.

1. Introduction

Effective vaccines exist for a number of viral diseases including Hepatitis B, polio, small pox, measles and rubella [1]. However, despite much effort there is no successful vaccine against Human immunodeficiency virus (HIV-1), and only strain specific vaccines against influenza are available which need to be updated annually to counter drifted viral strains in circulation. HIV-1 and influenza viruses are similar in a number of aspects: (i) Both are enveloped RNA viruses which attach to target cells through their envelope glycoproteins Env and HA respectively. (ii) Both viruses show exceptional sequence variation along with a number of other defense mechanisms to evade immune responses against them. They however, differ in terms of their infectivity and pathogenicity. HIV-1 virus shows a very low transmission probability per heterosexual act ranging from 0.0001 to 0.0032 for vaginal transmission [2,3] and infection takes on an average 9 years for progression to acquired immunodeficiency syndrome (AIDS) [4]. Despite its poor infectivity, HIV-1 has wreaked havoc world-wide, killing 25 million people. Currently ~34 million people are living with HIV-1 with approximately 2 million new infections occurring every year (UNAIDS, 2011).

In contrast, influenza virus is highly infectious but results in a self-limiting respiratory illness with a diverse array of symptoms including sudden onset of high fever, myalgia, non-productive cough, sore throat, and rhinitis, lasting one to two weeks in most individuals. However, among immunologically compromised individuals, the infection may lead to severe complications of underlying diseases, pneumonia and death [5]. Annual epidemics cause 250,000–500,000 deaths worldwide and pandemics remain a major public health threat (www.who.int). As traditional vaccine approaches fail to contain these pathogens effectively, new approaches are required to elicit effective protective responses against them.

2. HIV-1: An overview

HIV-1 is a member of *retroviridae* family from *lentivirus* genus with two copies of positive single stranded RNA as its genome [6]. HIV-1 has been phylogenetically classified into four groups: M, N, O and P, which may represent different cross-transmission events from non-human primates to humans [7,8]. Group M is responsible for ~97% of AIDS cases worldwide and is further categorized into nine different phylogenetic lineages or subtypes of which subtype A, B, C and D are most common. Subtype A and D are mainly found in Africa. Subtype B is common in North America and Europe whereas subtype C is largely found in Asia and Africa. In addition, superinfection to an infected host cell creates numerous circulating recombinant forms (CRF). Viral strains can vary up to 20% within a subtype whereas across subtypes, diversity can be as high as 38% [9–11]. Natural humoral immunity elicits largely strain specific and non-neutralizing antibody responses in most HIV-1 infected individuals [12–15]. The HIV-1 virus primarily infects host immune cells called CD4+ T cells. Envelope glycoprotein (Env) on the

Abbreviations: HIV-1, human immunodeficiency virus-1; Env, envelope; PNGS, potential N-linked glycosylation sites; bNAb, broadly neutralizing antibody; GL, germline; T/F, Transmitted/Founder virus; MPER, membrane proximal external region; OD, Outer domain; CD4bs, CD4 binding site; HA, Haemagglutinin

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HIV-1 virion surface confers tropism and binds to the CD4 receptor on CD4 + T cells. Binding of Env to the CD4 receptor triggers a series of conformational changes which lead to the exposure of co-receptor binding sites on Env. Subsequent binding of Env to the co-receptor (CXCR4 or CCR5) on T-cells initiates fusion of cellular and viral membranes. HIV-1 Env is synthesized as a gp160 precursor protein. During transport of gp160 to the cell surface, it is cleaved into the surface exposed gp120 and membrane anchored gp41 subunits (Fig. 1). The HIV-1 virion has relatively few (10–12) Env molecules on its surface [16,17]. Native, cleaved HIV-1 Env is a trimer of surface exposed gp120 and transmembrane gp41 heterodimers (Fig. 1) [18,19]. gp120 is the primary target for HIV-1 vaccine design as it is largely exposed on the virion surface, contains the binding sites for primary receptor (CD4) and co-receptors (CCR4/CXCR5) [20–23] and antibody response in an HIV-1 infected individual is largely directed against gp120 (Fig. 1C). HIV-1 poses a unique challenge for vaccine design. The primary reason for the difficulty in generating an effective neutralizing antibody response against HIV-1 lies in extensive Env sequence variability [24,25]. Env diversity observed in an HIV-1 infected individual six years post sero-conversion is comparable to the global variability of influenza hemagglutinin sequence for a given year [26]. Given the fact that influenza requires a new vaccine practically every year, it seems like an impossible task to have an HIV-1 vaccine which can elicit antibodies that simultaneously neutralizes viruses from all the different HIV-1 clades. Apart from sequence variation, HIV-1 deploys a number of defense mechanisms to evade the immune response against it. gp120 is one of the most heavily glycosylated viral proteins known with ~50% of its molecular mass contributed by glycans [27]. gp120 contains ~24 potential N-linked glycosylation sites (PNGS) as compared to ~6 PNGS on influenza HA [27–29]. This extensive glycosylation of gp120 helps to mask critical neutralization epitopes, although some bNAbs target glycan dependent epitopes to achieve virus neutralization [30,31]. In addition, the presence of long, variable immunodominant loops focuses the immune response away from conserved epitopes [32]. Certain conserved epitopes are cryptic and are exposed only after gp120 binds to CD4 [33]. Moreover, the highly flexible nature of gp120 and the lability of the gp120:gp41 complex make structural characterization extremely difficult [21,34]. Shed gp120 samples various non-native conformations that are likely to be absent in native Env and that elicit non-neutralizing antibodies. Despite these defense mechanisms, about 20–30% of HIV-1 patients do generate a broad neutralization response [35] and around 1% of patients dubbed as elite neutralizers, do so with high potency [36]. A large number of broadly neutralizing antibodies (bNAbs) against HIV-1 have been discovered in the last decade [37]. There are four major epitopes on the Env surface targeted by bNAbs: (i) CD4 binding site (CD4bs) for bNAbs such as b12 [38], VRC01 [39], NIH-45-46 [40], PGV04 [41], 3BNC117, 8ANC131 [40], HJ16 [42] and CH103 [43] (ii) V1/V2 loop bNAbs such as PG9/PG16 [44], PGT 145 [45], CH01-04 [46] (iii) V3 loop bNAbs such as PGT 121 and PGT 128 [45] (iv) gp41 MPER bNAbs 2F5 [47], 4E10 [48] and 10E8 [49] (Fig. 2). The very existence of such bNAbs shows that it is possible to elicit a neutralizing response against HIV-1 if conserved epitopes are targeted properly. Moreover, the passive transfer of these antibodies protects animals from challenge with HIV-1 virus [50–52]. Although these bNAbs and their epitopes have been identified, eliciting similar bNAbs through immunization is challenging. The CD4bs is discontinuous and the various conserved segments are interspersed with non-conserved ones. The epitopes in the V1/V2 and V3 loop regions are all glycan dependent. Eliciting antibodies that target specific glycans is difficult, both because of the large number of glycans on gp120 and because glycans are recognized as self-molecules by the immune system. The gp41 MPER epitopes are difficult to target because of their close proximity to the membrane. Some bNAbs targeting these epitopes have been shown to be polyreactive [53]. Another difficulty in HIV-1 vaccine research is that there are no animal models available except non-human primates to test the efficacy of an immunogen against HIV-1 pathogenic challenge. Typically, the efficacy of a HIV-1 immunogen is tested by in vitro measurement of the virus neutralization potential of sera elicited from immunized animals using a TZM-bl assay. TZM-bl cells express the CD4 receptor and the CCR5/CXCR4 coreceptors [54] and contain Tat-regulated reporter genes for firefly Luciferase and *Escherichia coli* β-galactosidase under regulatory control of an HIV-1 long terminal repeat sequence [55]. In the TZM-bl assay, virus neutralization is measured as a function of reduction in Tat-induced reporter gene expression (Luciferase) after a single round of viral infection [56]. The EC_{50} value (50% reduction in virus mediated luciferase activity) determined using the TZM-bl assay correlates well with the serum-neutralizing titers required to provide passive protection from SHIV challenge in macaques [57]. Hence, neutralization observed in a TZM-bl assay is a useful tool for assessing a vaccine candidate.

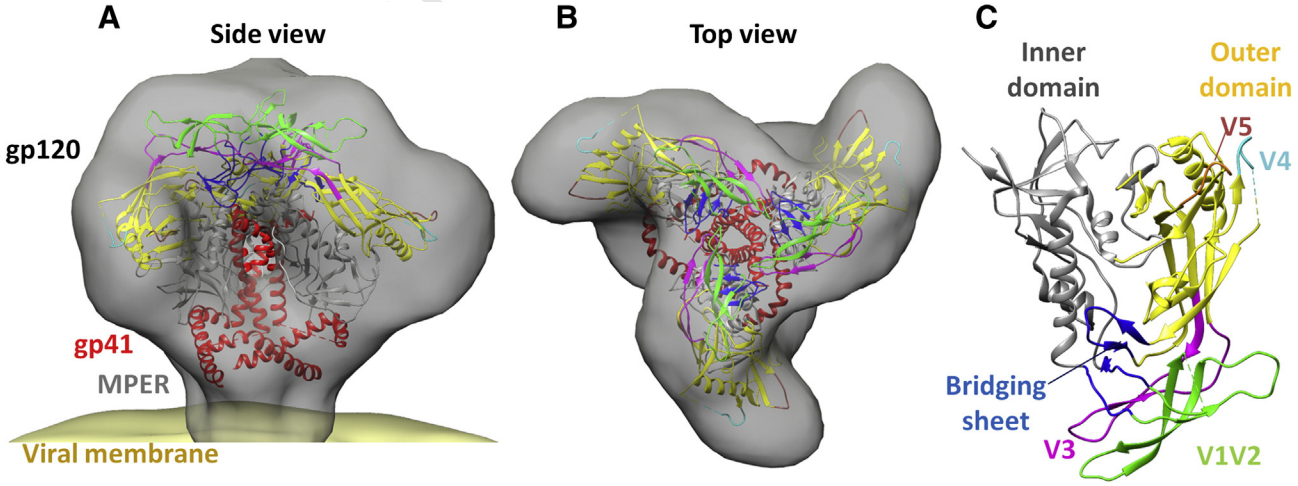

Fig. 1. Structural organization of HIV-1 envelope spike. HIV-1 envelope is a trimer of surface exposed gp120 (multi-colored) and membrane spanning gp41 (red) heterodimers. HIV-1 unliganded envelope spike (A) side view and (B) top view show cryo-EM density map (gray, EMD ID:5022 [18]) fitted with X-ray coordinates from gp120–gp41 trimeric structure (PDB ID: 4NC0 [58]). V1/V2 (green) and V3 (magenta) loops are located towards the apex of the viral spike whereas gp41 (red) has its C-terminal region proximal to the viral membrane. (C) Cartoon representation of HIV-1 gp120 monomer (PDB ID: 4NC0 [58]). gp120 can be broadly subdivided into outer domain (yellow), inner domain (gray), bridging sheet (blue), V1/V2 (green),V3 (magenta), V4 (cyan) and V5 (brown) loop. Outer domain (yellow) contains receptor binding sites and is the major target for a large number of bNAbs. Inner domain (gray) is largely unexposed on a native trimer, however it is accessible on an isolated monomer and thus exposes non-neutralizing epitopes. The figures were generated using Chimera [59].

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