Published in final edited form as:

Nature. 2014 October 23; 514(7523): 437–438. doi:10.1038/nature13926.

A stamp on the envelope

ROGIER W. SANDERS and **JOHN P. MOORE**

and **John P. Moore** are in the Department of Microbiology and Immunology, Weill Medical College, Cornell University, New York, New York 10065, USA. **R.W.S.** is also in the Department of Medical Microbiology, Academic Medical Centre, University of Amsterdam, 1105 AZ Amsterdam, the Netherlands. e-mails: r.w.sanders@amc.uva.nl; jpm2003@med.cornell.edu

Abstract

A high-resolution crystal structure of the HIV-1 Env trimer proteins, in their form before they fuse with target cells, will aid the design of vaccines that elicit protective immune responses to this protein complex.

The surface of the HIV-1 virus is studded with envelope glycoprotein (Env) spikes. The virus uses these trimeric complexes, which each contain three gp120 and three gp41 subunits, to fuse with cells and initiate infection. Sixteen years ago, Kwong and colleagues described the crystal structure of the core of the gp120 subunit¹, but the lability of the complex in its pre-fusion form meant that the trimer structure was not determined until last year, when an engineered, stabilized and soluble version was used to produce highly concordant structures by X-ray crystallography² and cryo-electron microscopy³. Now, on page 455 of this issue, Kwong and colleagues (Pancera *et al.*)⁴ report a crystal structure of the same Env trimer at higher resolution, providing a better picture, particularly of the gp41 subunits. Together, these structures^{2–4} help us to understand how the Env trimer functions, how antibodies recognize it (or not), and how vaccines exploiting this protein can be better designed.

HIV-1 fusion occurs when the gp120 components of Env trimers interact first with CD4 receptors on a cell's surface and then with a co-receptor (CCR5 or CXCR4). The sequential receptor engagements drive the concerted disentanglement of the intimate, but fragile, embrace between gp120 and gp41. The ectodomain of each gp41 subunit (the region that extends out from the viral membrane) contains six segments (A–F) that form two heptad-repeat regions (HR1 and HR2). These segments eventually become two long helices in the post-fusion structure, which is known as the six-helical bundle. Pancera and colleagues' pre-fusion structure shows that HR1 and HR2 are each split up into two smaller helices connected by loops; together, the four helices form a ring encircling the amino and carboxy termini of gp120 (Fig. 1). In turn, these gp120 regions act as a 'safety pin' to prevent gp41 from transiting to the energetically more favoured six-helical-bundle form.

The authors use their structure to make inferences about the conformational changes in Env proteins that take place during fusion, adding detail to the existing model of the process (Fig. 1b). When the cellular receptors are engaged, the safety pin is removed in a two-stage process. First, the top of the trimer opens up. The diminished constraints on the N-terminal segments of gp41 and the space vacated at the trimer axis allow segment B to undergo a loop-to-helix transition. The formation of the resulting long helix (HR1), now containing

segments A, B and C, punches the hydrophobic fusion peptide at the N terminus of gp41 into the target-cell membrane. The concomitant removal of one component of the four-helix ring weakens the links between gp120 and gp41. Co-receptor binding then fully removes the safety pin. The gp120 subunit probably now completely dissociates, eliminating any remaining steric constraints on the melding of the viral and cell membranes. In gp41, segments D–F extend to form the second long helix (HR2), and the formation of the six-helical bundle provides sufficient energy to fuse the membranes. Once enough individual trimers (probably around five⁵) have undergone these transitions, the resulting fusion pore in the cell membrane allows the viral core to enter the cell.

The human immune system can prevent HIV-1 from infecting cells by generating neutralizing antibodies that bind to various regions (epitopes) on the pre-fusion Env trimer. But the virus has evolved defences to evade both the generation and the binding of neutralizing antibodies. The most relevant are a dense array of protective glycans, which are less immunogenic than protein surfaces, and the ability to vary the trimer's amino-acid composition. Pancera and colleagues' structure displays just how efficient these obstacles are: one stunning image (see Fig. 6 in the Extended Data) shows the comprehensiveness of the shielding effect of its glycans compared with analogous proteins from influenza virus and respiratory syncytial virus.

Even so, humans can produce antibodies that have extremely broad reactivity against circulating HIV-1 variants. Most known epitopes targeted by such broadly neutralizing antibodies (many of these epitopes include the otherwise protective glycans) are present on the engineered BG505 SOSIP.664 trimers used to generate the existing and new Env structures^{2–4,6}. Because inducing such antibodies is a major goal of HIV-1 vaccine development, does this mean that the problem is solved? Unfortunately, no — or, at least, not yet.

There are two main elements in the development of vaccines based on Env: making immunogenic proteins with epitopes that could elicit broadly neutralizing antibodies, and forcing the immune system to respond to them. Current trimer-based immunogens, termed gp140s, are rendered soluble for vaccine development by deleting the transmembrane region of gp41 (ref. 7). However, doing so creates a loose end at the C terminus, which may destabilize inter-subunit interactions^{2–4}. For most gp140s, the cleavage site between gp120 and the gp41 ectodomain is deliberately eliminated to 'stabilize' the trimer⁷. However, that stability is illusory, because these 'uncleaved' gp140s predominantly adopt configurations in which semi-dissociated gp120 subunits dangle loosely from the gp41 six-helical bundle^{8–10}. The outcome is reminiscent of the conformational changes outlined above: the dissociation of gp120 from the gp41 ectodomain and the latter's transition to the six-helical bundle post-fusion conformation.

These stability problems, and the resulting loss of epitopes that elicit broadly neutralizing antibodies, are overcome in the BG505 SOSIP.664 trimers through the introduction of an amino-acid substitution (I559P)¹¹ that prevents the loop-to-helix transition in segment B and of an engineered disulphide bond between cysteine residues 501 and 605 (ref. 12) that pins the gp120 subunits to the four-helix ring of gp41 (Fig. 1). Cleavage of the trimers is

also promoted, which seems to strengthen the association between gp120 and gp41 (ref. 9). Together, these engineered changes maintain the soluble trimers in the pre-fusion conformation and preserve key epitopes for eliciting neutralizing antibodies^{2–4,6}.

The BG505 SOSIP.664 trimers, which are now vaccine candidates for human trials, are encouragingly immunogenic in animals, provoking a strong and consistent neutralizing-antibody response to the BG505 virus, a neutralization-resistant strain of HIV-1. But they do not elicit broadly neutralizing antibodies. Future strategies to increase the breadth of the antibody response may involve reverse-engineering the immunogens on the basis of antibody evolution¹³ and devising different ways of presenting them to the immune system, for example as particulate antigens¹⁴. Nowadays, structure-guided immunogen design is the best route to an effective vaccine¹⁵, and the new structural data will undoubtedly facilitate such improvements. By again placing their stamp on the envelope, the Kwong group has posted a frank warning to the virus.

References

1. Kwong PD, et al. *Nature*. 1998; 393:648–659. [PubMed: 9641677]
2. Julien J-P, et al. *Science*. 2013; 342:1477–1483. [PubMed: 24179159]
3. Lyumkis D, et al. *Science*. 2013; 342:1484–1490. [PubMed: 24179160]
4. Pancera M, et al. *Nature*. 2014; 514:455–461. [PubMed: 25296255]
5. Klasse PJ. *Virology*. 2007; 369:245–262. [PubMed: 17825343]
6. Sanders RW, et al. *PLoS Pathog*. 2013; 9:e1003618. [PubMed: 24068931]
7. Forsell MN, Schief WR, Wyatt RT. *Curr Opin HIV AIDS*. 2009; 4:380–387. [PubMed: 20048701]
8. Guttman M, Lee KK. *J Virol*. 2013; 87:11462–11475. [PubMed: 23966389]
9. Ringe RP, et al. *Proc Natl Acad Sci USA*. 2013; 110:18256–18261. [PubMed: 24145402]
10. Tran K, et al. *Proc Natl Acad Sci USA*. 2014; 111:E738–E747. [PubMed: 24550318]
11. Sanders RW, et al. *J Virol*. 2002; 76:8875–8889. [PubMed: 12163607]
12. Binley JM, et al. *J Virol*. 2000; 74:627–643. [PubMed: 10623724]
13. Medina-Ramírez M, Sanders RW, Klasse PJ. *Expert Rev Vaccines*. 2014; 13:449–452. [PubMed: 24606603]
14. Schiller J, Chackerian B. *PLoS Pathog*. 2014; 10:e1004254. [PubMed: 25101974]
15. McLellan JS, et al. *Science*. 2013; 342:592–598. [PubMed: 24179220]

Figure 1 |. A model of HIV-1 fusion to target cells.

HIV-1 Env proteins are trimers of three identical protomers, each with a gp120 and a gp41 subunit. The gp41 subunit comprises cytoplasmic (CT) and transmembrane (TM) domains, and an ectodomain that has six helix-forming segments (A–F), a fusion peptide (FP), a disulphide loop (DSL) and a membrane proximal external region (MPER). **a**, Pancera and colleagues' trimer structure⁴ (a single gp140 protomer is shown) contains the gp120 subunit (green) and most of the ectodomain of the gp41 subunit (orange and purple), but omits other gp41 domains. The cysteine amino-acid residues (501C–605C) forming the engineered disulphide bond¹² in the trimer are indicated. **b**, The structure, together with previous data, helps to build a model of viral fusion to target cells. In the pre-fusion protomer, helix segments A and C, and D and F, are interspersed by loop segments B and E, respectively. On binding to cell-surface receptors, a long helix comprising segments A, B and C forms, punching FP into the host-cell membrane. (The approximate location of the I559P amino-acid substitution, which blocks the loop-to-helix transition in segment B of engineered trimers¹¹ and thereby stabilizes the pre-fusion structure, is indicated.) A second long helix of segments D, E and F then forms and aligns with the other helix in a hairpin structure. The formation of the trimer of hairpins (called the six-helical bundle) pulls the viral and target-cell membranes together.