doi:10.1038/nature14205

HIV

Tied down by its own receptor

An engineered protein that binds to the envelope of HIV viruses protects monkeys against infection with a simian–human virus that causes AIDS. This gene-therapy approach might provide an alternative to elusive HIV vaccines.

NANCY L. HAIGWOOD

The past 30 years have been marked by a long and discouraging search for an effective HIV vaccine. In 2009, the 'Thai trial' of the candidate vaccine RV144 was the first to demonstrate any success, measuring a 31.2% reduction in the rate of infection, although efficacy decreased over the first year after vaccination[1]. The difficulty in developing a more effective vaccine has forced investigators to explore problems that are posed by other intractable pathogens, including persistence in the host, a high degree of variability of certain regions, masking of common regions, and pathogen-induced inhibition of host immunity. But in a paper published on *Nature*'s website today, Gardner *et al.*[2] describe research suggesting that protection against HIV infection may be achievable through a gene-therapy approach, rather than by relying on eliciting protective immune responses by vaccination.

The trimeric envelope protein that is found on the surface of the viral particle of nearly all HIV strains binds directly to the CD4 receptor protein on the surface of many human immune cells, such as T cells and macrophages. This binding event causes a major shift in the envelope conformation, allowing the virus to bind to other co-receptors and enter the cell. It has been known since 1984 that CD4 is the receptor for HIV[3,4], and various forms of stabilized CD4 tethered to human immunoglobulin molecules (CD4–Ig) have been proposed and tested as potential therapeutics — the idea was that viral binding to these constructs would 'neutralize' the virus by preventing it binding to and entering cells. But this approach failed. Gardner and colleagues' findings provide the first logical explanation for this failure, and suggest an elegant way of using human CD4 derivatives to prevent infection.

The researchers engineered CD4 by fusing it with a mimetic of the amino terminus of CCR5, the host-cell co-receptor used by most HIV-1 strains during infection and disease progression. The CCR5 terminus has two sulfated tyrosine amino-acid residues that bind to the HIV envelope and facilitate viral entry[5], so the peptide mimetic is a sulfopeptide. The mimetic was based on an antibody that binds to the CCR5 binding site of the viral envelope; the authors modified and positioned it in the CD4–Ig construct for maximum activity and fit.

This synthetic compound, named eCD4–Ig, has potent and broad neutralizing activity against all HIV isolates tested, including viral strains that are typically thought of as highly resistant to neutralization. It achieved these effects at lower concentrations than required when using the neutralizing monoclonal antibodies (NmAbs) that arise during the immune responses of some patients to HIV, and which are currently a major focus of attempts to develop HIV vaccines that prevent infection, rather than modulate viraemia once infection has occurred[6]. Furthermore, the construct was more effective than previous CD4–Ig constructs or the NmAb b12 at inducing immune killing of HIV-infected cells — a process known as antibody-dependent cellular cytotoxicity, which functions in concert with viral neutralization.

Gardner *et al.* went on to show that the eCD4–Ig construct imparted resistance to HIV-1 when infused into mice that model human HIV infections. As a further test of *in vivo* activity, the authors treated monkeys with an adenovirus-associated virus (AAV) that expressed the gene encoding a rhesus macaque version of the eCD4–Ig construct and with a separate AAV vector expressing a rhesus macaque enzyme to promote efficient sulfation. This gene-therapy vector allows continuous expression of the desired proteins in host cells by integrating into the host genome.

The animals expressed the transgene stably, although at different levels, and all were fully protected against repeated challenge with increasing doses of SHIV (a virus combining parts of the simian immunodeficiency virus (SIV) and HIV genomes). This protection was sustained for as long as 34 weeks after AAV transduction, and was achieved despite the monkeys receiving the virus intravenously, which is considered the infection route that provides the most stringent test of protection. These findings improve on an earlier test of the AAV transduction system to express a NmAb specific for SIV in monkeys[7], in which only a subset of monkeys that expressed the transgene were protected from SIV challenge.

Why did Gardner and colleagues' construct work? In a nutshell, it all comes down to the way that eCD4–Ig binds to the virus (Fig. 1). Human NmAbs that are able to neutralize a broad range of HIV-1 strains do so by

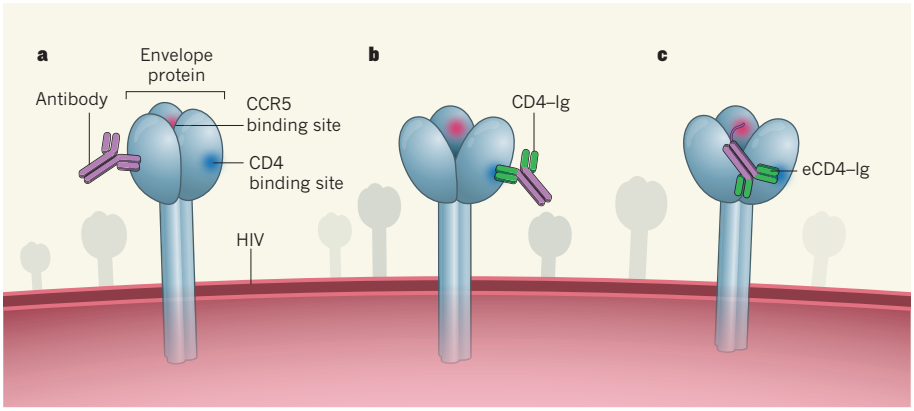

Figure 1 | Vaccination versus gene therapy. a, HIV infection begins with the virus's envelope protein binding to CD4 and CCR5 molecules on the surface of T cells. Most current strategies aimed at conferring protection against HIV focus on vaccines that are designed to prevent this binding by producing antibodies that bind to structures shared by the envelope of many HIV strains. However, antibodies bind only to small sections of the envelope, and the virus can evolve to shield these regions from antibody binding. **b**, An alternative approach proposed to stop virus binding to host cells is to use artificial constructs of human CD4 attached to immunoglobulin molecules. These CD4–Ig constructs will bind many viral strains, but they may expose the CCR5 binding site on the envelope protein and thus actually facilitate binding of the virus to CCR5 on the host cell. **c**, Gardner *et al.*[2] present an alternative construct, eCD4–Ig, which contains both CD4 and a mimetic of CCR5 and therefore blocks both points of viral binding.

binding with very high affinity to shared viral structures (epitopes) that have precise but relatively small footprints. However, HIV has a variety of tricks to shield these shared epitopes from the immune system, although some infected individuals — referred to as elite neutralizers — do produce NmAbs of this sort. By contrast, CD4 binds to the envelope of all HIV-1 strains, albeit at lower affinity than these 'super potent' NmAbs. However, CD4 binding leads to a conformational change in the envelope that exposes the CCR5 binding site, thus potentially promoting HIV-1 infection in CCR5-expressing cells[8]. The modifications introduced by Gardner *et al.* into their eCD4–Ig construct seem to overcome this problem by preventing the engagement of envelope proteins with CCR5, while at the same time engaging multiple parts of the viral envelope, thereby increasing the binding power of their construct.

The study raises several questions and a few caveats. First, the modified protein is not natural and required the co-expression of an enzyme to perform the efficient addition of the sulfate moiety onto tyrosine residues. Second, the sample size of the monkey studies was quite small, and larger experiments in non-human primates are warranted. Furthermore, the intravenous challenge route, although rigorous, is not representative of the vast number of HIV-1 exposures worldwide, and it remains to be seen how expression of eCD4–Ig would affect virus challenges at mucosal sites, which better mimic natural routes of infection. It is also not yet clear whether the construct needs to be expressed close to the challenge sites. This, too, could be tested in non-human primate models.

Another major question rests in understanding the safety of eCD4–Ig in humans. Immune responses against the protein were elicited in the monkeys, albeit less strongly than against human NmAbs, and such responses could undermine its efficacy. But perhaps the greatest caveat to clinical application of the construct is how it, or future derivatives, will be used in humans. Such a complex molecule is unlikely to be administered repeatedly to those at risk of HIV infection, although that might be considered if it could be applied topically. The risks of expressing the construct as a transgene, in a similar manner to Gardner and colleagues' monkey experiments, are not known, and this approach would require careful and stepwise clinical safety testing. However, in the absence of a vaccine that can elicit broadly protective immunity and prevent infection, and given the lack of major breakthroughs on the horizon to provide one, the idea of conferring potent, sustained vaccine-like protection against HIV infection through gene therapy is certainly worth strong consideration. ■

Nancy L. Haigwood *is in the Division of Pathobiology & Immunology, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, Oregon 97006, USA.*
e-mail: haigwoon@ohsu.edu

1. Rerks-Ngarm, S. *et al. N. Engl. J. Med.* **361,** 2209–2220 (2009).
2. Gardner, M. R. *et al. Nature* http://dx.doi.org/10.1038/nature14264 (2015).
3. Dalgleish, A. G. *et al. Nature* **312,** 763–767 (1984).
4. Klatzmann, D. *et al. Nature* **312,** 767–768 (1984).
5. Farzan, M. *et al. Cell* **96,** 667–676 (1999).
6. Hansen, S. G. *et al. Nature* **502,** 100–104 (2013).
7. Johnson, P. R. *et al. Nature Med.* **15,** 901–906 (2009).
8. Hoxie, J. A. *Annu. Rev. Med.* **61,** 135–152 (2010).