Neutralizing antibodies take a swipe at HIV *in vivo*

David C Montefiori

Developing an effective AIDS vaccine may hinge on understanding how to elicit antibodies that recognize and disable the HIV virus—but first, experiments in people must show that such antibodies are even useful in controlling infection. Information from a recent human trial provides hope that controlling the virus by neutralizing antibodies might be possible. And another study examines why the antibodies are so difficult to elicit (pages 615–622).

Neutralizing antibodies provide a major line of defense against many pathogens and are considered highly important for HIV-1 vaccines. What drives this notion for HIV-1 vaccines is the finding in multiple passive-transfer experiments that preexisting neutralizing antibodies can prevent AIDS virus infection in Rhesus macaques¹. Most vaccines, however, do not prevent the acquisition of infection; rather, they program the immune system for a rapid response that eliminates the pathogen before it can harm the host.

In the case of HIV-1 infection, genetic integration and early seeding of latent viral reservoirs make it highly unlikely that the virus can be eliminated, no matter how potent the immune response. This raises a fundamental question: can immune priming by vaccines that do not prevent the acquisition of HIV-1 infection still be effective in halting the spread of AIDS? In principle, vaccines that prime for rapid and potent immune-mediated suppression of HIV-1 could delay or prevent disease and make it less likely that an infected person will transmit virus². Thus, proof of this principle for neutralizing antibodies would add clarity to the goals for vaccination and lend support for antibody-based immunotherapy. Initial proof would come from knowing whether antibodies that neutralize HIV-1 *in vitro* can suppress the virus in infected individuals. Although this is often assumed to be the case, direct evidence has been lacking.

Figure 1 Passive monoclonal antibody treatment after ART cessation in HIV-1-infected individuals. Eight chronically and six acutely HIV-1-infected subjects, all of whom had undetectable levels of plasma viremia while on ART, received multiple infusions of a mixture of three neutralizing monoclonal antibodies over an 11-week period. All subjects harbored viruses that were highly sensitive

to at least two monoclonal antibodies *in vitro* before ART. Viral rebound in six chronic and two acute subjects rose to pre-ART levels in the presence of high concentration of neutralizing antibodies without any apparent delay (nonresponders, red). Viral rebound in another six subjects was judged to be delayed (responders); after the delay, viral rebound in two of these latter subjects (one chronic, one acute) rose to pre-ART levels in the presence of high plasma concentrations of monoclonal antibodies (purple). In two others (both acute), viral rebound peaked below pre-ART levels (2–4 logs) and remained low after the monoclonal antibodies cleared peripheral circulation (green).

A report by Trkola *et al.*³ in this issue provides the first piece of direct evidence. They show that a triple combination of neutralizing monoclonal antibodies given in high doses can delay viral rebound after cessation of antiretroviral treatment (ART). In a separate report by Haynes *et al.*⁴, two of these monoclonal antibodies are discovered to be autoreactive, possibly explaining why some key epitopes of the virus are poorly immunogenic.

Neutralizing antibodies are detected in nearly every individual infected with HIV-1; however, any potential effect these antibodies might have on the virus is soon thwarted by the emergence of escape variants⁵. HIV-1 has found multiple ways to escape neutralizing antibodies by making strategic altera-

tions in the sequence and structure of its surface gp120 and transmembrane gp41 envelope glycoproteins^{6,7}.

This enormous capacity of HIV-1 to diversify poses a difficult challenge for vaccine development. In particular, immunogens that elicit broadly cross-reactive neutralizing antibodies remain elusive^{8,9}. To solve this problem, much attention has focused on a small number of human monoclonal antibodies that have an unusual ability to neutralize many different strains of HIV-1 (refs. 10,11). These antibodies, and the conserved epitopes they recognize, are of considerable interest for passive immunotherapy and for new vaccine immunogens that aim to elicit an effective neutralizing antibody response.

The author is in the Department of Surgery, Laboratory for AIDS Vaccine Research and Development, Duke University Medical Center, Durham, North Carolina 27710, USA.
e-mail: monte@acpub.duke.edu

Trkola *et al.* studied three of these monoclonal antibodies. One monoclonal antibody, 2G12, is thought to recognize a cluster of sugar moieties on some of the approximately 25 asparagine-linked glycans that decorate the surface of gp120 (ref. 12). The two additional monoclonal antibodies, 2F5 and 4E10, bind peptide epitopes that lie adjacent to one another and in close proximity to the viral membrane on the ectodomain of gp41 (ref. 13). In principle, the combination of these three monoclonal antibodies could prove quite powerful in controlling the virus and impeding escape, much the same as when multiple classes of antiretroviral drugs are combined to treat HIV-1 infection.

To test this, the authors conducted a small clinical trial with eight chronically and six acutely HIV-1-infected individuals in whom plasma viremia was suppressed to undetectable levels by ART. Subjects were carefully selected on the basis of harboring viruses that were highly sensitive to neutralization *in vitro* by at least two and, in most cases, all three monoclonal antibodies before ART. Thus, these subjects were prime candidates to respond to antibody treatment. ART was stopped one day after commencing an 11-week course of treatment with the triple combination of monoclonal antibodies. Kinetics of viral rebound and emergence of neutralization-escape variants were monitored over a 24-week period to determine whether the antibody treatment had an effect on the virus.

Antibody treatment was not able to prevent viral rebound in a majority of cases; however, one subject had no detectable viral rebound, whereas another five subjects (two chronic, three acute) were judged as having a delayed rebound, suggesting that antibody treatment was partially effective in some individuals (Fig. 1).

What is surprising is that most of the effect of antibody treatment could be attributed to a single monoclonal antibody—2G12. Patients who experienced a delay in viral rebound had higher effective plasma concentrations of 2G12 (based on *in vitro* potency against pre-ART virus) compared to those who did not control their virus. Moreover, 2G12 was the only monoclonal antibody that rebound viruses escaped. The finding that rebound viruses remained highly sensitive to 2F5 and 4E10 suggests that either the plasma concentrations of these two monoclonal antibodies were too low to be effective or there is something fundamentally different about the neutralizing activity of these two monoclonal antibodies *in vitro* and their ability to affect HIV-1 *in vivo*.

So was this in fact an effect of a single antibody? Anecdotal, the longer plasma half-life of 2G12 permitted much higher concentrations of this monoclonal antibody to be achieved *in vivo* compared to 2F5 and 4E10. Specifically, plasma

levels of 2G12 in responders were an average of 394-fold higher than the *in vitro* 90% inhibitory dose. Closer inspection reveals that the mean effective dose of 2G12 achieved in responders was more than double the highest effective dose achieved for 2F5 and 4E10 in any single subject. This raises the possibility that plasma concentrations achieved for 2F5 and 4E10 were below a crucial threshold needed to control the virus.

This threshold might have been exceeded in two subjects in whom the most potent and sustained control of plasma viremia was observed. Effective concentrations of 4E10 in these subjects were higher than in all other subjects in the trial (plasma levels 150 and 152 times higher than the *in vitro* ID₉₀). Moreover, one of these subjects harbored a 2G12-resistant virus before ART and at the end of the trial, making it unlikely that this person would respond to 2G12. Of course, it is difficult to know whether these two acute subjects controlled their virus spontaneously, regardless of antibody treatment. Additional studies in larger numbers of individuals and with higher doses of the monoclonal antibodies will be needed to gain a more definitive answer regarding the antiviral effect of 2F5 and 4E10 in HIV-1-infected individuals.

Overall, these three monoclonal antibodies at the doses tested were no substitute for conventional ART in achieving potent and sustained control of HIV-1. Nevertheless, the combined results provide the first direct evidence that neutralizing antibodies can suppress HIV-1 in infected individuals. Moreover, they reinforce the widely held view that escape is readily achieved by the virus. In fact, escape might be achieved more rapidly than previously recognized.

It must also be kept in mind that these findings appear to be based on a single, rare monoclonal antibody that has an unusual specificity for sugar moieties. It remains to be seen whether other neutralizing antibodies, including true polyspecific antibody combinations, will have similar or greater effects, and if the magnitude of these effects will prove efficacious for vaccines that do not prevent the acquisition of HIV-1 infection. The outcome of the study by Trkola *et al.* offers hope that they will; however, antibody titers may need to be quite high.

In addition to the need for high titers, there can be little doubt that neutralizing antibodies will need to target a broad spectrum of viral variants to be effective for vaccination. The three broadly neutralizing human monoclonal antibodies studied here, plus a small number of additional human monoclonal antibodies with similar activity¹¹, are good examples of the types of antibodies vaccines should aim to elicit. Paradoxically, these antibodies are rarely detected in HIV-1-infected individuals. Moreover, all attempts to elicit these antibodies with candidate vaccine immunogens

have failed. Why these epitopes are such poor immunogens has long been a mystery. The recent discovery by Haynes *et al.*⁴ that 2F5 and 4E10 are autoreactive may begin to provide answers.

Previous studies^{14,15} showed that 2F5 and 4E10 possess unusually long, hydrophobic third complementarity-determining regions (CDR3)—structures that are found primarily in early developing B cells and are typical of natural polyspecific autoantibodies. Haynes *et al.* screened a panel of 33 HIV-1 envelope-specific monoclonal antibodies for reactivity with a series of autoantigens, and found 2F5 and 4E10 had multiple reactivities, most notably with cardiolipin. Another broadly neutralizing monoclonal antibody, IgG1b12, reacted with double-stranded DNA and several nuclear proteins, whereas 2G12 was nonautoreactive.

These are provocative findings that shed light on yet another mechanism that may be used by HIV-1 to evade the host immune response: antigenic mimicry. The autoreactive nature of 2F5 and 4E10 also suggests ways of improving the immunogenicity of their cognate epitopes. Normally, autoreactive B-cell clones are either deleted or made tolerant early in development to prevent the production of potentially harmful, self-recognizing antibodies. New adjuvants and immunogens might be discovered that can circumvent these pathways and allow full maturation into high affinity antibody-producing plasma B cells. Consequences for safety will be a key issue; however, at least for 2G12, 2F5 and 4E10, no adverse side effects have been observed in humans^{3,16}.

These two studies, although dealing with separate issues, converge at the intersection of immunogen design and vaccination goals. The findings promise to stimulate fresh new ideas in a field that has seen little success despite nearly two decades of intense effort to discover immunogens that elicit an effective neutralizing antibody response against HIV-1.

1. Mascola, J.R. *Curr. Molec. Med.* **3**, 211–218 (2003).
2. Quinn, T.C. *et al. N. Engl. J. Med.* **342**, 921–929 (2000).
3. Trkola, A. *et al. Nat. Med.* **11**, 615–622 (2005).
4. Haynes, B.F. *et al.* Published online 28 April 2005 (doi:10.1126/science.1111781).
5. Richman, D.D. *et al. Proc. Natl. Acad. Sci. USA* **100**, 4144–4149 (2003).
6. Wei, X. *et al. Nature* **422**, 307–312 (2003).
7. Kwong, P.D. *et al. Nature* **420**, 678–682 (2002).
8. Mascola, J.R. *et al. J. Infect. Dis.* **173**, 340–348 (1996).
9. Bures, R. *et al. AIDS Res. Hum. Retroviruses* **16**, 2019–2035 (2000).
10. Binley, J.M. *et al. J. Virol.* **78**, 13232–13252 (2004).
11. Burton, D.R. *et al. Nat. Immunol.* **5**, 233–236 (2004).
12. Scanlan, C.N. *et al. J. Virol.* **76**, 7306–7321 (2002).
13. Zwick, M.B. *et al. J. Virol.* **75**, 10892–10905 (2001).
14. Ofek, G. *et al. J. Virol.* **19**, 10724–10737 (2004).
15. Cardoso, R.M.F. *et al. Immunity* **22**, 163–173 (2005).
16. Armbruster, C. *et al. J. Antimicrob. Chemother.* **54**, 915–920 (2004).