MEDICINE

Amping up HIV antibodies

High serum titers of neutralizing antibody can protect humans against HIV

By **Dennis R. Burton**[1,2]

The recent results (*1*) from the Antibody Mediated Prevention (AMP) study are a landmark in AIDS research. They show that a broadly neutralizing antibody (bnAb) can protect humans against infection with sensitive strains of HIV. BnAbs, antibodies that can neutralize a large fraction of globally circulating HIV strains, are the focus of many HIV vaccine efforts and of strategies to prevent or treat HIV by passive immunization (infusion with antibodies). This focus results from the enormous strain variability of HIV—only bnAbs can hope to counter this variability. The results have profound implications for HIV vaccine design and for the use of passively administered bnAbs. Within the context of earlier animal model studies, the AMP study results provide insight into the factors that are important for antibody protection against HIV.

The AMP study involved two trials that enrolled nearly 3000 HIV-negative men and transgender men who have sex with men in the United States, South America, and Europe and nearly 2000 HIV-negative women in Africa to receive the bnAb VRC01 at two doses, 10 or 30 mg/kg, every 8 weeks for a total of 10 intravenous (IV) infusions. VRC01 is a bnAb, isolated in 2010 from an HIV-positive donor (*2*), that potently neutralizes a large proportion of circulating strains, although it is not as potent as some bnAbs isolated more recently. The dosing regime maintained serum bnAbs within defined limits. Overall, the study failed to show efficacy because the numbers of infected individuals in the treated groups were not significantly different from those in the placebo groups.

However, if the study was examined in terms of the neutralization sensitivity of viruses isolated from trial participants who became infected, then there was an important difference between treated and placebo groups. The frequency of infection by VRC01-sensitive viruses [defined as those that are 80% neutralized in vitro (80% inhibitory concentration, or IC_{80}) by less than 1 μg/ml of VRC01] was significantly lower in treated individuals than in placebos. In other words, there is evidence that the antibody offers protection against neutralization-sensitive viruses. The data can be translated into a protective efficacy against HIV infection of ~50% at very approximate serum neutralizing titers of 1:100, ~75% at 1:250, and ~90% at 1:500. A serum neutralizing titer of 1:100, denoted as 50% inhibitory dose (ID_{50}) = 100, indicates that the serum of the donor could be diluted 100-fold and it would produce 50% neutralization in an in vitro neutralization assay; higher ID_{50}'s (more potent neutralizing sera) are required to achieve higher levels of protection. In the AMP study, neutralization was measured in a high-throughput pseudovirus assay (*3*), and an important conclusion of the study was that, with some caveats, this assay could be used to predict bnAb-based protection against HIV infection in humans. The lack of overall efficacy in the study arose from an underestimate of the serum bnAb titers required for protection in humans. Estimates were made based in part on an incorrect assumption that bnAb titers that were lower than those required in studies of nonhuman primate (NHP) viral infection would suffice.

Many studies have investigated the ability of antibodies to protect against HIV infection in animal models. Passive immunization with IV transfusion of a monoclonal antibody was shown to protect a single chimpanzee against challenge with a laboratory-adapted neutralization-sensitive virus as early as 1992 (*4*). Antibody protection titration studies were initially carried out in severe combined immunodeficiency mice populated with human peripheral blood lymphocytes (hu-PBL-SCID mice) that could be infected with HIV. Such studies showed that passive immunization with a first-generation bnAb could protect against both laboratory-adapted, neutralization-sensitive and neutralization-resistant HIV challenge (*5*). The latter are much more representative of global circulating viruses. The concentrations of bnAbs required for protection in this crude mouse model were high; serum ID_{50}'s were typically on the order of 100. Similar ID_{50}'s were generally noted in a second animal model: high-dose mucosal challenge of NHPs with chimeric simian–human immunodeficiency viruses (SHIVs) that have the surface envelope (Env) glycoprotein of HIV (so are neutralized by HIV bnAbs) but the remaining gene segments of SIV (and thus replicate in NHPs).

A comprehensive examination (*6*) of available bnAb NHP SHIV protection data showed, in a logistic model that adjusts for bnAb epitopes and challenge viruses, that the serum ID_{50}'s to achieve 50, 75, and 95% protection were 91, 219, and 685, respectively. These numbers are similar to those estimated from the AMP study for protection in humans (see the figure). Excluding a small number of outliers, the NHP study suggested that protection was largely dependent on serum neutralizing antibody titer alone and was independent of antibody specificity (dose is adjusted to give similar neutralizing titers for antibodies of differing potency against the virus) and independent of challenge route.

Recent HIV Env vaccine-induced protection in NHPs was consistent with passive bnAb studies and showed ~90% protection at ID_{50}'s greater than ~500 (*7*). In that study, because bnAbs have not yet been induced through vaccination, the NHPs were immunized with an Env protein of a given strain and then challenged with the virus of the same strain, and the nAbs ("autologous" nAbs) provided protection. A common caveat that has been attached to animal model protection studies is the use of relatively high-dose viral challenge to ensure that all control animals become infected, which keeps the number of animals per study manageable. Consequently, the viral challenge dose typically used in NHPs was thought to be much higher than the average dose of HIV to which humans were exposed in sexual transmission. Thus, it had been assumed that lower bnAb titers than those derived from animal model studies would provide protection against HIV in humans, but the AMP study results do not support this assumption. In particular, protective titers (at least in terms of ID_{50}'s) from high-dose SHIV challenge in NHPs correlate well with those from passive bnAb VRC01 protection against HIV in humans.

Is a requirement for high bnAb concentrations for protection against HIV unusual? It is likely that bnAbs used in passive immunization to protect against HIV must provide sterilizing immunity, which is defined here as either neutralizing every transmitted virion directly or containing virus replication so that infection can be rapidly aborted without a primary infection. A requirement for sterilizing immunity is expected for a retrovirus that can readily establish latency. Thus, even a small number of virus particles could infect and become integrated into host chromosomes to be later activated and establish a full-blown infection. However, the requirement for high protective nAb concentrations in passive transfer studies is not unusual

[1]Department of Immunology and Microbiology, International AIDS Vaccine Initiative (IAVI) Neutralizing Antibody Center, Consortium for HIV/AIDS Vaccine Development, The Scripps Research Institute, La Jolla, CA 92037, USA. [2]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA. Email: burton@scripps.edu

for viral infections. For example, this is the case in the favored cotton rat model of respiratory syncytial virus (RSV) challenge, which was used to inform the dose of the RSV nAb palivizumab to protect at-risk infants from RSV lung disease (*8*). Of note, in passive immunization, nAbs are the sole immune mechanism of protection, whereas in vaccine-induced immunity, cellular immune responses may also be important and lower nAb titers may be highly beneficial.

Why are such high serum bnAb concentrations in humans relative to in vitro neutralization titer required for protection against HIV? Several potential contributors can be considered. Higher nAb concentrations may be required to prevent viral entry into cells in vivo than with in vitro assays. Indeed, in the AMP study, neutralization was measured using a pseudovirus and a target cell line, compared with the natural situation of a replicating virus targeting primary $CD4^+$ T cells or mononuclear phagocytes. However, although there are well-established differences in nAb sensitivity between the two virus target cell systems, they are not sufficient to explain the high serum bnAb concentrations needed for HIV protection. Another factor could be that serum may not be appropriate for nAb measurement; mucosal tissue has much lower nAb concentrations but may be more critical. An argument against this interpretation is that similar serum nAb concentrations are required for protection in hu-PBL-SCID mice and in IV challenge in NHPs (*9*). Alternatively, high nAb concentrations may be required in vivo to mediate critical functions other than neutralization, such as inhibition of cell-cell spread of virus or Fc-dependent effector functions of bnAbs. Neither of these mechanisms can currently be ruled out. Perhaps most notably, the neutralization curve is asymptotic, meaning that relatively high serum nAb concentrations are required to neutralize every virion if multiple viruses are transmitted and/or if some replication occurs after transmission. This could help explain the general nature of the observation that high serum nAb concentrations are required to completely protect against viruses from different families under widely varying conditions such as animal model and route of challenge and with antibodies of differing specificity.

The AMP study results have important implications for the use of bnAbs for the prevention of HIV infection and for HIV vaccine design. In terms of prevention, a first question is how extendable are the results to bnAbs other than VRC01 likely to be? The animal model studies suggest that most other bnAbs can be expected to offer protection in humans at similar neutralizing titers, although this remains to be determined and there may be outliers, as there are in NHP studies. In addition, a neutralization-resistant fraction of virus may reduce the efficacy of some antibodies against some HIV strains even though the virus is defined as sensitive in terms of an IC_{50} or an IC_{80} (*10*).

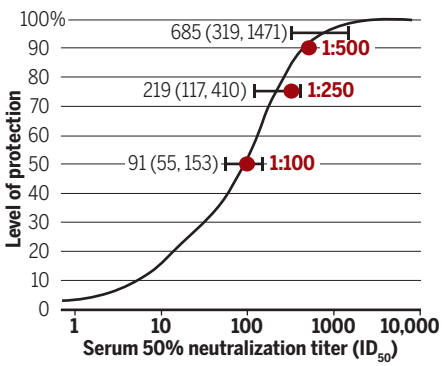
The results also suggest that, to achieve and sustain the proposed protective titers, attention should be focused on the most potent bnAbs, likely as cocktails and likely with half-life extension mutations. For example, the half-life of VRC01 in the AMP study was around 2 weeks, but this could be increased ~fivefold with antibody engineering. Should one incorporate antibody effector function–enhancing mutations into HIV prophylactic bnAbs? Current animal model studies suggest that the effect of such mutations is antibody dependent, and further research is required before such a step is taken (*11*). Major issues for the use of HIV prophylactic bnAbs include whether sufficient potency can be achieved to administer antibodies subcutaneously rather than intravenously, whether multivalent platforms such as bispecific or trispecific antibodies can be generated as effective reagents to replace single-specificity antibody cocktails (*12*), whether unit costs can be brought down to make bnAbs a feasible option for prevention in low- and middle-income countries (*13*), and how bnAbs compare with long-acting small-molecule drugs in terms of cost, efficacy, longevity of action, and other factors (*14*).

The AMP trial results pose a challenge for HIV vaccine design; the induction of sustained high serum bnAb titers will be difficult to achieve. If protection is simply related to serum antibody neutralizing titer irrespective of the antibody specificity involved, it may be easier to induce moderate titers against multiple sites than high titers against a single site given current difficulties in inducing bnAbs through immunization. Targeting multiple sites should also restrict virus neutralization escape. An alternative approach that might ameliorate a requirement for high serum bnAb concentrations is to try to induce protective concentrations of antibodies at mucosal surfaces through mucosal vaccination, although it is not clear that mucosal immunity alone could prevent HIV infection in humans. Lower serum bnAb titers may also be beneficial in the presence of effective cellular immunity. Indeed, a recent study in NHPs showed protection against SHIV infection for a vaccine that induced both cellular immunity and nAbs at lower concentrations than those associated with protection induced by a vaccine that induced only nAbs (*15*).

Overall, it is likely that data from the AMP study will be mined for some time and will doubtless provide new insights into HIV infection and the role of neutralizing antibody in containing virus infection. For example, infected donors in the AMP study provide a large number of contemporaneous viruses that have been sexually transmitted and their sensitivities to a range of bnAbs, thereby potentially improving existing virus panels used for assessing bnAbs. Notably, the study results are the most important development yet in establishing the goalposts for passive immunity through antibody transfusion as a prevention strategy and for bnAb-based HIV vaccine design. ■

Correlates of protection

The graph shows serum neutralization titers (ID_{50}, bootstrap confidence intervals are shown in parentheses) aggregated from studies of simian–human immunodeficiency virus (SHIV) in nonhuman primates (NHPs) [reproduced from (*6*)]. The red dots show the approximate serum neutralization titers for humans from (*1*). This suggests that similar titers are required to protect humans and NHPs against virus.



The figures for human 50% inhibitory dose (ID_{50}) were deduced from 80% inhibitory concentration (IC_{80}) and mean antibody serum concentration and multiplied by 3.

REFERENCES AND NOTES

1. L. Corey *et al.*, *N. Engl. J. Med.* **384**, 1003 (2021).
2. X. Wu *et al.*, *Science* **329**, 856 (2010).
3. M. Li *et al.*, *J. Virol.* **79**, 10108 (2005).
4. E. A. Emini *et al.*, *Nature* **355**, 728 (1992).
5. M. C. Gauduin *et al.*, *Nat. Med.* **3**, 1389 (1997).
6. A. Pegu *et al.*, *Cell Host Microbe* **26**, 336 (2019).
7. M. G. Pauthner *et al.*, *Immunity* **50**, 241 (2019).
8. S. Johnson *et al.*, *J. Infect. Dis.* **176**, 1215 (1997).
9. Y. Nishimura *et al.*, *J. Virol.* **76**, 2123 (2002).
10. L. E. McCoy *et al.*, *PLOS Pathog.* **11**, e1005110 (2015).
11. L. Hangartner *et al.*, *Sci. Transl. Med.* **13**, eabe3349 (2021).
12. N. N. Padte *et al.*, *Retrovirology* **15**, 60 (2018).
13. IAVI/Wellcome, "Expanding access to monoclonal antibody-based products: A global call to action" (2020); https://bit.ly/3vbFnDN.
14. R. M. Gulick, C. Flexner, *Annu. Rev. Med.* **70**, 137 (2019).
15. P. S. Arunachalam *et al.*, *Nat. Med.* **26**, 932 (2020).

ACKNOWLEDGMENTS

My laboratory is supported by funding from the National Institute of Allergy and Infectious Diseases, the Bill and Melinda Gates Foundation, the International AIDS Vaccine Initiative (IAVI), and the James B. Pendleton Charitable Trust. I am a consultant for IAVI. I thank R. Andrabi, P. Anklesaria, L. Hangartner, J. Jardine, and D. Sok for helpful feedback. I thank P. Gilbert for help in interpreting macaque and human protection data.

10.1126/science.abf5376

GRAPHIC: N. DESAI/*SCIENCE*

Amping up HIV antibodies

Dennis R. Burton

Science **372** (6549), 1397-1398.
DOI: 10.1126/science.abf5376