ment of enamel records the isotopic composition of the animal at the time of precipitation, and because enamel is not replaced, the record is permanent. Seasonal, climatically driven changes in food $^{18}O/^{16}O$ cause the $^{18}O/^{16}O$ of an animal to vary during the year, and these seasonal changes are recorded in a crown-to-root isotope variation of the enamel. Thus, the oxygen isotope zoning of a tooth provides a record of seasonal climate variations at the time the animal lived.

Seasonal climate records are important for a variety of reasons. They are fundamental observations that must be explained by global climate models, which predict the worldwide impact of factors such as increasing greenhouse gas concentrations or sea-surface temperatures. Seasonal records can also address whether different climates in the past were associated with different seasonalities and whether global warming will likely increase or decrease seasonality. Increased seasonality may even have helped drive our own evolution in East Africa (*9*). Finally, teeth provide climate records from vitally important continental interiors. Continents respond to global change differently from oceans, but quantitative continental climate records are more sparse and difficult to obtain.

In some ways, the future of stable-isotope biogeochemistry remains obscure. There are so many recent revelations and developments that today's liberal speculations will seem conservative tomorrow. Nonetheless, one major development that is directing current research is the ability to rapidly separate and analyze specific compounds from a single sample (*10*). Different dietary compounds are processed within the body to different degrees. Thus, analysis of essential versus nonessential amino acids or nonproteinaceous compounds may allow much more detailed investigation of diet than has been previously possible. Ultimately, our improved capability of identifying the sources of different dietary components will further our understanding of resource use, animal behavior, and climate.

References

1. M. J. DeNiro and S. Epstein, *Geochim. Cosmochim. Acta* **42**, 495 (1978).
2. Applications of Stable Isotope Techniques to Ecological Studies, Saskatoon, Saskatchewan, 19 to 22 April 1998; You Are What You Eat: Isotopic Ecology of Fossil Vertebrates, 58th Annual Meeting of the Society of Vertebrate Paleontology, Snowbird, UT, 30 September 1998.
3. P. L. Koch, M. L. Fogel, N. Tuross, in *Stable Isotopes in Ecology and Environmental Science*, K. Lajtha and R. H. Michener, Eds. (Blackwell Scientific, Oxford, 1994), pp. 63–92.
4. T. E. Cerling *et al.*, *Nature* **389**, 153 (1997).
5. H. Schwarcz and M. J. Schoeninger, *Yearb. Phys. Anthropol.* **34**, 283 (1991).
6. W. Dansgaard, *Tellus* **16**, 436 (1964); A. Longinelli, *Geochim. Cosmochim. Acta* **48**, 385 (1984); B. Luz, Y. Kolodny, M. Horowitz, *ibid.*, p. 1689.
7. J. D. Bryant and P. N. Froelich, *Geochim. Cosmochim. Acta* **59**, 4523 (1995); M. J. Kohn, *ibid.* **60**, 4811 (1996).
8. H. C. Fricke and J. R. O'Neil, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **126**, 91 (1996).
9. R. A. Foley, in *Seasonality and Human Ecology*, S. J. Ulijaszek and S. S. Strickland, Eds. (Cambridge Univ. Press, Cambridge, 1993), pp. 7–37.
10. J. M. Hayes, K. H. Freeman, B. N. Popp, C. H. Hoham, *Org. Geochem.* **16**, 1115 (1990).

PERSPECTIVES: HIV VACCINES

Magic of the Occult?

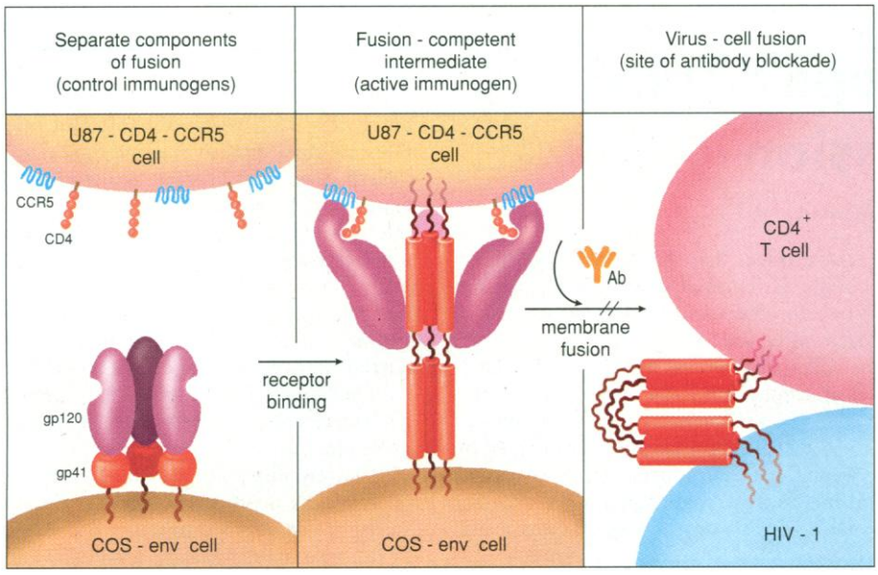
David C. Montefiori and John P. Moore

How do we know when an experimental vaccine is working? In the case of human immunodeficiency virus type 1 (HIV-1), success has usually been gauged by the ability of candidate immunogens to generate measurable immune responses in human volunteers and animal models. The two responses to watch for have been virus-specific $CD8^+$ cytotoxic T lymphocytes (CTLs), which attack and destroy infected cells, and neutralizing antibodies, which bind to the virus and prevent infection of new cells. For HIV-1, a combination of both responses is likely to be more effective than either one alone. Recombinant poxviruses containing HIV-1 proteins have generated low numbers of HIV-specific $CD8^+$ CTLs in a subset of volunteers, and these CTLs can sometimes cross-react with genetically diverse HIV-1 strains—a potentially useful property (*1*). These prototype vaccines can and likely will be improved to generate more potent and consistent CTL responses. The induction of neutralizing antibodies has been more problematic. Early enthusiasm slowly waned as it became increasingly clear that a "principal neutralizing domain" (the V3 loop of the surface glycoprotein gp120) present on HIV-1 laboratory strains, which was potentially a powerful immunogen (*2*), was a poor target on virus recently isolated from patients (or "primary isolates") (*3*). These viral strains closely reflect the viruses a vaccine will encounter in the real world, but because the V3 loop is a poor target in these strains, antibodies generated by the current repertoire of candidate HIV-1 vaccines do not neutralize them efficiently (*4*). This failure is principally because the functional, oligomeric envelope glycoprotein complex of these strains has evolved structural features that reduce the exposure of antibody epitopes (*5*).

Because of this impasse, designing an immunogen that can generate an antibody response able to neutralize a broad spectrum of primary isolates has been a major goal. Now, on page 357 of this issue La-Casse *et al.* (*6*) may have identified a solution to this problem that could eventually be exploited for vaccine development. They proposed that epitopes with superior immunogenicity might be exposed or created as HIV-1 begins to fuse with cell membranes. Fusion complexes were, therefore, tested for their ability to induce neutralizing antibodies in mice. The complexes were created by taking simian fibroblasts engineered to express functional envelope glycoproteins from the primary HIV-1 isolate 168P (COS-Env cells) and mixing them with human neuroblastoma cells stably expressing the CD4 and CCR5 fusion receptors for HIV-1 (U87-CD4-CCR5 cells) (see the figure). The two cell types gradually fuse with one another because the HIV-1 envelope glycoproteins on the COS cells bind the fusion receptors on the U87 cells. During the fusion process, the conformations of the envelope glycoproteins change, leading to the exposure of previously occult epitopes or the de novo formation of neo-epitopes. Fixation of the fusing cells, and hence the fusing envelope-receptor complexes, with mild concentrations of formalin permit the immunogenicity of these epitopes to be evaluated. Immunizations were performed in mice engineered to be transgenic for human CD4 and CCR5 to reduce the possibility that antibodies would be raised to the human CD4 and CCR5 proteins and interfere with the interpretation of the neutralizing antibody assays performed on the immune sera.

Perhaps to the surprise of all involved, this ambitious experiment worked. The mouse sera, and antibodies purified from them, inhibited the infectivity of an impressive array of diverse HIV-1 primary isolates, including viruses from multiple genetic subtypes. Furthermore, the potency of

D. C. Montefiori is at the Center for AIDS Research, Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA. E-mail: monte005@mc.duke.edu. J. P. Moore is at the Aaron Diamond AIDS Research Center, Rockefeller University, New York, NY 10021, USA. E-mail jmoore@adarc.org



Model of a fusion-competent immunogen. (Left) Continuous cultures of simian fibroblastoid cells (COS-7) are transfected to express the transmembrane glycoprotein gp41 and surface glycoprotein gp120 of a primary HIV-1 isolate (168P) as a fusion-competent, native complex (COS-Env cells). Human neuroblastoma cells (U87) are transfected to express human CD4 and CCR5 (U87-CD4-CCR5 cells), which are the major receptor and one of the coreceptors for this HIV-1 isolate, respectively. **(Middle)** The two cells types are mixed together, to permit gp120 to bind to CD4 and CCR5. A transitional intermediate of gp41 forms, protrudes from the COS-Env cell surface, and inserts into the U87-CD4-CCR5 cell membrane (*9, 10*). New epitopes on gp41 are probably created or exposed at this time; other neo-epitopes (not shown) may also be formed on gp120, perhaps dependent on the altered quaternary structure of the oligomeric glycoprotein complex. Formalin can "freeze" these fusion intermediates, permitting evaluation of their immunogenicity in mice. **(Right)** Virus-cell fusion occurs as the transitional intermediate of gp41 undergoes further changes to form a hairpin structure that draws the two membranes into close proximity. Antibodies raised in mice to the gp41 (or conceivably the gp120) component of the fusion intermediate structures may inhibit the formation or function of this structure, thereby preventing hairpin formation and membrane fusion in the neutralization assays. A broadly similar mode of action has been proposed for fusion-inhibiting gp41 peptides (*10*).

the neutralization was significant, with 10- to 100-fold reductions in infectivity being achieved by the key sera at dilutions around 1:50. In a crucial control experiment, the authors demonstrated that infection initiated by the simian immunodeficiency virus (SIV) and murine leukemia virus (MuLV) envelope glycoproteins was not inhibited by the immune sera. This result implies that the effective antibodies were binding to structures conserved among HIV-1 envelope glycoproteins but not shared by SIV and MuLV. Had the opposite result been observed, and SIV and MuLV been neutralized, significant questions would have been raised about just what the antibodies were actually reacting with.

Indeed, one of the major strengths of this study is the precaution taken to reduce (but not entirely eliminate) the possibility that neutralization was due to an antibody response directed against the cells. Immunizing with formalin-killed, SIV-infected cells and human cell–grown SIV was shown nearly a decade ago to protect monkeys from SIV infection, but the protective principle was later found to be antibodies directed against xenogeneic cellular antigens, not viral antigens (*7*). Thus, antibodies to cellular proteins on the surface of either the target cells or the virus itself may neutralize infectivity either directly by interfering with infection or through complement-mediated virolysis (*8*). Awareness of this potential problem prompted the inclusion of the many control experiments detailed in the study: the use of CD4 and CCR5 transgenic mice; of nonfusing cells as control immunogens; and of serum absorptions against the COS-Env cells and, to a lesser extent, against U87-CD4-CCR5 cells.

One can still imagine situations in which unidentified antibodies to the cell could be contributing to the antiviral effect. For example, neo-antigens might be induced on the U87-CD4-CCR5 cell surface during the hours over which fusion occurs, perhaps in consequence of the assult on membrane integrity that cell-cell fusion represents. Antibodies to such epitopes could have an adverse effect on target cells during neutralization assays; they would not necessarily be removed during the absorption experiments, or produced during the immunizations with control, nonfusing cells. Another potential concern is that the effective antibodies in the immune sera are actually directed against altered conformations of the CD4 and CCR5 receptors and not against the viral envelope glycoproteins. Any residual concerns will no doubt be alleviated if and when these experiments are repeated in larger animals—such as macaques. The greater yield of immune serum from these more relevant animals should allow identification of the particular antigens that are recognized by the effective antibodies. Whether the antibody response to these immunogens is strong enough to confer protection from infection can also be addressed in macaques by virus challenge experiments.

Can these observations be exploited to make a practical HIV-1 vaccine? This will not be straightforward, because cell-based vaccines are not ideal, especially those that require tumor cell lines in their generation. The difficulty of immunogen production and its safety are likely to be the major concerns. Gaining greater understanding of the nature of the fusion-related structures that are the most probable targets of the relevant antibodies might permit their expression as recombinant proteins, especially given the increased knowledge of envelope glycoprotein structure and function obtained over the past few years (*9*). Overall, the interesting experiments performed by LaCasse *et al.* should add weight to the perception that an effective neutralizing antibody component would be a worthwhile, and ultimately achievable, component of an effective HIV-1 vaccine.

References

1. C. A. Heilman and D. Baltimore, *Nature Med.* **4**, 532 (1998); G. Ferrari *et al., Proc. Natl. Acad. Sci. U.S.A.* **94**, 1396 (1997).
2. K. Javaharian *et al., Proc. Natl. Acad. Sci. U.S.A.* **86**, 6768 (1989).
3. D. C. Bou-Habib *et al., J. Virol.* **68**, 6006 (1994); J. P. Moore *et al., ibid.* **69**, 101 (1995); T. C. Vancott *et al., AIDS Res. Hum. Retroviruses* **11**, 1379 (1995).
4. C. V. Hanson, *AIDS Res. Hum. Retroviruses* **10**, 645 (1994); J. R. Mascola *et al., J. Infect. Dis.* **173**, 340 (1996); R. I. Connor *et al., J. Virol.* **72**, 1552 (1998); B. S. Graham *et al., J. Infect. Dis.* **177**, 310 (1998).
5. J. P. Moore and J. Binley, *Nature* **393**, 630 (1998).
6. R. A. LaCasse *et al., Science* **283**, 357 (1999).
7. D. C. Montefiori, V. M. Hirsch, P. R. Johnson *Nature* **354**, 439 (1991); A. J. Langlois *et al., Science* **255**, 292 (1992); M. P. Cranage *et al., AIDS Res. Hum. Retroviruses* **9**, 13 (1993).
8. L. O. Arthur *et al., Science* **258**,1935 (1992); D. C. Montefiori *et al., Virology* **205**, 82 (1994).
9. W. Weissenhorn, A. Dessen, S. C. Harrison, J. J. Skehel, D. C. Wiley, *Nature* **387**, 426 (1997); D. C. Chan *et al., Cell* **89**, 263 (1997); P. D. Kwong *et al., Nature* **393**, 648 (1998); R. Wyatt *et al., ibid.*, p. 705; C. D. Rizzuto *et al., Science* **280**, 1949 (1998).
10. C. Wild, T. Greenwell, T. Matthews, *AIDS Res. Hum. Retroviruses* **9**, 1051 (1993); J. M. Kilby *et al., Nature Med.* **4**, 1302 (1998).