90, 41 (1992); K. Nakai and H. Sakamoto, *Gene* **141**, 171 (1994).
3. R. Parker and P. G. Siliciano, *Nature* **361**, 660 (1993); G. Chanfreau, P. Legrain, B. Dujon, A. Jacquier, *Nucleic Acids Res.* **22**, 1981 (1994); A. Deirdre, J. Scadden, C. W. Smith, *EMBO J.* **14**, 3236 (1995).
4. I. J. Jackson, *Nucleic Acids Res.* **19**, 3795 (1991).
5. S. L. Hall and R. A. Padgett, *J. Mol. Biol.* **139**, 357 (1994).
6. I. Kiss *et al.*, *J. Biol. Chem.* **264**, 8126 (1989); R. N. Jenkins *et al.*, *ibid.* **265**, 19624 (1990); R. G. Larson *et al.*, *Cancer Commun.* **2**, 63 (1990); A. I. McClatchey *et al.*, *Hum. Mol. Genet.* **1**, 521 (1992); A. L. George Jr., G. S. Iyer, R. Kleinfield, R.G. Kallen, R. L. Barchi, *Genomics* **15**, 598 (1993); T. Ohshima *et al.*, *ibid.* **28**, 585 (1995); Q. Wang, Z. Li, J. Shen, M. T. Keating, *ibid.* **34**, 9 (1996).
7. The published 5′ splice site sequence of intron 2 of human CACNL1A1, the fibroblast voltage-gated L-type calcium channel, precisely matches the AT-AC consensus, whereas the reported sequences of two other calcium channels, CACNL1A2 and CACNL1A3, have GTATCC (rather than ATATCC) at the corresponding 5′ splice site; however, all three calcium channel introns reportedly end with the conventional AG 3′ splice site [N. M. Soldatov, *Genomics* **22**, 77 (1994); Y. Yamada *et al.*, *ibid.* **27**, 312 (1995); K. Hogan, R. G. Gregg, P. A. Powers, *ibid.* **31**, 392 (1996)]. The sodium and calcium channels are thought to derive from a common ancestral gene: they have considerable nucleotide and amino acid sequence homology, and the unusual intron interrupts a homologous position of the coding sequence in all five genes. Unless there are errors in some of the reported sequences, it will be interesting to determine whether the calcium channel transcripts are processed via the major pathway, the AT-AC pathway, or a hybrid pathway.
8. A portion of the human SCN4A gene was amplified by PCR from human total genomic DNA (Promega) with primers containing restriction sites and matching exons 2 and 3, to generate a fragment comprising nt 886 to 1229 (numbering according to GenBank accession number L04216). This fragment was digested with Hind III and Xba I and subcloned into the corresponding sites of pSP64 (Promega) to generate the pSP64-SCN4A plasmid. For construction of pSP64-SCN4AM, a different downstream PCR primer containing the mutations and an Eco RI site was used to amplify a mutant fragment from the cloned wild-type template, which was then subcloned as a Hind III–Eco RI fragment in pSP64. All constructs were confirmed by sequence analysis. pSP64-SCN4A and pSP64-SCN4AM were linearized with Xba I or Eco RI, respectively, for use as templates for in vitro transcription with SP6 RNA polymerase. The transcripts contain short extensions at both ends, derived from the vector.
9. Nuclear extract preparation and conditions for in vitro transcription and for β-globin splicing were as described [A. Mayeda and A. R. Krainer, *Cell* **68**, 365 (1992)]. The SCN4A in vitro splicing reaction was optimized by varying individual parameters. The optimum condition for SCN4A splicing was 60% (v/v) nuclear extract [giving final concentrations of 12% (v/v) glycerol, 12 mM Hepes–K^+ (pH 8.0), 60 mM KCl, 0.6 mM dithiothreitol, and 0.3 mM EDTA] plus an additional 32 mM Hepes–K^+ (pH 7.3), 3.5 mM $MgCl_2$, 0.5 mM ATP, 20 mM creatine phosphate, 2.6% (w/v) polyvinyl alcohol, and 1.6 nM SCN4A pre-mRNA, incubated at 30°C for 6 hours. No splicing was detectable when ATP or $MgCl_2$ was omitted. RNA was recovered and analyzed on 4.5% denaturing polyacrylamide gels, followed by autoradiography. Splicing efficiency, defined as the molar ratio mRNA/(pre-mRNA + mRNA), was estimated by phosphor image analysis (Fujix, BAS2000).
10. Q. Wu and A. R. Krainer, data not shown.
11. To sequence the authentic and cryptic spliced mRNAs across the spliced junctions, we amplified each gel-purified RNA by RT-PCR with exon 2 and exon 3 primers (TCATCGTACTCAACAAGG and TACTCCACATTCTTGGAC). The amplified fragment, subcloned into PCR2.1 (Invitrogen), was sequenced with T7 Sequenase 2.0 (USB).
12. P. J. Grabowski, R. A. Padgett, P. A. Sharp, *Cell* **37**, 415 (1984); B. Ruskin, A. R. Krainer, T. Maniatis, M. R. Green, *ibid.* **38**, 317 (1984).
13. W.-Y. Tarn and J. A. Steitz, *ibid.* **84**, 801 (1996).
14. A. Krämer, W. Keller, B. Appel, R. Lührmann, *ibid.* **38**, 299 (1984); A. R. Krainer and T. Maniatis, *ibid.* **42**, 725 (1985); D. L. Black, B. Chabot, J. A. Steitz, *ibid.*, p. 737; S. M. Berget and B. L. Robberson, *ibid.* **46**, 691 (1986); D. L. Black and J. A. Steitz, *ibid.*, p. 697.
15. For RNase H inhibition experiments, the nuclear extract was preincubated for 15 min under splicing conditions in the presence or absence of the appropriate oligonucleotides. The oligonucleotides were complementary to U1 snRNA position 2 to 11, U2 snRNA position 1 to 15, or U12 snRNA position 11 to 24. snRNA cleavage is catalyzed by the endogenous RNase H (*14*), and exogenous RNase H had no additional effect (*10*). All snRNA cleavage and splicing inhibition experiments were carried out at least three times, with reproducible results.
16. S. J. Baserga and J. A. Steitz, in *The RNA World*, R. F. Gesteland and J. F. Atkins, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1993), pp. 359–381; D. A. Wassarman and J. A. Steitz, *Mol. Cell. Biol.* **11**, 3432 (1991).
17. S. L. Hall and R. A. Padgett, *Science* **271**, 1716 (1996).
18. K. A. Montzka and J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8885 (1988); D. L. Black and A. L. Pinto, *Mol. Cell. Biol.* **9**, 3350 (1989).
19. K. M. Wasserman and J. A. Steitz, *Mol. Cell. Biol.* **12**, 1276 (1992).
20. R. R. Gontarek, M. T. McNally, K. Beemon, *Genes Dev.* **7**, 1926 (1993).
21. G. P. Shumyatsky, S. V. Tillib, D. A. Kramerov, *Nucleic Acids Res.* **18**, 6347 (1990).
22. Y. Zhuang and A. M. Weiner, *Cell* **46**, 827 (1986); P. G. Siliciano and C. Guthrie, *Genes Dev.* **2**, 1258 (1988); B. Seraphin, L. Kretzner, M. Rosbash, *EMBO J.* **7**, 1533 (1988).
23. B. L. Robberson, G. J. Cote, S. M. Berget, *Mol. Cell. Biol.* **10**, 84 (1990); H.-C. Kuo, F. H. Nasim, P. J. Grabowski, *Science* **251**, 1045 (1991); J. P. Kreivi, K. Zerivitz, G. Akusjärvi, *Nucleic Acids Res.* **19**, 6956 (1991).
24. The SCN4AM mRNA was accurately spliced, as verified by RT-PCR sequencing. No spliced products were detected in the absence of ATP or magnesium. The SCN4AM cryptic spliced product was also confirmed by sequencing. Cleavage of U4 and U6 snRNAs had the same effect as U2 cleavage. An additional pre-mRNA, in which the downstream 5′ splice site was deleted, had identical splicing and inhibition profiles as those of the SCN4AM substrate (*10*).
25. S. M. Berget, *J. Biol. Chem.* **270**, 2411 (1995).
26. M. D. Chiara and R. Reed, *Nature* **375**, 510 (1995).
27. M. Ohno, H. Sakamoto, Y. Shimura, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5187 (1987); E. Izaurralde *et al.*, *Cell* **78**, 657 (1994).
28. M. Niwa and S. M. Berget, *Genes Dev.* **5**, 2086 (1991); K. M. Wassarman and J. A. Steitz, *ibid.* **7**, 647 (1993); C. S. Lutz *et al.*, *ibid.* **10**, 325 (1996).
29. B. E. Hoffman and P. J. Grabowski, *ibid.* **6**, 2554 (1992); J. Y. Wu and T. Maniatis, *Cell* **75**, 1061 (1993); D. Staknis and R. Reed, *Mol. Cell. Biol.* **14**, 7670 (1994).
30. D. C. Kohrmann, J. B. Harris, M. H. Meisler, *J. Biol. Chem.* **271**, 17576 (1996).
31. We thank D. Horowitz for comments on the manuscript; A. Mayeda for sharing protocols and reagents; and M. Zhang, M. Murray, H.-X. Liu, and T.-L. Tseng for helpful discussions. Supported by grant GM42699 from NIH.

19 July 1996; accepted 3 September 1996

TECHNICAL COMMENTS

HIV-1 Evolution and Disease Progression

Steven M. Wolinsky *et al.* (*1*) studied the evolution of human immunodeficiency virus (HIV) in six infected patients with variable rates of disease progression and have represented their findings (*1*, *2*) as inconsistent with our evolutionary hypothesis of HIV disease progression, also known as "antigenic diversity threshold theory" (*3*–*6*). Their account of our model is, however, incorrect. Consequently their interesting and important data do not falsify our theory. We proceed to give a short review of our theory and show that the findings of Wolinsky *et al.* (*1*) provide further evidence that the major assumptions of our evolutionary theory of HIV infection are valid.

The central tenet of our theory can be stated as follows: (i) virus load causes disease; (ii) immune responses reduce virus load; (iii) virus evolution during infection weakens the effect of the immune response to HIV and increases virus load. The key result is a dynamic threshold condition that specifies whether or not the HIV population in a given patient is controlled by immune responses. The precise location or value of this threshold, which can be breached by virus evolution leading to increasing antigenic diversity, is likely to vary greatly among individual patients. In particular, weak immune responders should have a low antigenic diversity threshold and can therefore progress to disease rapidly and without significant antigenic variation (*3*–*6*). Admittedly, for simplicity our first papers presented analytic results for situations in which all patients had the same immune response, and all HIV strains had the same replication rate; these may have caused confusion. But even in these first papers, there is a discussion (and figures) for situations where diversity thresholds differ among infected individuals.

Wolinsky *et al.* (*1*) followed six HIV-1 infected patients longitudinally over up to 5 years after infection (Fig. 1). Two patients were rapid progressors and died within 36 and 42 months after infection. Genetic diversity (proviral DNA) was sampled in a region of the envelope protein, and cytotoxic T lymphocyte (CTL) precursor frequency was determined against the ENV, GAG, and POL proteins. The data were interpreted as rejecting our theory, apparently because high genetic diversity did not correlate with rate of CD4 cell loss and in particular

the two patients who progressed rapidly to disease had low genetic diversity. The statement that such findings refute our theory rests on the incorrect assumption that the magnitude of the diversity threshold is the same in different patients irrespective of their immune response against HIV.

In our previous publications it is stated that weak immune responders should have a low diversity threshold and may therefore develop disease with low antigenic diversity. In 1990 and 1991, we noted that if a patient has a very weak immune response against HIV (or if a particular virus strain is highly pathogenic) then even a single strain can overcome the threshold (3). In this case (4, p. S7)

> [T]he virus population replicates to high levels and may induce acute disease and death within a short time; no antigenic variation may be observed because of selection for the fastest growing strain.

In 1995, Nowak and McMichael emphasized (5, p. 62)

> If the combined immune responses to conserved and variant epitopes are too weak to control HIV replication from the start, AIDS should develop rapidly. In that situation, the original viral particles would proliferate without encountering much resistance, and so the virus would be under little pressure to generate mutants able to escape immune reconnaissance. Such patients might progress to AIDS even in the absence of significant viral diversity.

More recently, Nowak and Bangham (6) showed that a weak immune responder can have high virus load and low diversity, while a strong immune responder can have low virus load and high diversity [see figure 3 of (6)]. Thus, previously published results of our theory are in agreement with the findings of Wolinsky *et al.* (1), who show that fast progression is associated with weak CTL responses, high viral load, and comparable low genetic diversity (7). Nevertheless, Wolinsky *et al.* (1) and Miedema and Klein (2) do not acknowledge that this association is a result of our theory and misinterpret the finding of rapid progression with low diversity as contradicting the theory.

Our model of HIV pathogenesis is based on the assumptions that: (i) HIV replicates rapidly during infection; (ii) below the threshold (that is, during the majority of the incubation period) virus growth is controlled by immune responses; (iii) HIV can produce mutants that escape from current immune responses; and (iv) HIV impairs immune responses either directly or indirectly by depleting CD4 cells. Analysis of the mathematical model (3–6), which mirrors these assumptions, reveals a steady state between virus replication and immune responses where virus load, v, is given by the following equation:

$$v = \frac{nrb}{pc - nru} \quad (1)$$

Here r denotes the replication rate of the virus, b the rate of decline of anti-HIV immune responses in the absence of stimulation, p the rate of elimination of virus by immune responses, c the rate of stimulation of immune responses, u the rate at which HIV depletes CD4 cells, and n the antigenic diversity of the HIV population. Further analysis of the model leads to a dynamic threshold condition that specifies whether or not the virus is limited by immune responses or left to uncontrolled replication (only subject to availability of target cells). Specifically, if

$$nru > pc \quad (2)$$

then the virus escapes from control by the immune response. This condition specifies that a combination of parameters (nru) has to exceed the immune response (pc), which may be correlated to the concentration of CD4 cells. In the simplest version of our theory, the immune system provides selection pressure for antigenic variation and the threshold is breached by increasing antigenic diversity, n, while the other parameters, r, u, p, and c remain constant. This prompted the name "antigenic diversity threshold" (9). If a patient has a weak anti-HIV immune response, low p and c, then it is obvious from Eq. 2 that a low antigenic diversity, n, can overcome the threshold.

Such a theory of HIV disease progression is clearly testable. The direct approach is to study antigenic variation in defined epitopes which are seen by dominant immune responses. If CTL exert the major immune response against HIV, then it is important to study variation in CTL epitopes. Antigenic diversity in these epitopes should increase over time and eventually lead to reduced control of HIV replication, increasing virus load and declining CD4 cells.

Wolinsky *et al.* observe increasing genetic diversity in all patients, but they do not find a clear correlation between increasing diversity and increasing virus load if patients are followed longitudinally. Would we expect such a correlation? The main problem is that it remains unclear to what extent genetic variation in ENV represents antigenic variation. The sequenced ENV region is a potential target for antibody and CTL responses; the V3 region is known to be under positive selection for variation (11), which can be a consequence of immune responses or selection for cell tropism (12). The CTL data of Wolinsky *et al.*, however, suggest that only patient P4 has a significant response against ENV, while the five other patients have low or undetectable CTL precursors against ENV. The major CTL responses in these patients are directed against the GAG and POL proteins, which have not been sequenced. If the dominant immune response in these patients is exerted by CTL (which is also supported by the observation of Wolinsky *et al.* that weak CTL responses are associated with high virus load and rapid disease progression), then the wrong region has been sequenced for testing our theory. A test of our theory has to investigate antigenic variation in epitopes seen by major immune responses. Genetic variation has to be related to escape from dominant immune responses. Genetic diversity as such need not correlate with CD4 decline or virus load increase.

We conclude that the findings of Wolinsky *et al.* are consistent with a number of central results of our work, namely that the immune system provides positive selection for variation (13) and that weak immune responses are associated with high virus load and rapid disease progression (3–6). Their final conclusion that "HIV-1 quasispecies dynamics are compatible with an

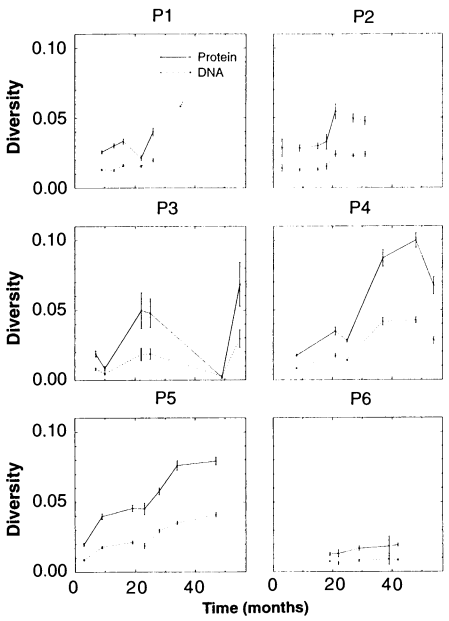

Fig. 1. Genetic variation in the V3 to V5 region of the envelope gene in six patients studied by Wolinsky *et al.* (1). Patients P1 and P2 rapidly progress to disease and die within, respectively, 36 and 42 months after infection. Patients P3 to P6 remain asymptomatic. Mean Hamming distance (that is, the number of point mutations) of DNA and protein sequences is shown. Other distance measures such as the Kimura 2-parameter distance or protein sequence distance based on the Dayhoff PAM-matrix give similar results. In all patients there is a trend of increasing viral diversity.

ideal Darwinian system" has been the central hypothesis of our previous papers on the subject.

Martin A. Nowak
Roy M. Anderson
Maarten C. Boerlijst
Sebastian Bonhoeffer
Robert M. May
Department of Zoology,
University of Oxford,
South Parks Road,
OX1 3PS Oxford, United Kingdom
Andrew J. McMichael
Institute of Molecular Medicine,
John Radcliffe Hospital,
Oxford OX3 9DU, United Kingdom

REFERENCES AND NOTES

1. S. M. Wolinsky *et al.*, *Science* **272**, 537 (1996).
2. F. Miedema and M. R. Klein, *ibid.*, p. 505.
3. M. A. Nowak, R. M. May, R. M. Anderson, *AIDS* **4**, 1095 (1990); M. A. Nowak *et al.*, *Science* **254**, 963 (1991).
4. M. A. Nowak and R. M. May, *AIDS* **7**, suppl. 1, S3 (1993).
5. M. A. Nowak and A. J. McMichael, *Sci. Am.* **273**, 58 (August 1995).
6. M. A. Nowak and C. R. M. Bangham, *Science* **272**, 74 (1996).
7. E. L. Delwart *et al.*, *J. Virol.* **68**, 6672 (1994); V. V. Lukashov *et al.*, *ibid.* **69**, 6911 (1995); B. F. Haynes, G. Pantaleo, A. S. Fauci, *Science* **271**, 324 (1996).
8. B. Asjo *et al.*, *Lancet* **ii**, 660 (1986); M. R. Tersmette *et al.*, *J. Virol.* **62**, 2026 (1988); M. Koot *et al.*, *Ann. Intern. Med.* **118**, 681 (1993); R. I. Connor *et al.*, *J. Virol.* **67**, 1772 (1993).
9. The effects of changes in the other parameters have also been studied: evolution of fast replicating, highly cytopathic variants, high r and u (*8*), or variants with reduced immunogenicity, low c and p, lead to a lower threshold. [R. J. de Boer and M. C. Boerlijst, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 544 (1994); M. A. Nowak and R. M. May, *J. Theor. Biol.* **159**, 329 (1992)].
10. R. E. Phillips *et al.*, *Nature* **354**, 453 (1991); M. A. Nowak *et al.*, *ibid.* **375**, 606 (1995).
11. S. Bonhoeffer, E. C. Holmes, M. A. Nowak, *ibid.* **376**, 125 (1995); E. C. Holmes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4835 (1992).
12. A. McKnight *et al.*, *J. Virol.* **69**, 3167 (1995).
13. In patient P4, there is increased variation in an HLA-B7 restricted epitope (although it has not been formally demonstrated that the patient's CTL indeed recognize this epitope). In patients P3, P4, and P5 there is a faster rate of accumulation of nonsynonymous substitutions than in patients P1, P2, and P6, which leads Wolinsky *et al.* to conclude that the increase in genetic diversity correlates with positive selection for change.

6 October 1995; revised 7 June 1996; accepted 14 August 1996

Response: The "diversity threshold theory" posits that the capability of the immune response is exceeded once antigenic diversity increases beyond a threshold (*1*, *2*). This central tenet is controversial. The high mutation rates inherent to the replicative processes and the short genome doubling time are major factors involved in rapid virus evolution (*3*). Furthermore, it is the high replicative capacity of the virus rather than the generation of diversity per se that destroys the host. Thus, within a particular host, diversity is driven by the collective sum of all the selective forces acting on the HIV-1 quasispecies virus population, rather than a specific immune parameter by or in itself (*4*).

The theory predicts two possible outcomes. First, infected individuals with diversity higher than their individual specific threshold lose immune control and rapidly progress to AIDS, while those below their individual specific threshold remain clinically stable. Second, an increase in antigenic diversity over time in the same individual gives rise to loss of immune control and faster progression to AIDS once the individual's specific threshold is crossed.

The fact that the magnitude of the diversity threshold could be different in different infected individuals is a result of the inherent plasticity of the mathematical model used to derive the antigenic diversity threshold theory (*1*, *2*). As a consequence, the model becomes virtually untestable. The actual model could encompass many different possible parameters that can trigger the trajectory to AIDS, all of which are sensitive to initial conditions (*5*) and better expressed as a nondimensional threshold condition (*6*). Thus, stochastic simulations of the infection process only partially characterize the model dynamics (*5*).

The results of our study (*7*) do not support a model that relates increasing antigenic diversity to pathogenic progres-

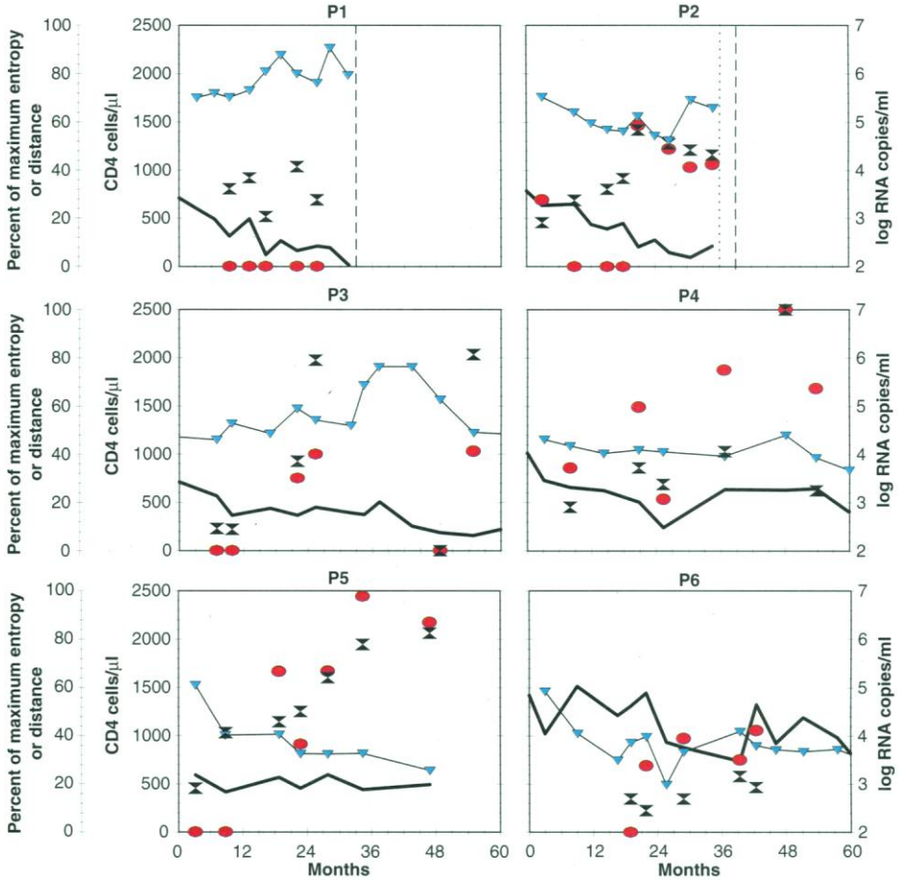

Fig. 1. Lack of correlation between progression to AIDS and HIV-1 diversity. Rate of CD4 T cell decline (heavy solid line, read against y-axis at left innermost) and the HIV-1 virion-associated RNA burden (light line with triangles, read against y-axis at right) in the six different patients [see Wolinsky *et al.* (*7*) for more details] correlates neither with the subjects' viral diversity, measured by Shannon entropy measurements (large circles, read against y-axis at left outermost) of the proviral gp120 sequences, nor with the median Hamming distance (filled hatches, read against y-axis at left outermost) between the sequences in each time point. Shannon entropy and median Hamming distance were calculated according to methods described in the report by Wolinsky *et al.* and are given as percentage of the maximum entropy (1.69 = 100%; P4 at 48 months) and median Hamming distance (9.91 = 100%; P4 at 48 months) among these patients. Dotted vertical lines represent the time of clinical AIDS diagnosis. Dashed vertical lines represent the time of death of the study subject. An increase in the viral burden or in the rate of CD4 T cell depletion during progression to disease of each subject is not necessarily correlated with an increase in viral diversity for that study subject. Rather, for several subjects, an increase in HIV-1 diversity occurs when the viral burden decreases and CD4 T cell count increases.

sion. Although Nowak *et al.* attribute the limited diversity observed for those people who had a more rapid rate of CD4 T cell loss after primary infection to the transgression of their individual specific threshold, such a threshold is nonexistent or negligible. A diversity threshold of "one strain" (*1*, *2*, *8*) cannot be considered a threshold because a lower value of diversity is not possible. Furthermore, a stochastic simulation of these "weak immune responders" models a viral burden that continues to escalate exponentially. This is not biologically plausible.

The model would also predict that, at least in some individuals, the apparent diversity of viral forms in a quasispecies virus population expands before a precipitous decline in the CD4 T cell count (*1*, *2*). We find that within infected individuals with slower rates of CD4 T cell loss, higher viral diversity did not correlate with an increase in the viral burden or CD4 T cell decline (Fig. 1). The results, while limited by a small sample size, are nevertheless not consistent with the outcome Nowak *et al.* predict (*1*, *2*, *7*).

We used a measure of protein diversity as a surrogate for antigenic diversity and tracked substitutions in well-characterized CTL epitopes (*7*). The relevant caveats concerning these analyses were considered and explained at length in the text and notes of our report (*7*). However, using the analytical measures and parameters put forth by Nowak *et al.*, which are more appropriately a measure of genetic distance than protein diversity, we are still unable to support the premise that breaching a specific antigenic diversity threshold is a likely precipitating event to the progression to disease (*7*, *8*).

Nowak *et al.* submit that a valid empirical test of the diversity threshold theory is to assess antigenic oscillations and shifting immunodominance in defined human leukocyte antigen (HLA) class I–restricted epitopes (*9*, *10*). We found the genetic characterization of unambiguously defined HLA class I–restricted CTL epitopes in *Env* and a significant response against *Env* in two patients (P4 and P5) rather than one, as stated by Nowak *et al.* Nowak *et al.* further contend that, because of other potentially confounding selective forces, *Gag* and *Pol* are more appropriate regions for analysis than is *Env*. Their reasoning and their interpretation of our results are difficult to rectify with the current state of knowledge about the biology of the cell-mediated immune response (*11*).

Nowak *et al.* concur with our final conclusion that "HIV-1 quasispecies dynamics are compatible with an ideal Darwinian system." We agree with Nowak *et al.*'s assertion that viral evolution is driven by selective forces. We suggest that the problem is with their interpretation of the model, specifically the statement that an "antigenic diversity threshold" has an important role in viral pathogenesis.

When judged by empiricism in the context of reasonable definitions that distinguish between antigenic and genetic diversity and the potential for applicability to other biological systems (*3*), the "diversity threshold theory" lacks relevance and sufficient experimental support. Specifically: (i) the theory does not explain the limited diversity observed for those people who have a rapid rate of CD4 T cell loss after primary infection, and (ii) existence of an "antigenic diversity threshold" for those people with a lower rate of CD4 T cell loss has yet to be demonstrated experimentally.

Steven M. Wolinsky
Kevin J. Kunstman
Northwestern University Medical School, 303 East Chicago, Tarry Building, 3-735, Chicago, IL 60611-3008, USA
E-mail: smw006@anima.nums.nwu.edu
Jeffrey T. Safrit
Bristol-Myers Squibb Pharmaceutical Research Institute, 3005 First Avenue, Seattle, WA 98103, USA
E-mail: safritj@bms.com
Richard A. Koup
Aaron Diamond AIDS Research Center, 455 1st Avenue, 7th Floor, New York, NY 10016, USA
E-mail: koup@phri.nyu.edu
Avidan U. Neumann
Bette T. M. Korber
Theoretical Biology, Los Alamos National Laboratory, Los Alamos, NM 87545, USA
and *the Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87505*
E-mail: aun@santafe.edu
E-mail: btk@t10.lanl.gov

REFERENCES

1. M. A. Nowak, R. M. May, R. M. Anderson, *AIDS* **4**, 1095 (1990); M. A. Nowak *et al.*, *Science* **254**, 963 (1991); M. A. Nowak and A. J. McMichael, *Sci. Amer.* **273**, 58 (August 1995).
2. M. A. Nowak and S. Bonhoeffer, *Nature* **375**, 193 (1995); S. Bonhoeffer, E. C. Holmes, M. A. Nowak, *Nature* **376**, 125 (1995); M. A. Nowak and C. R. M. Bangham, *Science* **272**, 74 (1996).
3. J. M. Coffin, *Science* **267**, 483 (1995).
4. R. A. Weiss, *ibid.* **272**, 1885 (1996).
5. N. I. Stilianakis, D. Schenzle, K. Dietz, *Math. Biosci.* **121**, 235 (1994).
6. R. J. de Boer and M. C. Boerlijst, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 544 (1994).
7. S. M. Wolinsky *et al.*, *Science* **272**, 537 (1996).
8. E. L. Delwart *et al.*, *J. Virol.* **68**, 6672 (1994).
9. M. A. Nowak *et al.*, *Nature* **375**, 606 (1995).
10. R. Phillips *et al.*, *ibid.* **354**, 453 (1991).
11. A. J. McMichael and B. D. Walker, *AIDS* **8S**, S155 (1994).

19 June 1996; accepted 14 August 1996