On 12 January 1995, David Ho and George Shaw presented results in the journal *Nature* which suggested a radical new understanding of HIV infection, one in which the immune system is in a constant battle with HIV from the moment of initial infection. According to a “News and Views” editorial by *Nature*’s editor John Maddox the following week, these “new developments are (or should be) an embarrassment for Duesberg.” Maddox even proclaimed that it may be time for “the Duesbergs of this world [sic]...to recant.”

It should be pointed out that the aforementioned papers presented in *Nature* largely provided the justification for the new phase of protease inhibitor and “cocktail” treatments, as well as for the expanded use of surrogate markers such as “viral load” and CD4 counts. Each of these represented a significant departure in terms of HIV/AIDS diagnosis, maintenance, treatment, and epidemiological reporting, so the validity of the results presented should have been crucial.

The models were developed by “teaming up with mathematicians” and involve some elementary differential equations. In point of fact, the models have now been discredited for many years, although the “significant departures” mentioned above remain prevalent in practice.

In Part I, the initial papers are presented along with criticisms which were made by many people almost immediately upon their publication, including mathematical criticisms by Mark Craddock, as well as biological criticisms by Duesberg and Harvey Bialy.

Part I should give sufficient documentation for the community to judge whether the acceptance of the papers of Ho and Shaw was warranted at the time. Readers may also wish to take note that a recent PBS documentary (*Frontline*) transmitted in May 2006 uncritically repeated this discredited model as fact.

DCB
Viral dynamics in human immunodeficiency virus type 1 infection

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The natural history and pathogenesis of human immunodeficiency virus type-1 (HIV-1) infection are linked closely to the replication of virus in vivo. Clinical stage is significantly associated with all measures of virus load, including infectious virus titres in blood, viral antigen levels in serum, and viral nucleic acid content of lymphoreticular tissues, peripheral blood mononuclear cells (PBMCs) and plasma (reviewed in ref. 18). Moreover, HIV-1 replication occurs preferentially and continuously in lymphoreticular tissues (lymph node, spleen, gut-associated lymphoid cells, and macrophages)19,20; virus is detectable in the plasma of virtually all patients regardless of clinical stage11,12,13,14; and changes in plasma viral RNA levels predict the clinical benefit of antiretroviral therapy (R. Coombs, unpublished results). These findings emphasize the central role of viral replication in disease pathogenesis.

Despite the obvious importance of viral replication in HIV-1 disease, relatively little quantitative information is available regarding the kinetics of virus production and clearance in vivo. The rapidity of virus and CD4+ cell population turnover, and the fixation rates of biologically relevant viral mutations22,23. This circumstance is largely due to the fact that previously available antiretroviral agents lacked sufficient potency to abrogate HIV-1 replication, and methods to quantify virus and determine its genetic complexity were not sufficiently sensitive or accurate. We overcame these obstacles by treating subjects with new investigational agents which potently inhibit the HIV-1 reverse transcriptase (nevirapine, NVP)24 and protease (ABT-538; L-735,524)25,26; by measuring viral load changes using sensitive new quantitative assays for plasma virus RNA24,25,26; and by quantifying changes in viral genotype and phenotype in uncultured plasma and PBMCs using automated DNA sequencing27 and an in situ assay of RT function28,29.

Virus production and clearance

Twenty-two HIV-1-infected subjects with CD4+ lymphocyte counts between 18 and 251 per mm3 (mean ± s.d. 102 ± 75 cells per mm3) were treated with ABT-538 (n = 10), L-735,524 (n = 8) or NVP (n = 4) as part of phase I/IIA clinical studies. The design and clinical findings of these trials will be reported elsewhere (K. Squires et al., and V.A. J. et al., manuscripts in preparation). Plasma viral RNA levels in the 22 subjects at baseline ranged from 106 to 107 molecules per ml (geometric mean of 106.5) and exhibited maximum declines generally within 2 to 4 weeks of initiating drug therapy (Figs 1 and 2a). For ABT-538- and L-735,524-treated patients, virus titres fell by as much as 105-fold (mean decrease of 104.9-fold) whereas for NVP-treated patients virus fell by as much as 104.8-fold (mean decrease of 104.4-fold). The overall kinetics of virus decline during the initial weeks of therapy with all three agents corresponded to an exponential decay process (Figs 1 and 2a).

The antiretroviral agents used in this study, despite their differing mechanisms of action, have a similar overall biological effect in that they block de novo infection of cells. Thus the rate of elimination of plasma virus that we measured following the initiation of therapy is actually determined by two factors: the clearance rate of plasma virus per se and the elimination (or suppression) rate of pre-existing, virus-producing cells. To a good approximation, we can assume that virus-producing cells decline exponentially according to y(t) = y0e−αt, where y(t) denotes the concentration of virus-producing cells at time t after the initiation of treatment and α is the rate constant for the exponential decline. Similarly, we assume that free virus v(t) is generated by virus-producing cells at the rate ky(t) and declines exponentially with rate constant v. Thus, for the overall decline of free virus, we obtain v(t) = v0[ue−vτ − ae−αt]/(v − α). The kinetics are largely determined by the slower of the two decay processes. As we have data only for the decline of free virus, and not for virus-producing cells, we cannot determine which of the two decay processes is rate-limiting. However, the half-life (t1/2) of neither process can exceed that of the two combined. With these considerations in mind, we estimated the elimination rate of plasma virus and of virus-producing cells by three different methods: (1) first-order kinetic analysis of that segment of the viral elimination curve corresponding to the most rapid decline in plasma virus, generally somewhere between days 3 and 14; (2) fitting of a simple exponential decay curve to all viral RNA determinations between day 0 and the nadir or inflection point (Fig. 1); and (3) fitting of a compound decay curve.
FIG. 1 Plasma viral RNA determinations in representative subjects treated with the HIV-1 protease inhibitors ABT-538 (a) and L-735,524 (b). Subjects had not received other antiretroviral agents for at least 4 weeks before therapy. Treatment was initiated at week 0 with 400-1,200 mg d⁻¹ of ABT-538 or 1,600-2,400 mg d⁻¹ of L-735,524 and was continued throughout the study. Viral RNA was determined by modified branched DNA (bDNA) (a) or RT-PCR (b) assay and confirmed by QPCR. Shown are the least-squares fit linear-regression curves for data points between days 0 and 14 indicating exponential (first-order) viral elimination.

that takes into account the two separate processes of elimination of free virus and virus-producing cells, as described. Method (1) gives a 1/2 of 1.8 ± 0.9 days; method (2) gives a 1/2 of 3.0 ± 1.7 days; and method (3) gives a 1/2 of 2.0 ± 0.9 days for the slower of the two decay processes and a very similar value, 1.5 ± 0.5 days, for the faster one. These are averages (±1 s.d.) for all 22 patients. Method (3) arguably provides the most complete assessment of the data, whereas method (2) provides a simpler interpretation (but slightly slower estimate) for virus decline because it fails to distinguish the initial delay in onset of antiviral activity due to the drug accumulation phase, and the time required for recently infected cells to initiate virus expression from the subsequent phase of exponential virus decline. There were no significant differences in the viral clearance rates in subjects treated with ABT-538, L-735,524 or NVP, and there was also no correlation between the rate of virus clearance from plasma and either baseline CD4⁺ lymphocyte count or baseline viral RNA level.

**Virus turnover**

**Direct population sequencing.** As an independent approach for determining virus turnover and clearance of infected cells, we quantified serial changes in viral genotype and phenotype with respect to drug resistance in the plasma and PBMCs of four subjects treated with NVP (Fig. 2). NVP potently inhibits HIV-1 replication but selects for one or more codon substitutions in the reverse transcriptase (RT) gene. These mutations result in dramatic decreases (up to 1,000-fold) in drug susceptibility and are associated with a corresponding loss of viral suppression in vivo. Genetic changes resulting in NVP resistance can thus serve as a quantifiable molecular marker of virus turnover. A rapid decline in plasma viral RNA was
observed following the institution of NVP therapy and this was associated with a reciprocal increase in CD4+ lymphocyte counts (Fig. 2a and 3b). Both responses were of limited duration, returning to baseline within 6–20 weeks in these four patients. The proportion of virus in uncultured plasma and PBMCs that contain NVP-resistance-conferring mutations (Fig. 2c) was determined by direct automated nucleotide sequencing of viral nucleic acid (Fig. 3), as previously described24. We first validated this method by reconstitution experiments, confirming its sensitivity for detecting RT mutants that comprise as little as 10% of the overall virus population. Defined mixtures of wild-type and mutant HIV-1 RT cDNA clones differing only at the second base position of codon 190 were amplified and sequenced (Fig. 3a).

Varying proportions of wild-type and mutant viral sequences present in the original DNA mixtures (mutant composition: 0, 10, 25, 50, 75 and 100%) were faithfully represented in the relative peak-on-peak heights (and in the relative peak-on-peak areas) of cytosine (C) and guanine (G) residues at the second base position within this codon. Ratios of (mutant)/ (mutant + wild type) nucleotide peak heights expressed in arbitrary fluorescence units were as follows (predicted/observed): 0/ < 10%; 10/18%; 25/29%; 50/49%; 75/71% and 100/94%.

We next determined the ability of direct population sequencing to quantify wild-type and mutant viral RNA genomes in clinical specimens. Figure 3b shows the sequence chromatograms of RT codons 179–191 from virions pelleted directly from uncultured plasma specimens of subject 1625 before (day –7) and after (days +28 and +140) the initiation of NVP therapy. At day –7, all codons within the amino-terminal half of the RT gene (codons 1–250), including those shown, were wild-type at positions associated with NVP resistance31,32. However, after only 28 days of NVP therapy, the wild-type plasma virus population was completely replaced by a NVP-resistant mutant population differing from the wild-type at codon 190 (glycine-serine substitution). After 140 days of drug therapy, this codon had evolved further such that the plasma virus population consisted of an equal mixture of two drug-resistant strains, one containing G190S and the other containing G190A. There were no other NVP-resistance-conferring mutations detectable within the viral RT gene.

FIG. 3 Quantitative detection of HIV-1 drug-resistance mutations by automated DNA sequencing. a, DNA sequence chromatograms of RT codon 190 from a defined mixture of wild-type (wt) and mutant (mut) HIV-1 cDNA clones differing only at the second base position of the codon. Sequences shown were obtained from, and therefore are presented as, the minus (non-coding) DNA strand. For example, the minus-strand TCC sequence shown corresponds to the plus-strand codon GGA (glycine, G). Similarly, the minus-strand TGC sequence corresponds to the plus-strand codon GCA (alanine, A). The single-letter amino-acid code corresponds to the plus-strand DNA sequence. Mixed bases approximating a 50/50 ratio are denoted as N. b, DNA sequence chromatograms of RT codons 179–191 (again displayed as the minus-strand sequence) derived from plasma-virus-associated RNA of subject 1625 before (day –7) and after (days +28 and +140) starting NVP therapy. Codon changes resulting in amino-acid substitutions at position 190 are indicated for the plus strand. For example, the GCC minus-strand sequence at position 190 (day –7) corresponds to GCC (glycine, G), and the GCT minus-strand sequence at position 190 (day +28) corresponds to AGC (serine, S) in the respective plus strands.

METHODS. Mixtures of wild-type and mutant cDNA clones (a) were prepared and diluted such that first-round PCR amplifications were done with 1,000 viral cDNA target molecules per reaction. HIV-1 RNA was isolated from virions pelleted from uncultured plasma specimens (b), as described24. cDNA was prepared using Moloney murine leukemia virus reverse transcriptase (GIBCO BRL) and an oligonucleotide primer corresponding to nucleotides 4,283 to 4,302 of the HXB2 sequence25. The full-length viral reverse transcriptase gene (1,680 bp) was amplified by means of a nested PCR using conditions and oligonucleotide primers (outer primers: nt 2,483–2,502 and 4,283–4,302; inner primers: nt 2,549–2,565 and 4,211–4,229), previously reported26. Subgenomic fragments of the RT gene were also amplified using combinations of the following oligonucleotide primers: (5) 2,585–2,610; (5) 2,712– 2,733; (3) 2,822–2,844; (3) 3,005–3,028; (3) 3,208–3,228; (3) 3,293–3,324; (3) 3,333–3,350; (3) 3,550–3,572; and (3) 3,904– 3,921. All 3′ primers incorporated the universal primer sequence for subsequent dye-primer sequence analysis. The HIV-1 copy number in every PCR reaction was determined (100–10,000 copies). A total of three to six separate PCR amplifications of primary patient material was done on each sample using different combinations of primers and representative chromatograms are shown. Rarely, codon interpretation was ambiguous. In the day +140 plasma sample from subject 1625 (bottom of panel b), the complementary (plus) strand could read: AGG (serine), GCN (alanine), ACN (threonine), AGA/AGG (arginine) or GGN (glycine). In this case, we sequenced 7 full-length RT molecular clones and found that they encoded only serine or alanine. For sequencing, an automated ABI 373A sequenator and the Taq Dye Primer Cycle Sequencing Kit (ABI) were used. Sequences were analysed using Sequencer (Gene Codes Corp.) and Microgenie (Beckman) software packages, and base-pair mixtures were quantified by measuring relative peak-on-peak heights27.
In all four subjects evaluated by direct viral population sequencing (Fig. 4), specific NVP-resistance-conferring mutations within the RT gene could be unambiguously identified and subsequently confirmed by molecular cloning, expression and drug susceptibility testing. In all cases, mutant virus increased rapidly in the plasma and virtually replaced wild-type virus after only 2–4 weeks of NVP therapy (Fig. 2c). By analysing the rate of accumulation of resistant mutants in the plasma population, we could obtain an independent estimate of the turnover rate of free virus. The rise of drug-resistant mutant virus is influenced substantially by the preceding increase in the CD4+ cell population (which provides additional resources for virus production) and therefore follows complex dynamics. However, we could obtain an estimate of these dynamics by making simplifying assumptions. We assume that wild-type virus declines exponentially with a decay rate $\alpha$, and that the drug-resistant mutant increases exponentially with the rate $\beta$. Thus, the ratio of mutant to wild-type virus increases exponentially at the combined rate $\alpha + \beta$. Our genetic RNA (cDNA) data allow us to estimate this sum. Knowing $\alpha$ from our data on virus decline, we get $\beta \approx 0.27$, or a 32% daily virus production (average over 4 patients). Assuming that mutant virus rises exponentially, this corresponds to a doubling time of $\approx 2$ days, which is in excellent agreement with the measured elimination half-life of 2.0±0.9 days for plasma virus (Figs 1 and 2a). Turnover of viral DNA from wild-type to drug-resistant mutant in PBMCs was delayed and less complete compared to plasma virus, reaching levels of only 50–70% of the total PBMC-associated viral DNA population by week 20 (Fig. 2c). Measurement of the time required for resistant virus to spread in the PBMC population allowed us also to estimate the half-life of infected PBMCs. After complete turnover of mutant virus in the plasma pool, we may assume that PBMCs infected with wild-type virus decline exponentially at a rate $d$, whereas cells infected by mutant virus are generated at a constant rate, but also decline exponentially at rate $d$. With these simplifying assumptions, the rate at which the frequency of resistant virus in the PBMC population increases provides an estimate for the parameter $d$ and hence for the half-life of infected PBMCs. We obtained a half-life of $\approx 50–100$ days. This means that the average half-life of infected PBMCs is very long and of the same order of magnitude as the half-life of uninfected PBMCs. Based on the long half-life of PBMCs, and the fact that these cells harbour predominantly wild-type virus at a time (days 14–28) when most virus in plasma is mutant, we conclude that most PBMCs contribute comparatively little to plasma virus load. Indeed, other cell populations, most probably in the lymphoreticular system, must be the major source of virus production.

Direct sequence analysis of viral nucleic acid revealed not only rapid initial turnover in viral populations but also continuing viral evolution with respect to drug resistance mutations. In subject 1625 (Fig. 4, top panel), wild-type virus in plasma was completely replaced after 28 days of NVP therapy by mutant virus.

![Figure 4](image-url)

**FIG. 4** Quantitative detection of HIV-1 drug resistance mutations: automated DNA sequencing in plasma viral DNA (cDNA) and PBMC-associated viral DNA populations before and after the initiation of NVP on day 0. As in Fig. 3, minus-strand sequences are shown together with single-letter amino-acid codes of the corresponding plus-strand sequence. Mixed bases approximating a 50/50 ratio are denoted as $N$.

**METHODS.** HIV-1 cDNA was prepared from virions pelleted from uncul- tured plasma as described for Fig. 3. Viral DNA was isolated from uncul- tured PBMCs, as described. The full-length viral reverse transcriptase genes as well as subgenomic fragments were amplified and sequenced as described for Fig. 3. The HIV-1 copy number in every PCR reaction was determined (100–10,000 copies). Some sequences were deter- mined from both coding and non-coding DNA strands to ensure the accuracy of quantitative measurements.
TABLE 1. \emph{In situ} functional analysis of HIV-1 RT clones

<table>
<thead>
<tr>
<th>Subject</th>
<th>Specimen</th>
<th>Functional clones</th>
<th>NVP-sensitive clones</th>
<th>NVP-resistant clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>1625</td>
<td>Plasma</td>
<td>day -7</td>
<td>80</td>
<td>80 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+14</td>
<td>72</td>
<td>27 (38%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+28</td>
<td>57</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+14</td>
<td>57</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+140</td>
<td>86</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1625</td>
<td>PBMC</td>
<td>-7</td>
<td>163 (100%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+14</td>
<td>121 (100%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+28</td>
<td>258 (134%)</td>
<td>52%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+84</td>
<td>133 (43%)</td>
<td>90 (68%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+140</td>
<td>261 (65%)</td>
<td>196 (75%)</td>
</tr>
<tr>
<td>1624</td>
<td>Plasma</td>
<td>-7</td>
<td>19</td>
<td>19 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+14</td>
<td>34</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+28</td>
<td>79</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+140</td>
<td>27</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>1624</td>
<td>PBMC</td>
<td>-7</td>
<td>24</td>
<td>24 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+14</td>
<td>34</td>
<td>29 (85%)</td>
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<td></td>
<td></td>
<td>+28</td>
<td>52</td>
<td>42 (81%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+140</td>
<td>87</td>
<td>26 (30%)</td>
</tr>
<tr>
<td>1605</td>
<td>PBMC</td>
<td>-7</td>
<td>31</td>
<td>31 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+140</td>
<td>31</td>
<td>31 (100%)</td>
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<tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>+28</td>
<td>41</td>
<td>41 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+140</td>
<td>38</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Full-length RT genes were amplified by PCR from uncultured plasma and uncultured PBMCs as described in Fig. 3 legend. DNA products were cloned into the EcoRII and HindIII sites of the bacterial expression plasmid pGL18-1 (refs 29, 30). The expression plasmids were screened for the presence of functional RT and tested in situ for susceptibility to NVP inhibition at 3,000 nM (~50–75 fold greater than the IC50). To ensure accuracy in distinguishing RT genes encoding NVP-resistant versus sensitive enzymes, and to confirm the identification of specific NVP-resistance-conferring mutations obtained by direct sequencing (Figs 3 and 4), we determined the complete nucleotide sequences of 21 cloned RT genes which had been phenotyped in the \emph{in situ} assay (V.A.J. and G.M.S., submitted). There was complete concordance between the phenotypes and genotypes of these 21 clones with respect to NVP-resistance-conferring mutations, as well as complete concordance between direct viral population sequences and clone-derived sequences at NVP-resistance-conferring codons.

(G190S), which in turn evolved by day 140 into a mixture of G190S and G190A. In subject 1624 (Fig. 4, middle panel), two codon changes conferring NVP resistance occurred. A G190A substitution appeared in plasma virus at day 14 and a Y181C appeared at day 42. Similarly, in subject 1605 (not shown), a Y181C mutation appeared in plasma at day 14 and a Y188L mutation at day 28. The sequential changes in virus populations were monitored by similar changes in PBMCs at later time points. In subject 1619, the pattern of resistance changes was even more complex (Fig. 4, bottom panel). By day 14, approximately 70% of plasma virus contained a G190A mutation. By day +28, this mutant population was largely replaced by virus containing a Y188F/L substitution. By day 84, still another major shift in the viral quasispecies occurred, this time resulting in a population of viruses containing mutations at both Y181C and G190A. Finally, by day 288 the viral population in plasma consisted exclusively of a mutant expressing a single tyrosine-to-isoleucine substitution at position 181 (Y181I); mutations at codons 188 and 190 were not present in this virus population. All of these amino-acid substitutions at RT codons 181, 188 and 190 were shown in our \emph{in situ} expression studies and by others 31,32 to confer high-level NVP resistance. The direct sequence analyses thus demonstrate that major changes in the HIV-1 quasispecies occur quickly and continuously in response to selection pressures and that these changes are reflected first and most prominently in the plasma virus compartment.

\textbf{In situ RT gene expression and drug susceptibility testing.}

Because direct sequence analysis of viral mixtures provides only semiquantitative information and does not distinguish between viruses with functional rather than defective RT genes, we employed another method for quantifying virus turnover in uncultured plasma and PBMC compartments. Full-length RT genes were amplified by polymerase chain reaction (PCR), cloned into pGL18-1, expressed in \textit{Escherichia coli}, and tested individually for enzymatic function and NVP susceptibility by \emph{in situ} assay 29,30 (Table 1). For subject 1625 at day -7, 100% (80/80) of RT clones from plasma and 100% (163/163) of RT clones from PBMCs expressed enzyme that was sensitive to NVP inhibition. By day 14, however, 62% of plasma-derived clones expressed enzyme that was resistant to NVP, and by days 28, 84 and 140, 100% were resistant. Conversely, at day 14, 0% of PBMC-derived clones expressed NVP-resistant enzyme, and even after 28, 84 and 140 days, only 48–75% of clones were resistant. Similar results were obtained for the other study subjects (Table 1). Thus, the kinetics of virus population turnover determined by a quantitative RT \emph{in situ} expression assay corresponded closely with those determined by direct population sequencing (Fig. 2c).

\textbf{Infectious virus drug susceptibility testing.} Plasma and PBMCs are known to harbour substantial proportions of defective or otherwise non-infectious virus 34,37. To determine whether the viral genomes represented in total viral nucleic acid (Fig. 4 and Table 1) corresponded to infectious virus with respect to NVP-resistance-conferring mutations, we co-cultivated PBMCs from three of the study subjects (1605, 1624, 1625) with normal donor lymphoblasts in order to establish primary virus isolates. The RT genes of these cultured viruses, obtained before and after therapy, were cloned (Fig. 3 and Table 1 legends) and sequenced in their entirety (V.A.J. and G.M.S., submitted). RT codons associated with NVP susceptibility were completely concordant in cultured and uncultured virus strains. Furthermore, the virus isolates exhibited NVP susceptibility profiles consistent with their genotypes.

\textbf{CD4+ lymphocyte dynamics}

Changes in CD4+ lymphocyte counts during the first 28 days of therapy could be assessed in 17 of our patients (Fig. 2b and data not shown). CD4+ cell numbers increased in every patient between 41 and 830 cells per mm$^3$. For the entire group, the average increase was 186 ± 199 cells per mm$^3$ (mean ± s.d.), or 268 ± 319% from baseline. As CD4+ lymphocytes increase in numbers because of (1) exponential proliferation of CD4+ cells in peripheral tissue compartments, and/or (2) constant (linear) production of CD4+ cells from a pool of precursors, we analysed our data based on each of these assumptions. The average percentage increase in cell number per day (assumption (1)) was 5.0 ± 3.1% (mean ± s.d.). The average absolute increase in cell number per day (assumption (2)) was 8.0 ± 7.8 cells mm$^{-3}$ d$^{-1}$. Given that peripheral blood contains only 2% of the total body lymphocytes 38, and that the average total blood volume is ~5 litres, an increase of 8 cells mm$^{-3}$ d$^{-1}$ implies an overall steady-state CD4+ cell turnover rate (where increases equal losses) of $(50 \times (5 \times 10^7 \text{mm}^3)) \times (8 \text{ cells mm}^{-3} \text{d}^{-1})$, or $2 \times 10^9$ CD4+ cells produced and destroyed each day.

\textbf{Discussion}

Previously, it was shown that lymphoreticular tissues serve as the primary reservoir and site of replication for HIV-1 (refs 11, 19, 20) and that virtually all HIV-1-infected individuals, regardless of clinical stage, exhibit persistent plasma viraemia in the range of $10^6$ to $10^8$ virions per ml. However, the dynamic contributions of virus production and clearance, and of CD4+ cell infection and turnover, to the clinical 'steady-state' were obscure, although not anticipated 32,33,39. We show by virus quantitation and mutation fixation rates that the composite lifespan of plasma virus and of virus-producing cells is remarkably short ($t_{1/2} = 2.0 ± 0.9$ days). This holds true for patients with CD4+ lymphocyte counts as low as 18 cells per mm$^3$ and as high as 355 cells per mm$^3$ (Figs 1 and 2; G.M.S., unpublished). These findings were made in patients treated with three different antiretroviral agents having two entirely different mechanisms of action and using three different experimental approaches for assessing virus turnover. The viral kinetics thus cannot be
explained by a unique or unforeseen drug effect or a peculiarity of any particular virological assay method. Moreover, when new cycles of infection are interrupted by potent antiretroviral therapy, plasma virus levels fall abruptly by an average of 99%, and in some cases by as much as 99.9% (10,000-fold). This result indicates that the vast majority of circulating plasma virus derives from continuous rounds of de novo virus infection, replication and cell turnover, and not from cells that produce virus chronically or are latently infected and become activated. The identity and location of this actively replicating cell population is not known, but appears not to reside in the PBMC pool, consistent with prior reports1.;19,20. Nevertheless, PBMCs traffic through secondary lymphoid organs and to some extent are in equilibrium with these cells9. It is thus possible that a small fraction of PBMCs9,19,21, like a small fraction of activated lymphoreticular cells9, could make an important contribution to viremia.

The magnitude of ongoing virus infection and production required to sustain steady-state levels of viremia is extraordinary: based on a virus t1/2 of 2.0 days and first-order clearance kinetics (k(t) = c(t)Ee(-αt), where α = 0.693/t1/2), 30% or more of the total virus population in plasma must be replenished daily. For a typical HIV-1-infected individual with a plasma virus titre equaling the pretreatment geometric mean in this study (10^5.3 RNA molecules per ml; 2 RNA molecules per virion = 10^9 virions per ml) and a plasma volume of 3 litres, this amounts to (0.30) x (10^5.3) x (3 x 10^1) = 1 x 10^8 virions per day (range for all 22 subjects, 2 x 10^7 to 7 x 10^8). Even this may be a substantial underestimate of virus expression because virions may be inefficiently transported from the interstitial extravascular spaces into the plasma compartment and viral protein expression alone (short of mature particle formation) may result in cytopathy or immune-mediated destruction. Because the half-life of cells producing the majority of plasma virus cannot exceed 2.0 days, at least 30% of these cells must also be replaced daily. In our patients, we estimated the rate of CD4+ lymphocyte turnover to be, on average, 2 x 10^7 cells per day, or about 5% of the total CD4+ lymphocyte population, depending on clinical stage. This rapid and ongoing recruitment of CD4+ cells into a short-lived virus-expressing pool probably explains the abrupt increase in CD4+ lymphocyte numbers that is observed immediately following the initiation of potent antiretroviral therapy, and suggests the possibility of successful immunological reconstitution even in late-stage disease if effective control of viral replication can be sustained.

The kinetics of virus and CD4+ lymphocyte production and clearance reported here have a number of biological and clinical implications. First, they are indicative of a dynamic process involving continuous rounds of de novo virus infection, replication and rapid cell turnover that probably represents a primary driving force underlying HIV-1 pathogenesis. Second, the demonstration of rapid and virtually complete replacement of wild-type virus by drug-resistant virus in plasma after only 14-28 days of drug therapy is a striking example of the capacity of the virus for biologically relevant change. In particular, this implies that HIV-1 must have enormous potential to evolve in response to selection pressure exerted by the immune system.9 Although other studies10-20 have provided some evidence that virus turnover occurs sooner in plasma than in PBMCs, our data show this phenomenon most clearly. A similar experimental approach involving the genotypic and phenotypic analysis of plasma virus could be helpful in identifying viral mutations and selection pressures involved in resistance to other drugs, immune surveillance and viral pathogenicity. Third, the difference in lifespan between virus-producing cells and latently infected cells (PBMCs) suggests that virus expression per se is directly involved in CD4+ cell destruction. The data do not suggest an 'innocent bystander' mechanism of cell killing whereby uninformed or latent-infected cells are indirectly targeted for destruction by adsorption of viral proteins or by autoimmune reactivities. Although we have emphasized that most virus in plasma derives from an actively replicating short-lived population of cells, latently infected cells that become activated or chronically producing cells that generate proportionately less virus (and thus do not contribute substantially to the plasma virus pool) may nonetheless be important in HIV-1 pathogenesis. Based on in situ analysis20, these cells far outnumber the actively replicating pool and the diversity of their constituent viral genomes represents a potentially important source of clinically relevant variants, including those conferring drug resistance. In future studies, it will be important not only to discern the specific elimination rates of free virus and of the most actively producing cells, but also the dynamics of virus replication and cell turnover in other cell populations and in patients at earlier stages of infection. Such information will be essential to developing a better understanding of HIV-1 pathogenesis and a more rational approach to therapeutic intervention.
Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection

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Treatment of infected patients with ABT-538, an inhibitor of the protease of human immunodeficiency virus type 1 (HIV-1), causes plasma HIV-1 levels to decrease exponentially (mean half-life, 2.1 ± 0.4 days) and CD4 lymphocyte counts to rise substantially. Minimum estimates of HIV-1 production and clearance and of CD4 lymphocyte turnover indicate that replication of HIV-1 in vivo is continuous and highly productive, driving the rapid turnover of CD4 lymphocytes.

In HIV-1 pathogenesis, an increased viral load correlates with CD4 lymphocyte depletion and disease progression19, but relatively little information is available on the kinetics of virus and CD4 lymphocyte turnover in vivo. Here we administer an inhibitor of HIV-1 protease, ABT-538 (refs 10, 11), to twenty infected patients in order to perturb the balance between virus production and clearance. From serial measurements of the subsequent changes in plasma viroemia and CD4 lymphocyte counts, we have been able to infer kinetic information about the pretreatment steady state.

ABT-538 has potent antiviral activity in vitro and favourable pharmacokinetic and safety profiles in vivo10. It was administered orally (400-1,200 mg per day) on day 1 and daily thereafter to twenty HIV-1-infected patients, whose pretreatment CD4 lymphocyte counts and plasma viral levels ranged from 36 to 490 per mm³ and from 15 x 10³ to 554 x 10³ virions per ml, respectively (Table 1). Post-treatment CD4 lymphocyte counts were monitored sequentially, as were copy numbers of particle-associated HIV-1 RNA in plasma, using an ultrasensitive assay (Fig. 1 legend) based on a modification of the branched DNA signal-amplification technique12,13. The trial design and clinical findings of this study will be reported elsewhere (M.M. et al., manuscript in preparation).

Kinetics of HIV-1 turnover

Following ABT-538 treatment, every patient had a rapid and dramatic decline in plasma viraemia over the first two weeks. As shown using three examples in Fig. 1a, the initial decline in plasma viraemia was always exponential, demonstrated by a straight-line fit to the data on a log plot. The slope of this line, as defined by linear regression, permitted the half-life (t½) of viral decay in plasma to be determined (Fig. 1 legend): for example, patient 409 was found to have a viral decay slope of −0.47 per day, yielding a t½ of 1.5 days (Fig. 1a). Hence the rate and extent of decay of plasma viraemia was determined for each patient. As summarized in Table 1, in every case there was a rapid decline, the magnitude of which ranged from 11- to 275-fold, with a mean of 66-fold (equivalent to 98.5% inhibition).

The residual viraemia may be attributable to inadequate drug concentration in certain tissues, drug resistance, persistence of a small long-lived virus-producing cell population (such as macrophages), and gradual activation of a latently infected pool of cells. As summarized in Table 1, the viral decay slopes varied from −0.21 to −0.54 per day, with a mean of −0.34 ± 0.06 per day; correspondingly, t½ varied from 1.3 to 3.3 days, with a mean of 2.1 ± 0.4 days. The latter value indicates that, on average, half of the plasma virions turn over every two days, showing that HIV-1 replication in vivo must be highly productive.

The exponential decline in plasma viraemia following ABT-538 treatment reflects both the clearance of free virions and the loss of HIV-1-producing cells as the drug substantially blocks new rounds of infection. But although drug inhibition is probably incomplete and virus-producing cells are not lost immediately, a minimum value for viral clearance can still be determined (Fig. 1 legend) by multiplying the absolute value of the viral decay slope by the initial viral load. Assuming that ABT-538 administration does not affect viral clearance, this estimate is also valid before treatment. As the viral load varies little during the pretreatment phase (Fig. 1a, and data not shown), we assume there exists a steady state and hence the calculated clearance rate is equal to the minimum virus production rate before drug therapy. Factoring in the patient's estimated plasma and extracellular fluid volumes based on body weight, we determined the minimum daily production and clearance rate of HIV-1 particles for each case (Table 1). These values ranged from 0.05 to 2.07 x 10³ virions per day with a mean of 0.68 ± 0.13 x 10³ virions per day. Although these viral turnover rates are already high, true values may be up to a few-fold higher, depending on the t½ of virus-producing lymphocytes. The precise kinetics of this additional parameter remains undefined. However, the mean t½ of virus-producing cells is probably less, or in any case cannot be much larger, than the mean t½ of 2.1 days observed for plasma viraemia elimination, demonstrating that turnover of actively infected cells is both rapid and continuous. It could also be inferred from our data that nearly all (98.5%) of the plasma virus must come from recently infected cells.

Examination of Fig. 1b shows that the viral decay slopes (clearance rate constants) are independent of the initial viral loads. The slopes do not correlate with the initial CD4 lymphocyte counts (Fig. 2a), another indicator of the disease status of patients. Therefore these observations strongly suggest that the viral clearance rate constant is not dependent on the stage of HIV-1 infection. Instead, they indicate that viral load is largely a function of viral production, because clearance rate constants vary by about 2.5-fold whereas the initial loads vary by almost 40-fold (Table 1).

Kinetics of CD4 lymphocyte turnover

After ABT-538 treatment, CD4 lymphocyte counts rose in each of the 18 patients that could be evaluated. As shown in three examples in Fig. 3, some increases were dramatic (patient 409, for example) whereas others (such as patient 303) were modest. Based on the available data, it was not possible to determine
with confidence whether the rise was strictly exponential (Fig. 3, top) or linear (Fig. 3, bottom). An exponential increase would be consistent with proliferation of CD4 lymphocytes in the periphery, particularly in secondary lymphoid organs, whereas a linear increase would indicate cellular production from a precursor source such as the thymus. Given that the thymus involutes with age and becomes further depleted with HIV-1 infection, it is more likely that the rise in CD4 lymphocytes is largely due to proliferation. Nevertheless, as both components may contribute, we analysed the observed CD4 lymphocyte data by modelling both exponential and linear increases.

The slope of the line depicting the rise in CD4 lymphocyte counts on a log plot was determined for each case (Fig. 3, top). Individual slopes varied considerably, ranging from 0.004 to 0.088 per day, with a mean of 0.047 per day (Table 1), corresponding to a mean doubling time of ~15 days (Fig. 3 legend). On average, the entire population of peripheral CD4 lymphocytes was turning over every 15 days in our patients during the pretreatment steady state when CD4 lymphocyte production and destruction were balanced. Moreover, the slopes were inversely correlated with baseline CD4 lymphocyte counts (Fig. 2b) in that patients with lower initial CD4 cell counts had more pro-
## TABLE 1 Summary data of HIV-1 and CD4 lymphocyte turnover during the pretreatment steady state

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD4 cell count (mm-2)</th>
<th>Plasma viremia (virems per ml x 10^3)</th>
<th>Slope</th>
<th>I_1/2 (days)</th>
<th>Minimum production and clearance (virems per day x 10^6)</th>
<th>Slope</th>
<th>Blood production and destruction (cells per day x 10^6)</th>
<th>Total (cells per day x 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>301</td>
<td>76</td>
<td>193</td>
<td>-0.30</td>
<td>2.3</td>
<td>0.56</td>
<td>0.070 (6.9)</td>
<td>21.7 (28.1)</td>
<td>1.1 (1.4)</td>
</tr>
<tr>
<td>302</td>
<td>209</td>
<td>80</td>
<td>-0.27</td>
<td>2.6</td>
<td>0.26</td>
<td>0.004 (0.5)</td>
<td>4.3 (2.7)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>303</td>
<td>293</td>
<td>41</td>
<td>-0.21</td>
<td>3.3</td>
<td>0.11</td>
<td>0.005 (1.4)</td>
<td>9.9 (9.5)</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td>304</td>
<td>174</td>
<td>121</td>
<td>-0.28</td>
<td>2.5</td>
<td>0.54</td>
<td>0.019 (1.9)</td>
<td>22.2 (13.0)</td>
<td>1.1 (0.6)</td>
</tr>
<tr>
<td>305</td>
<td>269</td>
<td>88</td>
<td>-0.33</td>
<td>2.1</td>
<td>0.50</td>
<td>0.055 (21.5)</td>
<td>108.0 (157.0)</td>
<td>5.4 (7.8)</td>
</tr>
<tr>
<td>306</td>
<td>312</td>
<td>175</td>
<td>-0.52</td>
<td>1.3</td>
<td>1.27</td>
<td>0.058 (25.7)</td>
<td>105.0 (150.0)</td>
<td>5.3 (7.5)</td>
</tr>
<tr>
<td>308</td>
<td>386</td>
<td>185</td>
<td>-0.46</td>
<td>1.5</td>
<td>1.48</td>
<td>0.020 (9.1)</td>
<td>55.9 (65.8)</td>
<td>2.8 (3.3)</td>
</tr>
<tr>
<td>309</td>
<td>49</td>
<td>554</td>
<td>-0.29</td>
<td>2.4</td>
<td>1.85</td>
<td>0.088 (11.8)</td>
<td>20.7 (56.6)</td>
<td>1.0 (2.8)</td>
</tr>
<tr>
<td>310</td>
<td>357</td>
<td>15</td>
<td>-0.26</td>
<td>2.7</td>
<td>0.05</td>
<td>0.038 (15.6)</td>
<td>71.0 (81.9)</td>
<td>3.6 (4.1)</td>
</tr>
<tr>
<td>311</td>
<td>107</td>
<td>130</td>
<td>-0.29</td>
<td>2.4</td>
<td>0.51</td>
<td>0.064 (11.0)</td>
<td>38.9 (62.8)</td>
<td>2.0 (3.1)</td>
</tr>
<tr>
<td>312</td>
<td>59</td>
<td>70</td>
<td>-0.30</td>
<td>2.3</td>
<td>0.30</td>
<td>0.048 (4.5)</td>
<td>17.0 (26.9)</td>
<td>0.8 (1.4)</td>
</tr>
<tr>
<td>313</td>
<td>47</td>
<td>100</td>
<td>-0.54</td>
<td>1.3</td>
<td>0.88</td>
<td>0.077 (5.9)</td>
<td>24.7 (40.5)</td>
<td>1.2 (2.0)</td>
</tr>
<tr>
<td>401</td>
<td>228</td>
<td>101</td>
<td>-0.40</td>
<td>1.7</td>
<td>0.47</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>402</td>
<td>169</td>
<td>55</td>
<td>-0.28</td>
<td>2.5</td>
<td>0.21</td>
<td>0.014 (3.1)</td>
<td>13.4 (17.4)</td>
<td>0.7 (0.9)</td>
</tr>
<tr>
<td>403</td>
<td>120</td>
<td>126</td>
<td>-0.32</td>
<td>2.2</td>
<td>0.74</td>
<td>0.015 (2.4)</td>
<td>13.8 (18.7)</td>
<td>0.7 (0.9)</td>
</tr>
<tr>
<td>404</td>
<td>46</td>
<td>244</td>
<td>-0.27</td>
<td>2.6</td>
<td>1.06</td>
<td>0.080 (8.5)</td>
<td>24.6 (57.5)</td>
<td>1.2 (2.9)</td>
</tr>
<tr>
<td>405</td>
<td>490</td>
<td>18</td>
<td>-0.31</td>
<td>2.2</td>
<td>0.08</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>406</td>
<td>36</td>
<td>23</td>
<td>-0.25</td>
<td>2.8</td>
<td>0.08</td>
<td>0.059 (3.4)</td>
<td>12.5 (19.7)</td>
<td>0.6 (1.0)</td>
</tr>
<tr>
<td>408</td>
<td>67</td>
<td>256</td>
<td>-0.47</td>
<td>1.5</td>
<td>2.07</td>
<td>0.073 (5.9)</td>
<td>35.3 (115.0)</td>
<td>1.8 (5.7)</td>
</tr>
<tr>
<td>410</td>
<td>103</td>
<td>99</td>
<td>-0.36</td>
<td>1.9</td>
<td>0.53</td>
<td>0.051 (5.6)</td>
<td>32.4 (34.5)</td>
<td>1.6 (1.7)</td>
</tr>
</tbody>
</table>

Range 36-490  15-554  0.05-2.07  0.0044-0.088 (0.5-25.7)  4.3-108.0 (2.7-157.0)  0.2-5.4 (0.1-7.8)

Mean 180±46  134±40  0.34±0.06  2.1±0.4  0.68±0.13  0.047 (8.6)  35.1 (53.2)  1.8 (2.6)

* The results for the kinetics of CD4 lymphocyte turnover generated by an exponential growth model are shown without parentheses; results generated by a linear production model are shown in parentheses.

† Each viron contains two RNA copies.

‡ Calculated using plasma and extracellular fluid volumes estimated from body weights, and assuming that plasma and extracellular fluid compartments are in equilibrium.

§ Calculated using blood volumes estimated from body weights.

||
|---|---|
|FIG. 2 a. Lack of correlation between viral decay slopes and disease status as indicated by baseline CD4 cell counts. Correlation coefficient = 0.05 (P value <0.1). b. Inverse correlation between the exponential CD4 increase slopes and baseline CD4 cell counts. Correlation coefficient = -0.57 (P value <0.01). Such an inverse correlation would be expected if T-cell proliferation were governed by a density-dependent growth function (logistic, for example), in which the growth rate decreases with increasing population level, if T cells were produced from precursors at a constant rate or from a combination of these two effects. | 125 |
The increase in CD4 lymphocyte counts following ABT-538 administration was also modelled linearly (Fig. 3, bottom). The slope of the line depicting the increase for each case was determined and the values varied from 0.5 to 25.7 cells per mm$^3$ per day, with a mean of 8.6 cells per mm$^3$ per day (Table 1). Using the same argument as for the exponential case, minimum estimates of total CD4 production (or destruction) rates at baseline were determined to vary from $0.1 \times 10^4$ to $7.8 \times 10^5$ cells per day, with a mean of $2.6 \times 10^5$ cells per day.

Although our two sets of CD4 lymphocyte analyses do not yield identical numerical results, they are in close agreement and emphasize the same qualitative points about HIV-1 pathogenesis. The number of CD4 lymphocyte destroyed and replenished each day is of the order of $10^9$, which is strikingly close to estimates of the total number of HIV-1 RNA-expressing lymphocytes in the body determined using in situ polymerase chain reaction and hybridization methods. In addition, CD4 replenishment appears to be highly stressed in many patients in that the faster production rates are $25-78$-fold higher than the slower rates (Table 1), which is presumably still higher than the as-yet-undefined normal CD4 turnover rate. The precise mechanisms of CD4 lymphocyte repopulation, however, will have to be addressed in the future by studies on phenotypic markers and functional status of the regenerating cells. Nonetheless, the rapid CD4 lymphocyte turnover has several implications. First, the apoptosis commonly observed in the setting of HIV-1 infection may simply be an expected consequence of an active lymphocyte regenerative process. Second, the CD4 lymphocyte depletion seen in advanced HIV-1 infection may be likened to a sink containing a low water level, with the tap and drain both equally wide open. As the regenerative capacity of the immune system is not infinite, it is not difficult to see why the sink eventually empties. It is also evident from this analogy that our primary strategy to reverse the immunodeficiency ought to be to target virally mediated destruction (plug the drain) rather than to emphasize lymphocyte reconstitution (put in a second tap).

**Discussion**

We believe our new kinetic data have important implications for HIV-1 therapy and pathogenesis. It is self evident that, with rapid turnover of HIV-1, generation of viral diversity and the attendant increased opportunities for viral escape from therapeutic agents are unavoidable sequelae. Treatment strategies, if they are to have a dramatic clinical impact, must therefore be initiated as early in the infection course as possible, perhaps even during seroconversion. The rapid turnover of HIV-1 in plasma also suggests that current protocols for monitoring the acute antiviral activity of novel compounds must be modified to focus on the first few days following drug initiation. Our interventional approach to AIDS pathogenesis has shown that HIV-1 production and clearance are delicately balanced but highly dynamic processes. Taken together, our findings strongly support the view that AIDS is primarily a consequence of continuous, high-level replication of HIV-1, leading to virus- and immune-mediated killing of CD4 lymphocytes.
HIV: Science by press conference

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One of the most disturbing aspects of what passes for AIDS research these days, is the separation between what researchers actually find, what they tell the press conference and what the media tells the public. To assume that these three are identical or even similar would be pure folly. The press conference has a venerable history in AIDS science. When Robert Gallo’s group ‘found’ that 26 out of 72 AIDS patients had small amounts of newly discovered retrovirus in their bodies, this interesting but unconvincing work was transformed by the magic of an officially sanctioned press conference into the announcement of the ‘probable cause of AIDS’. And Gallo’s modest findings became ‘compelling evidence’ that this new retrovirus was the cause of AIDS. Ten years later nothing has changed.

In 1993 another press conference announced that large amounts of HIV had been found in lymph glands in HIV positive people. The HIV skeptic’s claims that HIV cannot cause AIDS because it is present in only minute quantities was finally laid to rest. The lymph glands were ‘packed’ with HIV in HIV positive people. It is just a pity that the facts did not match the hype. The press conference where the findings on HIV in lymphoid tissue were announced, was given by Dr. Anthony Fauci and Dr. Ashley Haase, and the results appeared in Nature on March 25 1993 in back to back papers. Oddly enough if you read these papers, the results do not actually seem to be what was claimed at the press conference. In the paper by Fauci’s group (Pantaleo et al., ibid) it is stated that 12 patients were studied and results for 3 of them are hidden away in the caption to figure 1. They actually found HIV in only 1 in 1000 T cells in 2 of the three, and between 1 in 10 and 1 in 100 in the third, a patient with full blown AIDS. In other words they found no more HIV than anybody else ever has. How then do we explain the extravagant claims at the press conference? It is probably safer not to try.

Skip forward 2 years and we are back at another press conference. This time it is January 12, 1995 and David Ho and George Shaw are presenting the results of a study which seems to turn the last 10 years of HIV research on its head. Using new drugs and new techniques Ho and Shaw have found that HIV is an incredibly active virus, producing billions of offspring every 2 days, and killing billions of T4 cells in a battle to the death with the immune system. Once more the HIV sceptics have been and the answered. The journalists, lead by Lawrence K. Altman of the New York Times rush to tell us that a massive battle is going on between the virus and the immune system from the beginning of infection, a battle that lasts 10 years and that the immune system can only win if we pump healthy HIV positive people full of experimental drugs with no demonstrated benefits.

But stop for a minute. It is all too easy to be swept away by the hyperbole of journalists and the enthusiasm of scientists for their own work. (I say this as a scientist, but the easiest way to engage a scientist in conversation is to ask about their work. And scientists are extremely bad at judging the quality of their own work). We have to ask fundamental questions here. Does what Ho and Shaw say actually make any sense? Are their experimental techniques sound? Do their conclusions follow from their results? Is their mathematical analysis sound? If we are to evaluate the worth of this work we have to answer these questions. In fact we have to answer these questions for just about any scientific paper ever written, so we should certainly not spare HIV researchers. Particularly as we can be certain they will not ask themselves these questions. These are the issues that I will address now. My conclusion will be that this new work is about as convincing as a giraffe trying to sneak into a polar bears only picnic by wearing sunglasses (as Ben Elton might say).
To begin with, what were these people trying to do? They wanted to measure the rate at which both HIV and T cells are produced in infected people. The idea is deceptively simple. You measure the viral load in a patient at a given time, then you pump them full of antiviral drugs. The drugs reduce the amount of virus present in the blood by some factor. (Claimed to be an implausible 98.5% in these papers. Implausible because there is no possible way that viral load can be measured as accurately as the figure of 98.5% suggests). You then wait till HIV magically mutates into 'drug resistant' strains, and wait till the viral load returns to pre-treatment levels. This gives an estimate, through some relatively simple mathematics, for the rate at which the virus replicates. Both Ho and Shaw's groups found that in the absence of viral clearance, the total amount of virus in the body should double every 2 days. So suddenly the low levels of viral replication found in the past decade are thrown out the window, and HIV is now the cause of a relentless battle, a battle that takes place over a decade or more. The measurement of T cell production can be made in much the same way.

So our question must be whether or not we should believe these results? After all at the last big press conference, Ashley Haase's group (Embreton et al., Nature, March 25, 1993) found low levels of HIV RNA in the T cells of patients studied (4 people, one of whom had no HIV proviral DNA at all) indicating 'low levels' of viral replication. So what do we do when one press conference seems to contradict the other? Clearly we have to examine both studies carefully.

As a mathematician, I was intrigued by the claim of John Maddox, editor of Nature, that the new results provide a new mathematical understanding of the immune system. Unfortunately, my confidence in this claim was badly shaken when it turned out that on the very first page of the Shaw paper (Wei et al., p 117, Nature, Jan 12, 1995) they make an appalling mathematical error. And in the same paragraph make an assumption which turns out, by their own admission to have no basis in observation, and which they give no justification for. The authors of Wei et al. are attempting to give a mathematical formula for the amount v of free virus at time t. They state that virus is produced by virus producing cells y, at rate k, and decays exponentially at rate u. These two statements are mutually contradictory but that is not a real problem. If they change the word 'decays' to 'is cleared' then all is well. This leads to what is known as a differential equation for v which may be solved easily. (Craddock, letter to Nature unpublished)). They state a formula for v based on their assumptions, which unfortunately is completely wrong. Confidence that anything good will come out of this paper plummets at this point. Their result for v is not only wrong, but it does not even look right. You do not have to be a mathematician to realise that if the rate at which v is produced depends on ky(t), where y(t) is the number of virus producing cells at time t, then v is going to depend upon ky(0), where y(0) is the initial size of the virus producing cell population. So one wonders how they manage to produce a formula for v which does not depend upon ky(0) at all?

And they state in the same paragraph that virus producing cells can to 'a good approximation' be assumed to decline exponentially. They then state a few lines further down that they 'have data only for the decline of free virus, and not for virus producing cells'. If they have no data for virus producing cells then how can they possibly know that these cells decline exponentially? They might do anything. That is the whole point of not having any data. You do not know what is happening.

The problems that these errors entail are self evident. They are trying to estimate viral production rates by measuring viral loads in different times and trying to fit the numbers to their formula for free virus. But if their formula is wrong, then their estimates for viral production will be wrong too.

As bad as these mistakes are, they are only the beginning. It turns out that the whole basis for the measurements given in both papers is an unvalidated technique. Namely Quantitative Competitive Polymerase Chain Reaction. QC-PCR is based upon a deceptively simple idea. PCR mass produces fragments of DNA. You start with a small amount of DNA and after each PCR cycle the amount of DNA you have is between 1 and 2 times the amount at the beginning of the cycle. Thus the amount of DNA you have to study increases exponentially. The fact that the PCR is an exponential growth process means that experimental errors will also grow exponentially, so that you need to be very careful about what you do with the process. A number of people have decided that is should be possible to estimate the amount of DNA present in a sample by using PCR. This is so called quantitative competitive PCR. The idea is to add to the sample to be estimated a known amount of similar but distinguishable DNA and amplify both together. The assumption is that the relative amounts of the two products should stay the same, and hence you can work out the size of the sample you started with by knowing the ratio of the two,
determined by observation when PCR has produced enough of both to measure, and how much control DNA was added. What is absolutely crucial is that the relative sizes of the test DNA and your known control must remain EXACTLY equal. Close is not good enough. Because the slightest variations will be magnified exponentially and can produce massive errors in your estimate. The difficulties in using PCR quantitatively were pointed out by Luc Raemaekers in the journal Analytical Biochemistry in 1993. He demonstrated that published papers on QC-PCR contain data that show the fundamental assumption that the relative sizes of the samples remains constant is not met in practice. Despite this HIV researchers continue to use PCR to quantify viral load. The bottom line in all of this is that data obtained using QC-PCR must be treated with extreme caution. There is simply no way of knowing whether a given estimate is correct or is 100,000 times too high!

So we have an extraordinary problem already. We do not know whether or not the data that Shaw and Ho's groups obtained is actually meaningful. And the mathematical basis of the analysis used by Shaw's group is to say the least questionable. But these groups actually manage to do a lot worse than this. Neither group compared the rate of T4 cells generated in the HIV positive patients with HIV negative controls! Both groups assert that in HIV infected individuals, up to 5% of the circulating T4 cells are replaced every 2 days. This information is hardly new, Peter Duesberg says something similar in a paper in the proceedings of the National Academy of Sciences from 1989. Except he states that 5% of the bodies T cells will be replaced every 2 days, in healthy people.

The logic here is remarkable. It is claimed that HIV sends the immune system into overdrive as measured by a supposedly accelerated production of T4 cells. Between 100 million and 2 billion are produced each day in the patients that were studied. But where are the healthy controls? How can this production of T cells be ascribed to HIV if there is no comparison made with healthy people? And even if there were a comparison, how can the production by unambiguously attributed to the 'battle' with HIV? The patients in both study groups were being treated with new drugs such as Nevirapine (we are naturally told nothing of possible toxic side effects of these drugs) whose effects are largely unknown. So how can these results be extrapolated to HIV positive people who are not taking these drugs? It must surely be admitted that the system they are trying to study, namely the interaction of HIV with T4 cells, might behave substantially differently in people who are not being pumped full of new drugs, in addition to 'antiretrovirals' like Zidovudine?

Yet HIV 'science' has declined so far that these elementary questions are addressed neither by the research groups themselves, nor the referees at Nature whose job it is to critique the papers before publication. Is nobody at Nature bothered by the fact that neither paper contain any hard data which can be independently analysed? And Wei et al., use a technique for measuring viral load known as branched DNA. (bDNA). Yet their data for bDNA does not appear in the paper. The reader is given absolutely no explanation of how this assay of viral load is supposed to be carried out, and no indication of how reliable it is. All questions on bDNA are referred to 3 papers in preparation. But nobody in the HIV research community is at all bothered by this. They seem to have learned like the mad hatter to believe 6 impossible things before breakfast and so one more makes no difference. One gets a remarkable sense of being disassociated from the real world when entering the realm of AIDS research. Am I mad or are they?

We are to believe from Wei et al., that variation at a single place in the HIV genome can confer immunity to the virus against the new drugs. (In which case what is the point of administering them?). Yet currently PCR uses the DNA polymerase (the enzyme in cells that makes new DNA from old) from Thermus Aquaticus. This 'Taq' polymerase has not proof reading capacity. We then have to ask: are changes in the genome of HIV that appear after drug therapy due to the virus mutating, or are they an artifact of the PCR technique's inability to correct errors? There are no prizes for guessing how much attention the authors of both studies pay to this question. None.

Other people have made powerful objections to these papers based upon biochemical considerations. Duesberg and Bialy have written a superb critique, as have Eleni Eleopulos-Papadopoules, Val Turner and John Papadimitrou (although astonishingly their letter to Nature was rejected. What a shock!) As I have made clear, there are many problems that I see with these studies, but us as a mathematician, I have left what I consider to be the worst till last. The problem is this. If there is so much HIV present, and it is replicating so fast, why does 'HIV disease' take ten years to progress to AIDS?

In Ho et al., they use the analogy of a sink with the drain open, and the water pouring in from a tap at a slightly lower rate that it drains away. So you get a
slow steady decline in CD4 cells. Ho et al. have a few equations that are supposed to describe the changes in virus levels and CD4 cells over time. What do these equations actually predict, as opposed to what Ho et al. say they predict? In order to make them work you have correctly formulate them, which Ho et al. do not. When correctly formulated (Craddock, Ibid) what emerges is stunning. Ho et al.’s observations combined with their simple model for T cells and virus, predict that the T cell count should reach an equilibrium state quickly. Meaning exponentially fast. It is actually difficult to understand exactly what the equation on p 126 of Ho et al. is supposed to mean, but it definitely predicts that equilibrium is approached exponentially. When you add terms to the equation to describe the effects of Virus (inexplicably, they do not include the effects of the virus on the T cell population in their model. I thought HIV was supposed to be killing these cells somehow), then include the expression for the amount of virus that they give on p 124, you get a picture of ‘HIV disease’ that bears no relation to what happens in actual patients. AIDS should develop in days or weeks. There is no possible way it can take ten years. This emerges from Ho et al.’s., own model. They seem blissfully unaware of the prediction that their own results give. They probably have not bothered to look at tedious questions like ‘do our results correspond with what we observe in patients?’

Science is about making observations and trying to fit them into a theoretical framework. Having the theoretical framework allows us to make predictions about phenomena that we can then test. HIV ‘science’ long ago set off on a different path. It seems as if nobody bothers to check the details in this field. Nobody is asking the fundamental questions, and nobody wants to. People who ask simple, straightforward questions are labelled as loonies who are dangerous to public health. Yet we desperately need people to ask the questions. And it does not matter what the answers to the questions are as long as they are asked. My question is this? Just what exactly will it take to get the people doing HIV research to turn away from high tech, unproven methods, arcane speculations about molecular interactions etcetera etcetera and ask themselves ‘Do any of us have the faintest idea what we are doing?’
Supplementary Notes for HIV: Science by Press Conference

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In my article HIV: Science by Press Conference[1] I made a series of criticisms of the so-called Ho/Shaw model of HIV dynamics ("The new view of HIV", as it has been called) which first appeared in Nature in 1995 [2]. In it I referred to a number of what I considered to be mathematical problems with the Ho/Shaw model, including some curious modelling and apparent errors in the mathematics. However the article was aimed at a general audience and consequently it was not possible to detail the precise nature of some of my problems with the Ho/Shaw papers. The purpose of this note is to elaborate upon some points mentioned in the earlier piece for the benefit of a mathematically literate audience.

The purpose of the work undertaken by the Ho and Shaw groups was to obtain estimates for the rate of production of HIV in seropositive individuals by fitting data to a mathematical model of HIV infection. They also obtained estimates for the rate of production of CD4 cells.

It is important to understand that there does not exist any satisfactory mathematical model of HIV infection [3]. By satisfactory I mean a model which is

1) A natural consequence of a minimal set of assumptions;
2) Predicts with high accuracy the development of immune system collapse and the development of AIDS in seropositive individuals;
3) Remains stable under small perturbations of initial conditions and parameters;

The nonexistence of such a model does not mean that no such model is possible of course. It does suggest however that after fifteen years of effort, our understanding of so-called "HIV disease" is rather poor. The fact that HIV does not lend itself to effective mathematical modelling might be taken as evidence that perhaps its role in the development of AIDS may not be as is commonly thought. [4] This possibility is of course extremely controversial to say the least. Perhaps however the time is approaching when it will have to be taken seriously.

Examples of attempts to mathematically model HIV infection abound. Well known examples include Nowak et al’s Antigenic Diversity model[5] which was shown to be flawed by Stillianakis, Schenzle and Dietz [6].

A common defect of all such models is the failure to include the toxic effects of HIV chemotherapy regimes on T cell population and production. Kirschner in a recent model [3], assumes that AZT will only act on infected T cells. This is possibly related to the article of faith, supported by no evidence I am aware of that AZT preferentially inhibits the activity of retroviral Reverse Transcriptase (RT). AZT was developed years before RT was even discovered. It is a nucleoside analogue drug, essentially 'fake' Thymidine, one of the nucleic acids that make up DNA. During DNA synthesis, if AZT is taken up instead
of genuine thymidine, DNA synthesis terminates and the cell dies. Hence AZT is known as a DNA chain terminator. RT is an enzyme that translates RNA, commonly but not exclusively, in retroviruses, [7], into DNA. Why AZT should preferentially inhibit RT is far from clear. Yet it is generally assumed to be the case and modellers consistently ignore the effects of chemotherapy on the status of a patients immune system, instead concentrating solely upon the supposed effects of HIV, despite the fact that it is not at all clear how HIV depletes the T cell population. [8]

If we keep the lack of a good mathematical model for HIV infection in mind, then when we examine the Ho/Shaw publications we have reason for a-priori scepticism. Ho and Shaw were fitting data to a mathematical model for HIV infection. In fact a mathematical model of a rather elementary nature. Since no elementary model describes HIV infection as it is believed to behave, then one must wonder if fitting data to a model which does not describe what is clinically observed is a valid approach? Yet this is exactly what Ho and Shaw did and few people seem to have questioned it.

In [1] I referred to an "appalling mathematical error". The precise nature of what I considered this error to be I shall now elaborate. The comment referred to the Shaw paper, and in particular the second column of page 117. This is where the model for HIV activity is set up.

Shaw's group use a very simple model for HIV, a single linear ordinary differential equation, rather than the more commonly used system of equations. As such it is difficult a-priori to believe that they will get a model that accurately reflects what is taking place in seropositive individuals. This is compounded by some rather dubious assumptions and sloppy presentation that raises considerable questions.

Shaw et al tell us that they are assuming that free virus is produced at a rate $ky(t)$, where $y(t)$ is the number of virus producing cells at time $t$ and $k$ is a constant. Also they assume the virus is cleared at a rate proportional to $v$, the viral load at time $t$. Thus before drug treatment the viral load is governed by

$$\frac{dv}{dt} = ky(t) - uv(t)$$

Where $u$ is a constant. Clearly in order to solve this equation we need to know $y(t)$. It is here that the problems arise in my view. Shaw et al state that "To a good approximation, we can assume that virus-producing cells decline exponentially according to $y(t) = y(0)e^{-\alpha t}$" [Following treatment with the experimental drugs]. Then a few lines below we read "we have data only for free virus and not for virus producing cells". This renders the preceding statement absurd. "To a good approximation we can assume " that virus declines exponentially! A good approximation to what? Shaw and his group are assuming something that they are trying to prove, namely the efficacy of the experimental protease inhibitors they are testing. Protease inhibitors are supposed to suppress HIV production. Shaw and his group simply assume here that they work.

If one then solves the above equation for $v$, assuming that $u$ is constant, then plotting observed viral load levels against time will give an estimate for the clearance rate
However there are considerable problems with this. Firstly, it is far from clear that $u$ should remain constant following treatment with experimental drugs. It might, it might not. It might increase. The measurements undertaken by both Ho's group and Shaw's group occurred once a week. If the timescale for variation in $u$ was a matter of hours or days, then this will not be picked up by their measurements.

This is an important fact. The published estimates for viral production are in fact estimates for viral clearance rates. It is also claimed that they have obtained minimal estimates for viral production. Yet if $u$ has increased because of the perturbation of the system by the experimental protease inhibitors, then they have in fact obtained over estimates for viral production.

Assuming that $u$ is constant we obtain for $v(t)$, the solution

$$v(t) = \frac{k y(0)}{u - \alpha} (e^{-\alpha t} - e^{-u t}) + v(0)e^{-u t}$$

Curiously Shaw et al present a different solution which is independent of $ky(0)$.

$$v(t) = v(0)[ue^{-\alpha t} - \alpha e^{-u t}] / (u - \alpha)$$

These solutions are quite different. How have Shaw et al. obtained their solution? By putting $ky(0) = uv(0)$. But where has this come from? Well it comes from the assumption that is not mentioned that viral load is in a steady state and hence initially $\frac{dv}{dt} = 0$. We the readers are not told this, so Shaw et al's presented solution does not follow from the assumptions stated.

This may appear to be mathematical pedantry. Yet it is an important point. Nowhere in the Shaw paper do we see any evidence that viral load is in fact in a steady state before drug treatment. Indeed patient 5004 for example Fig1, p118 seems to have increasing viral load. Moreover Wain-Hobson in his accompanying commentary notes that "longitudinal studies have shown that the overall viral load, of both free virus and that within infected cells, increases slowly but inexorably"[9]. This would suggest that $\frac{du}{dt} > 0$. So the steady state assumption is an important one that needs to be not only stated explicitly but justified experimentally. After all, if the virus is not in a steady state then measurement of $u$ tells us nothing about viral production. All in all this is a far from satisfactory piece of work.

Other features of my commentary in [1] which may be expanded upon are the question regarding Quantitative Competitive PCR and the claim that Ho et al's model predicts rapid disease development.

I begin with the question of QC-PCR, since the all the observations of Ho and Shaw and much else in HIV research are based upon this unvalidated technique.

The idea of QC-PCR is that coamplification of a gene product with an internal control will allow quantification of the target to take place given knowledge of the initial quantity of the control. So the assumption is that after $n$ PCR cycles the ratio of
derived products remains fixed. Attempts to justify this theoretically have been extremely unconvincing [10] and Raeymaekers [11] has pointed out that published papers on the method include data which suggests that the critical assumption about constant ratios is in fact false. As I pointed out in [12], even tiny deviations in the critical ratio can lead to large errors in quantification.

It is important to remember that no large scale validation of QC-PCR has ever been undertaken. In papers on the subject we typically are given one or two reconstruction experiments with no information on how many times they ran experiments which did not work. A large scale validation would involve testing hundreds of samples by QC-PCR and comparing the results to known data and the results of other assays.

It is even more important to be aware of the fact that it is well known that QC-PCR the so called viral load test, does not measure viral load in any sense at all. PCR amplifies a short segment of DNA, and does not distinguish between DNA from the desired target and DNA from another source.

Thus for example, in Pitak et al [13], of the 9800 base pairs in the proviral DNA of HIV, the PCR assay amplified a short segment in the gag gene of length 260 base pairs. In other words Pitak et al, in common with essentially all other studies involving PCR "detection" of HIV, accepted the detection of three percent of the HIV genome as "proof" of the presence of HIV. This is precisely what the so called "viral load" test that is now widely used does. In this way a tiny fragment of genetic material is confused with infectious virus.

Look at the accompanying table from Piatak et al. (Table 1) The final column gives the quantity of infectious virus detectable in the patient. The third column gives the number of copies of the short segment of HIV RNA detected by QC-PCR. The numbers for HIV RNA could of course be off by orders of magnitudes if there were even small experimental errors present [12], but it is clear that they do not resemble in any way shape or form the actual amount of virus present.

This is even clearer when one looks at the fourth and fifth columns which give levels of the protein p24. Every HIV virion should have hundreds of copies of p24. Yet Piatak's data makes it plain people with hundreds of thousands of HIV RNA copies as measured by QC-PCR have no infectious virus and no p24. Patient MILA0284, for example supposedly had 815,100 HIV RNA molecules as measured by QC-PCR, but two assays found no p24 and no infectious virus either. Indeed over half the subjects have no p24 or infectious virus. Something is clearly wrong with this picture.

Yet in Ho and Shaw's work they simply equated the numbers produced by QC-PCR with viral load. Curious considering that Shaw is one of the authors of the Piatak paper. The consequences of this are obvious. If the numbers that QC-PCR produces, to say nothing of the branch DNA assay, have nothing to do with actual viral load, then the Ho and Shaw papers are utterly irrelevant to the question of HIV dynamics.

Now let us consider the second issue. I remarked that the equations in Ho et al. had not been correctly formulated. What did I mean by that? Ho et al.
the assumption of a steady state explicit unlike Shaw et al, but also give no compelling evidence for it. They have a model for HIV production that is given by

\[
\frac{dV}{dt} = P - cV
\]

where \( P \) is the viral production rate. Here the presentation is decidedly obscure. What is \( P \)? Ho et al state that in the pretreatment steady state \( P = cV \). Does this mean that they are assuming viral production to be proportional to \( V \)? This would make sense. The more free virus, the more infected cells, the more virus produced. But if this is the case, then their equation for \( V \) predicts exponential growth rates for viral load as soon as the production rate constant exceeds the clearance rate constant. It is clearly impossible to believe that production rates will precisely match clearance rates in individuals for more than a decade. Thus Ho et al's model would seem to predict that virus levels will eventually grow exponentially, and disease progression should be rapid.

Yet they talk of a pretreatment steady state. Clearly there is something peculiar taking place here. If we let \( P \) be a constant, then there is a steady state solution to their equation for \( V \), namely \( P = cV \). So it would seem that they have taken viral production levels to be constant, rather than dependent on the amount of virus produced. Or more precisely dependent upon the number of virus infected cells. It is self evident that constant production rates are absurd, and Ho et al are aware of this as their model in Science [14] uses a production rate proportional to the number of infected cells, as does Shaw's model.

The reason constant production rates are absurd is that if one cells produces a thousand virions, and 99.5 percent are cleared, then 5 virions will remain and we will have 5 infected cells, producing 5000 virions, leading to 25 infected cells etc. Clearly the number of virions produced must depend upon the number of infected cells, and so indirectly upon the quantity of free virus present.

However nonconstant production rates predict that sooner or later the virus will grow exponentially. In the numerical example in the preceding paragraph, if instead of 99.5 percent being cleared, we had 99.9 percent being cleared, we get a steady state. But as soon as the clearance rate drops below 99.9 percent, say to 99.5 percent, then we get exponential growth. Ho and Shaw say that the generation time of HIV is approximately 2 days. So in this example the viral load would increase fivefold every two days. Clearly this cannot continue for a decade. It would produce more virions than there are particles in the universe.

In fact since the generation time is two days then one would expect that the virus would cause a disease on a timescale commensurate with the generation time, namely weeks to a few months, like all other diseases that are absolutely known to be viral in origin. (It is claimed that certain diseases are caused by viruses over long timescales, but these are far from certain and not comparable in any regard to what is claimed for HIV infection. Oft cited examples like Shingles are in fact very different what is supposed to take place in HIV infection). Yet HIV is said to take ten years to cause disease, while all
this time replicating furiously. No plausible explanation of how this could possibly happen has ever been presented.

In their article in Science [14] Ho et al present a more sophisticated mathematical model for HIV infection which they claim predicts even higher rates of production. They state p1582 "Before drug treatment, the dynamics of cell infection and virion production are represented by

\[
\frac{dT^*}{dt} = kVT - \delta T^*
\]

\[
\frac{dV}{dt} = N\delta T^* - cV
\]

\( V \) is the number of viral particles in plasma, \( \delta \) is the rate of loss of virus producing cells, \( N \) is the number of new virions produced per infected cell, \( c \) is the rate constant for virion clearance". \( T \) are the target cells and \( T^* \) are the infected cells.

This model as presented is meaningless since we are not told how \( T \) behaves. Does it decline, oscillate increase? However Ho et al treat \( T \) as a constant in their own analysis (p1583). If \( T \) is a constant then the solutions are exponential functions, with for example

\[
V(t) = Ae^{\alpha t} + Be^{\beta t}
\]

where \( A \) and \( B \) are constants depending on \( V(0) \) and \( T^*(0) \) and

\[
\alpha = \frac{-(c + \delta) + \sqrt{(c - \delta)^2 + 4NkT\delta}}{2}
\]

and

\[
\beta = \frac{-(c + \delta) - \sqrt{(c - \delta)^2 + 4NkT\delta}}{2}
\]

It is thus apparent that a steady state is possible in this case if and only if \( NkT = c \). That is four independent parameters must remained balanced in this way for a decade. As soon as the parameters change in such a way as to violate this equality, then exponential growth or decay of virus levels must follow. Similar comments can be made for \( T^* \). Thus this model with constant \( T \) also seems to predict rapid disease progression.

A more general criticism is that Ho et al. give no evidence that this model does indeed describe the dynamics of HIV infection. They do not tell us what \( T \) looks like, other than briefly mentioning that it can sometimes be treated as a constant. How is one then to know if it does in fact model HIV dynamics? No other model does. If this model is in fact not a good description of "the dynamics of cell infection and virion production", then why should anybody believe that fitting data to it will give any information about those dynamics?

The conclusion is inescapable. The Ho and Shaw models for HIV infection are highly suspect and should be treated with a good deal of scepticism. It is certainly unwise
to base strategies for HIV chemotherapy on them, when the underlying mathematical models remain to be proven valid.

<table>
<thead>
<tr>
<th>Table 1. From Piatka et al. 1993</th>
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<td><strong>Table 1.</strong> Viral and clinical summary for 66 consecutively studied HIV-1–infected patients.</td>
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<th>Patient ID</th>
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<th>HIV RNA† (copies/ml)</th>
<th>HIV p24 Ag (pg/ml)</th>
<th>Plasmal culture (TCID50/ml)</th>
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*CD4+ cells determined by flow cytometry within 3 weeks of sampling for virologic assessments. HIV RNA copy number per milliliter of plasma, as determined by DC-PGR (3,17). Immune complex–associated HIV p24 antigen (19). Tissue culture infectious dose of virus per milliliter of plasma (19). *Centers for Disease Control classification system for staging of HIV-1 infection (2). *Three days previously, plasma cultures were positive at 10 TCID50/ml with HIV-1 RNA level of 1,350,600 copies/ml. **Eight
References


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S.L.
Duesberg and the right of reply according to Maddox-Nature

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In 1993 John Maddox, the editor of Nature, commissioned a commentary refuting the hypothesis that drugs cause AIDS (Ascher et al., 1993). The piece described 215 patients each of which had used drugs (Duesberg, 1993a; Duesberg, 1993b; Duesberg, 1993c). In view of this Duesberg sent a letter to Nature arguing that the perfect correlation between drug use and AIDS confirmed, rather than refuted, the drug hypothesis. Maddox censored the letter and wrote an editorial ‘Has Duesberg a Right of Reply?’ (Maddox, 1993). The editorial pointed out that the world’s oldest science journal could not afford an open scientific debate on the cause of AIDS because of the perceived dangers of infectious AIDS.

In an editorial on January 19, 1995, Maddox promised to lift the censorship to give ‘Duesberg and his associates an opportunity to comment’ on two Nature studies that in his opinion prove the HIV-AIDS hypothesis.

In the following we document how Maddox-Nature honors its commitments. Our documentation includes
(i) photocopy of Maddox’s News and Views article of January 19, 1995,
(ii) summary of phone conversation between Maddox and Bialy, on January 12, 1995,
(iii) our letter to Maddox and commentary on the two new Nature studies,
(iv) Maddox response to our commentary,
(v) our third letter to Maddox published by Nature with editorial comments, on May 18, 1995,
(vi) our letter to Maddox stating that Nature’s comments are erroneous.
(vii) Nature’s final letter.

References
Duesberg and the new view of HIV

This journal has offered Dr Peter Duesberg and his associates an opportunity to comment on last week's publications suggesting that the immune system reacts hyperactively to HIV infection.

The publication last week of two important articles on the dynamics of the infection of people by HIV is agreed to have been a major landmark in the process of understanding the disease called AIDS, but not everybody will be aware of that. Reporting of the event has been curiously selective, in particular, the British newspaper The Sunday Times, which as recently as a year ago was replete with accounts of how HIV can have little or nothing to do with the cessation of AIDS, chose not even to mention the new developments in last Sunday's edition.

Is it planning a major account of how it came to be so misled, thus to mislead its readers? Or is it waiting for a sign from Professor Peter Duesberg, of Berkeley, California, who started the hare the newspaper followed eagerly for two years?

The reasons why the new developments are (or should be) an embarrassment for Duesberg are simply put. Almost from the outset of AIDS as a recognized disease in the early 1980s, the objective index of an infected person's state of health has been the concentration in the blood of T lymphocytes carrying the CD4 antigen. The more advanced the infection, the smaller the concentration of CD4 cells.

But Duesberg was quick to point out a paradox in the observations: although the concentration of CD4 cells might decline with the persistence of infection, there was no dramatic increase in the frequency of infected T cells as the disease gave way to overt disease. Cell death by inter-cellular infection could hardly be consistent with that state of affairs.

In essence, the new developments resolve the paradox: by showing that the T cells in an infected person's blood are likely to have been created only in the few days previously. There will not have been time enough for more than a small proportion of them to have become infected, while those that harbour virus will be killed off very soon. So the scarcity of T cells from which virus can be recovered in test-tube experiments is consistent with the assertion that the immune system is in overdrive from the moment of infection by HIV.

On this (new) view, the progressive decline of the CD4 concentration with the duration of infection is rather a symptom of the underlying infection than the crux of its mechanism. What seems to matter is that there should be cells (including T cells) somewhere in the body (the lymph nodes are likely candidates) from which virus particles continue to leak into the blood plasma.

In other words, Duesberg is right to have argued all along that the usually slow decline of CD4 cells is not consistent with what one would expect from a specific cytolytic T cell mechanism, but the explanation is that the CD4 population in the blood at any time has been freshly created.

Despite that, the journal's severe line, some months ago, on Duesberg's right of reply to critics of his position, is now in the general reader's mind. And his associates' views on the new developments should be made public. Duesberg was not available to take a single telephone call one day last week, nor able to return it, but one of his associates appeared to welcome the idea of a comment on the articles by Wei et al. and Ho et al. (Nature 373, 117-122 & 123-126; 1995).

That will be eagerly awaited and will be published with the usual proviso — that it is not libellous or needlessly rude, that it pertains to the new results and that it should not be longer than it needs to be.

Meanwhile, one important question stands out like a sore thumb: why, after more than a decade of research, has it only now emerged that the response of the immune system to infection by HIV is hyperactivity rather than the opposite? Simon Wain-Hobson, writing in News and Views last week (Nature 373, 102; 1995), remarked that the investigators were able to reach their startling conclusions "by teaming up with mathematicians".

Intuitively, the sharp recovery of CD4 cells in the first few weeks after the administration of antiviral drugs pointed to their rapid production by the immune system. But in retrospect the good fortune of the investigators is clear. Only with the advent of highly specific drugs directed against HIV was it possible to cut off viral production so abruptly that the decline in plasma viraemia could form the basis for a model of viral production. New techniques for assessing the levels of virus involved were also necessary; had the drugs been available only a few years earlier, these studies would have been impossible on that account.

In retrospect, the dynamics of the immune system would seem to be central to any consideration of the body's response to infection, by measles virus as well as HIV. And modelling of such processes as the production of lymphocytes (B as well as T cells) in the immune response should be a relatively easy task (compared with, say, the appearance of endless molecular species in the evolution of a molecular cloud).

To be sure, immunologists are no strangers to quantization in this spirit. And the involvement of mathematicians is simply explained by the authors' desire to be sure that even experts in this area approved of their data analysis. But the rarity of such studies says something depressing about the state of biology, for all its modernity. Despite the explosion in molecular knowledge (including molecular knowledge of viruses), the information to perform this kind of quantization is almost never available.

In this case, the relevant data have emerged only after a decade of intensive research, fuelled by intense public interest in a most unpleasant pathogen. But virology is not the only field in which biology would benefit from more quantitative methods.

What more is to come? Now that the basis for the low CD4 T-cell count in AIDS patients is clear, further studies of the viral dynamics will be eagerly awaited. How much virus is produced by each productively infected cell? How fast is the virus produced by the lymph nodes? And what is responsible for killing the CD4 T cells? If these last are indeed being destroyed by the CD8 cells of the immune system, as Wain-Hobson suggests (and this remains to be seen), it will undeniably lend further support to the idea that individuals who are repeatedly exposed to HIV while remaining unaffected are protected by cytotoxic T lymphocytes (Rowland-Jones et al., Nature Medicine 1, 59-64; 1995).

The search for effective antiviral therapy will also benefit. Already Wei et al. have followed the emergence of mutants resistant to one drug, and studies of others, alone and in combination, will surely follow. Here too, improved quantization of the size of viral pools in different tissues, and their respective replication rates, will be vital.

What does this mean for basic research on AIDS, the cause eloquently advocated a year ago by Dr Bernie Fields (Nature 369, 95; 1994)? Wei et al. and Ho et al. have provided the basis for a much more pointed programme of investigation from which, no doubt, a complete picture of the dynamics of this hitherto perplexing disease will emerge. A return to basics seems almost to have happened. The prospects of therapy are much more difficult to tell, but has a fuller understanding ever failed to deliver improvements of technique? The danger for the Duesbergs of this world is that they will be left behind if they fail to keep a cause that will have few adherents at time passes. Now may be the time for them to recant.

John Maddox
Summary of phone conversation between John Maddox and Harvey Bialy on January 12, 1995

On the afternoon of January 12, the day the *nature* issue containing the Ho and Wei et al. papers appeared, and one day after the press conference announcing these landmark publications, I received a rare telephone call from my colleague, the newly knighted, Sir John Maddox, editor of *nature*. The essence of the ensuing conversation is summarized below.

After congratulating John on his recently acquired honorific, I asked to what did I owe the pleasure of his call. He then asked me what I thought of the ‘HIV-1 dynamics’ papers. I replied by thanking him for publishing them, as they were so transparently bad, they would convince any reasonable scientist who had the endurance to read them that the HIV-AIDS hypothesis was absolutely intellectually bankrupt. I also chided him by saying that even Wain-Hobson didn’t know what to make of them, judging by his incoherent news & views piece that accompanied their publication.

To my surprise, his response to these remarks was remarkably devoid of any outrage. We discussed in a cursory manner some of the obvious criticisms of the papers, such as their lack of controls, and the methodological and biological problems with their estimates of free infectious virus. I also mentioned that I thought it ironic that after years of denying that T cells turned over at the rate of 5% in two days, the HIV-AIDS protagonists were now at last admitting this well known fact. He responded by asking how did I explain the “dramatic increase in T cells after treatment with the protease inhibitor”. I replied that this transient, hardly dramatic, increase was also a well known phenomenon called lymphocyte trafficking, which occurs in response to many chemical insults.

The conversation then changed direction and John said that he had, without success, been trying to reach Peter (Duesberg) to inform him that he was, in this instance, willing to rescind his previous ‘refusal of the right of reply’ and would welcome a correspondence from Peter (and myself) addressing what we perceived as the shortcomings of Ho and Wei et al. He promised me that if the piece was relevant, succinct and not personally rude, he would publish it ‘unslagged’. When I asked him what this meant, he said that it would be published as received, without prior review and without a response appearing in the same issue. I said ‘do you mean it will be allowed to generate its own replies?’, and he said yes. I congratulated him on his willingness to open a proper scientific debate, and said I would communicate our conversation to Peter.

I was a bit surprised to see his editorial in the following week’s *nature* in which he went much further than our conversation in offering the pages of *nature* to uncensored debate. I was, however, not surprised to discover, some weeks later, that the response which appears unedited in this monograph, was deemed ‘too long by half and too unfocussed’ to warrant publication in his own highly esteemed journal.
February 7, 1995

Sir John Maddox
Nature, Macmillan Publishing
4 Little Essex St.
London, WC2R 3LF
England

Dear John,

As per your invitation, published in News and Views "Duesberg and the new view of HIV", and your invitation to Harvey Bialy over the phone, "to comment on last week's publications" by Wei et al. and Ho et al. we submit "Responding to Duesberg and the new view of HIV" by Duesberg & Bialy. We are delighted that after years of editorials, News & Views, and letters and censored letters we have been invited at last to make our case in our own words.

As you can see, our report meets your criteria of "not libellous or needlessly rude, that it pertains to the new results and that it should not be longer than it needs to be". The length of our commentary is compatible with the results presented in the two papers covering 10 pages, and the challenges delivered by the two accompanying News & Views from you and Wain-Hobson.

We both respect your courage and integrity to undertake an uncensored debate on the HIV-AIDS hypothesis.

Best regards

Peter Duesberg
Harvey Bialy
Responding to ‘Duesberg and the new view of HIV’

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The editor of Nature, John Maddox, has issued a published invitation to ‘Peter Duesberg and his associates ... to comment’ on two new studies by Wei et al. (1) and Ho et al. (2) that he feels lend strong support to the hypothesis that HIV causes AIDS (3). Maddox credits us for having identified two paradoxes of this hypothesis, (i) ‘Duesberg was quick to point to a paradox ... [that] there was no dramatic increase of the frequency of infected T-cells as infection gave way to overt disease’, and that (ii) ‘Duesberg is right to have argued all along that the usually slow decline of CD4+ cells [T-cells] is not consistent with... a specific cytotoxic viral mechanism’ (3).

According to Maddox, ‘the new developments are (or should be) an embarrassment for Duesberg’, because they ‘resolve the paradox’. But we do not see any reason why a scientist should be embarrassed for having pointed out paradoxes in the past, which ever way these paradoxes are subsequently solved. We also object to rhetoric personalizing a scientific debate. However, it is embarrassing that in the name of science clinical, public health, journalistic, and political decisions have been made in the past, based on a hypothesis that – we all agree now – was unproven at that time.

Since the HIV-AIDS hypothesis makes many assumptions that are paradoxical, if not bewildering, for pre-HIV virologists, and since the new studies do not clearly define the HIV hypothesis, we shall first state the hypothesis and then explain why, in light of these ‘new’ studies, it remains paradoxical.

In 1984 it was proposed that the retrovirus HIV can cause such diametrically different diseases as Kaposi’s sarcoma, pneumonia, dementia, diarrhea, and weight loss (4, 5). All of these diseases and over two dozen more are now collectively called acquired immuno-

deficiency syndrome (AIDS) (6), if antibody to HIV is present. But many of these diseases, including Kaposi’s sarcoma, lymphoma, dementia and weight loss, are neither consequences of, nor consistently associated with immunodeficiency (7, 8). For example Kaposi’s sarcoma and dementia have been diagnosed in male homosexuals whose immune systems were normal (9–13). As a cause of these diseases HIV was proposed to follow an entirely unprecedented course of action:

1) HIV was proposed to cause immunodeficiency by killing T-cells. But retroviruses do not kill cells (14, 15).

2) Within weeks after infection, HIV would reach moderate to high titers of 10–10^4 infectious units per ml blood (16), sufficient to induce antiviral immunity and antibodies (a positive ‘AIDS-test’). According to Shaw, Ho and their collaborators, HIV activity is ‘rapidly and effectively limited’ by this antiviral activity (17, 18). Prior to antiviral immunity, HIV would neither kill T-cells nor cause AIDS (16, 19). But all other viruses are primarily pathogenic prior to immunity; the reason vaccination protects against disease. Not one virus exists that causes diseases only after it is neutralized by antiviral immunity (20, 21).

3) On average 10 years after HIV is neutralized, the virus is postulated to cause AIDS diseases (5, 22). But all other viruses typically cause disease within days or weeks after infection, because they replicate exponentially with generation times of 8 to 48 h (20, 23, 24).

4) As a consequence of antiviral immunity, the virus titer is typically undetectably low prior to and even during AIDS (25–29). Only in rare cases HIV titers are as high as in the asymptomatic, primary infection (16, 30). But in all other viral diseases the virus

*Revised version (see 7 March letter from Duesberg and Bialy to Maddox).
titer is maximally high when viruses cause disease (20, 21).

5) Antiviral immunity would typically restrict HIV-infected lymphocytes to less than 1 in 500 – prior to and even during AIDS (14, 26, 27, 30–32). But all other viruses infect more cells than the host can spare or regenerate when they cause disease (20, 21).

6) The hypothesis fails to shed any light on the causation of non-immunodeficiency AIDS diseases, like Kaposi’s sarcoma, dementia, lymphoma and weight loss which make up 39% of all American AIDS cases (8, 33).

Today this HIV-AIDS hypothesis stands unproven and has failed to produce any public health benefits (34–36).

The new studies are claimed by two News and Views articles from Maddox (3) and Wain-Hobson (43) to resolve the paradox, (I) how HIV kills T-cells, (II) how HIV causes AIDS, and (III) why HIV needs 10 years to cause AIDS. But we argue that the new studies have failed to resolve any of these paradoxa, in fact they have added new ones:

(I) How HIV kills T-cells. Until HIV appeared on the scene, retroviruses did not kill their host cells. This is the reason they were considered possible tumor viruses. Since retroviruses integrate their genes into the chromosome of the host, they can only replicate as long as the host survives integration and remains able to express integrated viral genes. Therefore a cytocidal retrovirus would be suicidal. Indeed, HIV proved to be non-cytocidal. It is mass-produced for the “AIDS-test” in immortal T-cells in culture at titers of 10^6 infectious units per ml (37, 38). Luc Montagnier and others have confirmed that HIV does not kill T-cells (39–42). Hence the claim that HIV causes AIDS by killing T-cells is paradoxical.

The new papers have indeed resolved this paradox by shifting the paradigm: According to Maddox, T-cells ‘that harbour virus will be killed off very soon’ – but not by HIV – by the immune system. Also consistent with a non-cytocidal virus, Wei et al. report that ‘the average half-life of infected PBMCs [peripheral blood mononuclear cells] is very long and of the same order of magnitude as the half-life of uninfected PBMCs’. But, paradoxically, the same investigators also report that ‘the life span of virus-producing cells is remarkably short (11/2 = 2 ± 0.9 days)’, although these cells are in the same system as their long-lived HIV-infected peers (1). Ho et al. state that ‘there is virus- and immune-mediated killing of CD4 lymphocytes’ (2). But, according to the News and Views article by Simon Wain-Hobson, ‘an intrinsic cytopathic effect of the virus is no longer credible’ (43).

It is consistent with this ‘new view of HIV’ that there is no correlation between virus titers and T-cell counts in the patients that Wei et al. and Ho et al. have studied. In some of Ho et al.’s patients, i.e., # 303 and # 403, a 100-fold variation in virus titers corresponds to no changes in T-cell counts. In Wei et al.s patients 100-fold variations in virus titers correspond to only 0.25 and 3-fold variations in T-cell counts – hardly a correlation to prove that HIV kills T-cells.

Since HIV is no longer viewed as a T-cell killer, the above paradox is solved. However, if T-cell killing via antiviral immunity were the cause of AIDS, we would have a bigger HIV-AIDS paradox than before. Since only 1 in 500 T-cells are ever infected, and most of these cells contain latent HIV not making viral proteins (25, 26, 30, 44), only less than 1 in 500 T-cells could ever be killed by antiviral immunity.

(II) How HIV causes AIDS. Until HIV appeared on the scene, the pathogenicity of a virus was a direct function of the number of virus-infected cells: the more infectious virus there was, the more cells were infected, and the more pathogenic an infection was.

But in typical AIDS patients HIV is so rare, that even leading AIDS retrovirologists from the US, like Robert Gallo, and the UK, like Robin Weiss, failed for years to isolate HIV from AIDS patients (45, 46). Likewise, virus-infected cells are so rare that they could not be found by George Shaw, the senior investigator of the new study by Wei et al., Gallo and their collaborators in most AIDS patients (27) – until the rare proviral DNA could be amplified with the polymerase chain reaction (PCR) (31, 44, 47).

Although the new studies never mention the percentage of infected T-cells, Maddox confirms the status quo: ‘the scarcity of T-cells from which virus can be recovered in test-tube experiments is consistent with the assertion that the immune system is in overdrive from the onset of infection by HIV’. But the new studies claim on average 10^5 units of ‘free virus’ (1) or ‘plasma virion’ per ml blood (2) in AIDS patients. That should be enough virus to eliminate all remaining T-cells of these patients, 10^6 per ml, within the two days HIV needs to replicate (48) – unless, as Maddox suggests, the ‘new techniques for assay ing the low levels of virus involved were also necessary’ (3) (amplifying
viral RNA with the polymerase chain reaction) possibly because no infectious HIV could be detected by conventional infectivity tests.

Indeed, Wei et al. acknowledge 'substantial proportions of defective or otherwise non-infectious virus'. To determine whether the viral genomes represented in total viral nucleic acid correspond to infectious virus... they had to resort to the same techniques that the 'old HIV hands', as Wain-Hobson calls them (43), had used to isolate HIV from rare infected lymphocytes of AIDS patients: 'We cocultivated PBMCs ... with normal donor lymphoblasts in order to establish primary virus isolates'. Shaw together with some of the investigators of Wei et al. had shown in 1993 how to convert 'plasma viral RNA' to infectious virus. They concluded that the 'quantitative competitive PCR' is 'as much as 60,000 times more sensitive' (49) than infectious virus (16, 19). Divide 10^5 'plasma viral RNA' units by 60,000 and you have 1.6 infectious units per ml, a number that is consistent with numerous previous reports (see above). Ho and a different group of collaborators just published a paper in which they show that over 10,000 'plasma virions', detected by the 'branched DNA signal-amplification assay' used in their Nature paper, correspond to less than one (!) infectious virus (50). Thus Wei et al. and Ho et al. both reported titers of 10^5 biochemical virus-units that really correspond to one or even less than one infectious virus. However, infectivity is the only clinically relevant criterion of a virus.

In other words, there is no evidence for infectious virus in Wei et al.'s and Ho et al.'s patients. Wei et al. and Ho et al. had apparently detected non-infectious virus that had been neutralized by the immune system (that) reacts hyperactively to HIV infection – just as Maddox suggests. Infectious virus was only obtained by activating latent HIV from a few infected cells out of millions of mostly uninfected cells from a given AIDS patient. Such virus activation is only achieved by growing cells in culture away from the hyperactive immune system of the host, just as the 'old HIV hands' used to do it, when they tried to isolate HIV from AIDS patients (45, 46). Thus the paradox of too few viruses to cause immunodeficiency remains unresolved.

In view of the evidence that there are no more than 1.6 infectious HIVs per ml blood in Wei's and Ho's patients, one wonders whether the 10^5 viral RNAs per ml are real or are an artifact reflecting inherent difficulties in quantifying the input number of 'plasma viral RNA' molecules after many rounds of amplification by the PCR. The problem with the quantification of input RNAs – after 30 to 50 rounds of amplification by the PCR (51) – is like calculating the number of the original settlers in America, from the current number of Americans and their current growth rates. But even if the 10^5 'plasma viral RNAs' per ml were real, it is hard to guess where they came from in view of 'the scarcity of T-cells from which virus can be recovered...' acknowledged by Maddox (3).

However, the apparent lack of infectivity of the 'free virus' or 'virions' (2) resolves the paradox of the coexistence of 10^5 T-cells with 10^5 plasma viral RNAs per ml blood in Ho et al.'s and Wei et al.'s AIDS patients (1). Even HIV cannot kill T-cells that it cannot infect. The fact that over 99% of T-cells in persons with AIDS are not infected by HIV (14, 26, 27, 31, 32, 44), is definitive evidence that there is no infectious HIV in typical AIDS patients. Clearly, in AIDS patients with 1.6 infectious HIV units per ml something other than HIV must cause AIDS.

In earlier efforts to resolve the paradox, that there is too little HIV in AIDS patients to cause AIDS, both groups have observed huge discrepancies between virus titers and AIDS symptoms. In 1993, Shaw and colleagues have described otherwise identical AIDS patients of which 5 contained 0 infectious HIV per ml, and 22 contained between 5 and 10^5 (16, 19). In 1989, David Ho et al. have also described 40 AIDS patients with virus titers ranging from less than 1 to 10^5 infectious units per ml (30). In 1993, Ho et al. even reported 12 AIDS patients, including 8 who had AIDS 'risk factors', who were totally HIV-free: 'Specific antibody assays, viral cultures, and polymerase chain reaction (PCR) techniques for HIV were all negative. Their T-cell counts ranged from 3 to 308 per μl (52).

There is only one consistent hypothesis to reconcile the bewildering ranges of HIV titers in Ho's and Shaw's patients with the role of the virus in AIDS – HIV is a passenger virus, rather than the cause of AIDS. Indeed, non-correlation between the titers of a virus and disease, and between the very presence of a virus and disease – is one of the hallmarks of a passenger virus. Both Ho et al. and Shaw et al. have failed to understand that rare correlations between a virus-at-high-titer and a disease are the hallmark of a passenger virus, and that consistent correlations between a virus-at-high-titer and a disease are the hallmark of causative virus (8, 53, 54). Therefore they have, contrary to their claims, established HIV as a passenger virus of AIDS patients.

(II) Why HIV needs 10 years to cause AIDS. Until HIV appeared on the scene, the latent period from
infection to disease was a function of the generation time of a virus. A virus that replicates in 2 days and produces 100 viruses per generation would cause disease in about two weeks – provided there is no antiviral immunity. This is because 100 viruses infect 100 cells producing 100 × 100 or 10,000 viruses 2 days later. Within 14 days of such exponential growth 10^{14} cells – the equivalent of a human body – would be infected. Therefore the latent periods of pathogenic retroviruses, like Rous sarcoma virus, and non-retroviruses like flu, measles, mumps, herpes, hepatitis, mononucleosis, chicken pox are all 7 to 14 days (23). Since HIV replicates in 2 days, like all other retroviruses (48), and since according to Ho an infected cell produces over 1000 viruses per 2 days (32), HIV should cause AIDS, – if it could cause AIDS – just as fast as other viruses.

Yet, as Maddox points out, the failure of HIV to cause AIDS within weeks after infection presents another paradox for the HIV-AIDS hypothesis, ‘... the usually slow decline of CD4+ cells is not consistent with what one would expect from a specific cytototoxic viral mechanism’. Indeed, both studies confirm the paradox. Since the AIDS patients contain 10^6 ‘free viruses/virions’ and 10^5 T-cells per ml plasma, the plasma of these patients should be T-cell free within 2 days, the generation time of HIV. But Ho et al. report that the T-cells of AIDS patients are either steady or even increasing over 1 month, and Wei et al. report that the T-cells of their patients remain either steady or decline slowly over 5 to 8 months (1, 2).

Even if there are 50-times more T-cells in hidden reservoirs – as Ho et al. report –, they, too, should be infected within two weeks, because according to Wei et al., the ‘plasma viral RNA’ titer can rise two orders of magnitude within two weeks. In fact, the ability of HIV to increase from 10^3 ‘plasma viral RNA’ units to 10^5 units per ml described by Wei et al. should only be a fraction of the real ‘dynamics of the infection of people by HIV’ (3), since it occurred despite the presence of two DNA chain terminators, AZT and ddI, used as anti-HIV drugs in addition to a new coded antiviral drug.

Therefore it remains paradoxical that – dated from the time of HIV infection – AIDS occurs at entirely unpredictable times, currently estimated to average 10 years (5). To determine whether the currently unpredictable time from HIV infection to AIDS can be reconciled with a viral mechanism at all, one needs to know whether HIV kills T-cells, how much infectious virus there is, and the percentage of infected cells at a given time. Since the new studies by Wei et al. and Ho et al. provide none of these data, all new calculations ‘on the dynamics of the infection of people by HIV ... in the process of understanding the disease called AIDS’ are worthless.

However, the hypothesis that HIV is a passenger virus provides a consistent explanation for the unpredictable time intervals between HIV infection and AIDS. It is one hallmark of a passenger virus, that the time of infection is unrelated to, and independent of the time when a disease occurs – just as with HIV and AIDS. Another hallmark of a passenger virus is that its titer and even its presence are not correlated with disease – just as was shown above for HIV and AIDS.

The simplest interpretation of the slow decline of T-cells in Ho’s and Wei’s AIDS patients is a non-viral cause, e.g. long-term intoxication (7). Take for example the slow decline of liver cells in long-term alcoholics or of lung cells in long-term smokers.

Maddox seems concerned that ‘reporting of the new event has been curiously selective’. Perhaps even science reporters begin to wonder how much further the virus-AIDS hypothesis can be stretched to explain its most obvious failures and inconsistencies: Why is there no vaccine? Why does American/European AIDS stay in the classical risk groups, male homosexuals, intravenous drug users and transfusion recipients? Why do AZT-treated HIV-positives get AIDS (55, 56)? Why do 918 HIV-positives male homosexuals who had ‘avoided experimental medications on offer’ and ‘chose to abstain or significantly reduce their use of recreational drugs...’ remain AIDS-free, long-term survivors (57)? Why did the T-cells of 29% of 1020 HIV-positive male homosexuals and former intravenous drug users from the placebo arm of a clinical AZT trial increase up to 22% over two years – despite the presence of HIV (58)? Why did the T-cells of 14 out of 31 HIV-positive hemophiliacs treated with highly purified factor VIII increase up to 25% over three years – despite the presence of HIV (59)? Why is there not a single study showing that HIV-positive 20 to 50-year-old men or women who are not drug users or recipients of transfusions ever get AIDS (60)?

Why did neither Ho et al. nor Wei et al. identify the risk groups their patients came from or indicate whether they had Kaposi’s sarcoma, dementia, or diarrhea or lymphoma? Can they exclude that recreational drugs used by AIDS risk groups, like nitrite inhalants, amphetamines, and cocaine are immunotoxic or carcinogenic (61)? Why is it that among 10 long-term (10
to 15 years) survivors of HIV recently described by Ho et al. (50) "none had received antiretroviral therapy..."? Can Wei et al. and Ho et al. exclude that the DNA chain terminators, AZT and ddl, that their patients received in addition to the new experimental drugs, do not play any role in the 'slow decline of CD4+ cells'? Are they aware that the manufacturer of AZT says in the Physician's Desk Reference that "it was often difficult to distinguish adverse events possibly associated with zidovudine [AZT] administration from underlying signs of HIV diseases..." (62)? Are they aware that the DNA chain terminators were developed 30 years ago to kill growing human cells for chemotherapy, not as anti-HIV drugs?

It seems to us that the "new developments" of Wei et al. and Ho et al. are a Mayday of AIDS virologists – rather than a 'virological mayhem' (43).

Acknowledgements

We thank Serge Lang (Yale University), Siggi Sachs (UC Berkeley) and Russel Schoch (UC Berkeley) for critical comments. Supported by the Council for Tobacco Research, USA, and private donations.

References

2 March 1995

Peter H Duesberg
Department of Molecular
and Cell Biology
University of California
Berkeley, CA 94720

Dear Peter:

First, the good news: we shall publish the essence of what you have to say. But there are obvious snags. Let me retail my original conversation with Harvey Bialy: What format? he asked. A letter, I said. That's too little, he said; what about 1,000 words. I said I was not prepared to negotiate the length of a letter not yet written. But what you have sent would take at least 3 pages of Nature.

Second, I resent the way in which you appear to have alerted the world's press to the existence of your piece. Why do that?

Third, and this may not be such good news, I plan to go through your piece with a fine-tooth comb with the intention of ridding it of repetitions and various misrepresentations.

Let me illustrate the last point by reference to your page 3 and the continuation of the main paragraph on page 4. You start with the phrase "HIV was proposed to follow an entirely unprecedented course of action", you document various misconceptions about the functioning of HIV and then you conclude with the phrase "this HIV-hypothesis". Frankly, that smacks of the old Goebbels technique, that of creating a straw man from a farrago of indefensible propositions and then knocking it down. I know of nobody who, in the past decade, has put forward your points (1) to (5) as a unified statement of the conventional position. (Even you have to assemble the position with 20 references.) On the contrary, the "HIV-hypothesis" is much simpler: "HIV causes AIDS, in some manner not understood, most of those infected will develop the disease".

Nor is it a fair representation of Wei et al. and Ho et al. to say that they "claim" to resolve the three specific "paradoxes" you list. In truth, they do nothing of the kind. The only conceivable reference to the 10-year latency period, for example, is in Ho et al., and consists of the simile of the tap and drain. To quote from Wei et al., "The kinetics of virus and CD4 lymphocyte replication imply "First, continuous rounds of de novo virus infection, replication and rapid cell turnover... probably represent a primary driving force in HIV pathogenesis...Second... a striking capacity of the virus for biologically relevant change. Third... that virus production per se is directly involved in CD4 cell destruction." Ho et al. go...
Peter H Duesberg
2 March 1995
page 2

further, but only to this extent: "... our findings strongly support the view that AIDS is primarily a consequence of continuous high-level replication of HIV-1, leading to virus and immune-mediated killing of CD4 lymphocytes."

My position as an editor is that straight misrepresentations such as these have no place in a journal like this. You complain at an earlier stage that I have improperly "personalized" this argument. How do you suppose that Wei et al. and Ho et al. would feel if we were to publish your travesty of what they have said?

My suggestion, therefore, is that you throw away the first four pages of your introduction, and devise a less inflammatory introduction in which you state that the two papers have not changed your view, and go on to give the reasons. Please let me know whether that is acceptable. I have some other less radical comments on the remainder of the text, but there's no point in sending them at this stage if you cannot agree to something along the lines I have suggested.

Yours sincerely,

John Maddox
Editor

cc: Harvey Bialy
March 7, 1995
Sir John Maddox
Nature, Macmillan Publishing
4 Little Essex St., London, WC2R 3LF
England

Dear John,

After you have invited us with an editorial "to comment" on "the new view of HIV" (Nature, 19 January 1995), we are surprised to learn that you only want to "publish the essence of what [we] have to say".

We have followed your advice that "it should be no longer than it needs to be". Since neither of the two new Nature studies nor the two accompanying News and Views by you and Wain-Hobson have explained the old view of HIV, we had to explain the old view first for the reader of Nature to understand our comments on "the new view of HIV". We are not interested in a discussion between experts restricted just to titers of HIV. Therefore we can not accept your suggestion to "throw away the first four pages" of our commentary.

Moreover, if our commentary comes out to be 3 pages in Nature, as you say, that would only be a fourth of the space you have already dedicated to the "new view of HIV" — 10 pages for the two papers and 2 pages for the two editorials. A 3-page commentary on 12 pages in Nature, supplemented by an international press release, is hardly a convincing argument that "it is longer than it needs to be".

You write that you "resent the way in which [we] appear to have alerted the world press to the existence of [our] piece". However, we are afraid, if alerting the world’s press is a reason for resentment, we should resent you. After all, you have alerted the world’s press using the power of your office about the "embarrassment for Duesberg" and that you "eagerly awaited" our "comment". But you did not respond to our commentary from February 7 until March 2. As a result of your activities the world’s press has called us, and some callers were given our commentary, weeks after you had received it, with the proviso that it may not be published in its present form by Nature. Indeed, the exchange of opinions is protected by the free-speech amendment in this country.

Are you aware that both Wei et al. and Ho et al. gave their papers to John Coffin and David Baltimore prior to publication in Nature to write editorials for Science (267, 483, 1995) and NEJM (332, 259–260, 1995) respectively?

If you plan to meet your published commitment that "his [Duesberg’s] and his associates’ views on the new developments should be made public" by first cutting, and then editing our commentary with a "fine-tooth comb with the intention of ridding it of... various misrepresentations", we do not see a basis for an open debate with you.

In response to your letter we resubmit our manuscript with some revisions:

1) page 2, third paragraph:
Replace "despite these ‘new studies’" by "in light of these new studies”.

2) page 2, fourth paragraph:
Insert after "immunodeficiency syndrome (AIDS)", "if antibody to HIV is present".

3) page 3, item (2):
According to Shaw, Ho and their collaborators, HIV activity is "rapidly and effectively limited" by this antiviral activity17,18

4) page 4, second paragraph:
Replace the sentence "The new studies claim to resolve..." by "The new studies are claimed by two News and Views articles from Maddox (3) and Wain-Hobson (43) to resolve the paradox, (I) how HIV kills T-cells, (II) how HIV causes AIDS, and (III) why HIV needs 10 years to cause AIDS."

5) page 6, end:
Insert the following paragraph after "... numerous previous reports (see above)":
"Ho and a different group of collaborators just published a paper in which they show that over 10,000 "plasma virions", detected by the "branched DNA signal-amplification assay" used in the Nature paper correspond to less than one (!) infectious virus50. Thus Wei et al. and Ho et al. both reported titers of 10^5 biochemical virus-units that really correspond to one or even less than one infectious virus. However, infectivity is the only clinically relevant criterion of a virus."

6) page 11:
Insert after "are immunotoxic or carcinogenic?", "Why is it that among 10 long-term (10 to 15 years) survivors of HIV recently described by Ho et al.50 ‘none had received antiretroviral therapy...’?"

References:

Sincerely,

Peter Duesberg, Harvey Bialy (faxed)
CC: Confirmation conv
AIDS pathology unknown: HIV infection provokes hyperactivity of the immune system, but the causes of that are far from understood

The second arresting feature of this correspondence is the letter from Dr. Peter Duesberg and his colleague, Dr. Harvey Bialy, which has been published without change. Sadly, there seems no way in which the authors concerned can be persuaded that ‘free and fair scientific debate’ is ordinarily understood to mean a progressive process, one in which each of two sides learns from what the other says. A restatement of earlier and well-known positions is not that at all. On this occasion, Duesberg and Bialy’s citation of Loveday in their cause is especially inappropriate given Loveday’s name among the authors of a letter supporting Wei et al. and Ho et al. But no further solicitation of Duesberg’s opinion is called for’.

[Nature 375, p. 167 (1995)]

HIV an illusion

SIR – In an editorial (1) in the 19 January issue of Nature, John Maddox invited ‘Duesberg and his associates’ to comment on the ‘HIV-1 dynamics’ papers published the previous week, indicating that these new results should prove an embarrassment to us. Although we do not think that a scientist should be embarrassed for pointing out that inconsistencies and paradoxes in a hypothesis that have only been reportedly resolved 10 years later, we nonetheless prepared a fully referenced, approximately 2,000-word critique or the Ho et al. (2) and Wei et al. (3) papers that we believed met the criteria of ‘not being longer than it needs to be, and pertaining to the papers at hand’ that Maddox set out in his widely read challenge.

Unfortunately, he did not share our view and agreed to publish only a radically shortened version, and only after he had personally ‘gone over it with a fine-tooth comb’ to remove our perceived misrepresentations of the issues. We found these new conditions so totally at variance with the spirit of free and fair scientific debate that we could not agree to them.

Readers of Nature who are interested in these questions, and feel that they do not need to be protected by Maddox from our ill-conceived logic, can find the supplement to the most recent issue of Genetica (4).

Here we would point out only that the central claim of the Ho et al. (2) and Wei et al. (3) papers — that 10^5 HIV virions per ml plasma can be detected in AIDS patients with various nucleic-acid amplification assays — is misleading. The senior author of the Wei et al. paper has previously claimed that the PCR method they used overestimates by at least 60,000 times the real titre of infectious HIV (5): 100,000/60,000 is 1.7 infectious HIVs per ml, hardly the ‘virological mayhem’ alluded to by Wain-Hobson (6). Further, Ho and a different group of collaborators have just shown (7) that more than 10,000 ‘plasma virions’, detected by the branched-DNA amplification assay used in their Nature paper, correspond to less than one (!) infectious virus per ml. And infectious units, after all, are the only clinically relevant criteria for a viral pathogen.

Finally, in view of Wain-Hobson’s statement (6) that ‘the concordance of their [Wei and Ho’s] data is remarkable’, note that Loveday et al. (8) report the use of a PCR-based assay and find only 200 HIV ‘virus-on RNAs’ per ml of serum of AIDS patients — 1,000 times less than Ho and Wei. So much for the ‘remarkable concordance’.

Peter Duesberg
Department of Molecular and Cellular Biology,
University of California,
Berkeley, California 94720, USA

Harvey Bialy
Bio/Technology, New York,
New York 10010, USA

References

• Peter Duesberg was offered space in Scientific Correspondence for 500 words of his own choice, but declined. – Editor, Scientific Correspondence. [Nature 375, p. 197 (1995)]

TO: Sir John Maddox
    Editor, nature
    Porters South, Crinnan St.
    London, England
FROM: Peter Duesberg
Date: July 10/95

Dear John,

Following publication of the Duesberg-Bialy letter on May 18, Nature added: "Peter Duesberg was offered space in Scientific Correspondence for 500 words of his own choice but declined."

Since our letter used up that "space", Nature's comment is erroneous and should be retracted.

Could you please confirm or un-confirm our conclusion. I have faxed to you twice before requesting an answer to this question (June 20 and June 22, 1995) but have not received a reply.

Sincerely, Peter Duesberg

cc Harvey Bialy
In reply please quote:
DUESBERG MC/eg

19 July 1995

Dr P Duesberg
Dept Molecular & Cell Bio, c/o
Stanley/Donner Admin Services Ut
University of California
229 Stanley Hall
Berkeley CA 94720

Dear Dr Duesberg,

Thank you for your various faxes. We offered to publish a 500-word version of your response to Ho/Shaw in our issue in which we published other comments on those papers. You declined, and instead send us a complaint that we would not publish your long manuscript. We published that complaint. As far as we are concerned, the matter rests.

Yours sincerely,

[Signature]

Dr Maxine Clarke
Executive Editor
1. The letter from Duesberg and Bialy p. 197 is followed by a statement from the editor: "Peter Duesberg was offered space in Scientific Correspondence for 500 words of his own choice, but declined -- Editor, Scientific Correspondence."

The sentence is false. Duesberg did not decline. The words of his choice constituted the letter printed above this sentence, which gives one more \textit{prima facie} example of \textit{Nature} editor's inability to report facts correctly.

2. There is an editorial note stating that the letters printed represent a selection of correspondence received. Be it noted that the letter from Mark Craddock was not printed.
HIV results in the frame

The following is a selection of the correspondence received about two papers published on 12 January on the dynamics of HIV infection in vivo.

Results confirmed

SIR—Elegant studies using early, frequently sampled patients on nevirapine and protease inhibitors (ABT-538 and L-735,524) have now made it possible to calculate the rapid turnover of HIV-1 viruses in infected patients1; the rate of plasma retroviral decay revealed a half-time of about 2 days for free virus particles or virus-producing cells, indicating that about 30% of the plasma virus population in infected patients is replaced every day. These observations have important implications for our understanding of HIV pathogenesis, and here, using a similar analysis in our previously analysed cohort of 11 AZT-treated patients2,3, we confirm the earlier findings1,2.

Our patients were homosexual men with CDC group IV disease, who have hence the estimated half-time, being a function of the survival of virus particles in the serum and the decay of virus-producing cells, can represent only the upper limit of viral half-time, assuming immediate and absolute inhibition of viral synthesis, which would seem implausible.

The table shows the analysis of our patients. For each patient the initial viral load (in copies per ml), the minimum virus load (and the day when it was reached), the rate of decline, the daily turnover, the half-life time and the data points used for the fit of the exponential decay curve are shown. The average rate of virus decline is 0.46 ± 0.31 (days), the daily turnover is 35 ± 17%, and the half-life time is 1.9 ± 1.1 days. (For these averages we leave out patient 1, who never achieved a 50% decline in serum viral load and whose viral half-life time is about 9 standard deviations.

Patient | Initial virus load | Minimum virus load | Rate of decline | Daily turnover | Half-life time | Data points used for fit
---|-------------------|--------------------|-----------------|----------------|----------------|-----------------------------
1 | 2,300 | 1,480 (7) | 0.06 | 0.06 | 11.0 | 2
2 | 30,000 | 3,000 (8) | 0.27 | 0.24 | 2.6 | 3
3 | 18,000 | 80 (7) | 1.10 | 0.67 | 0.6 | 3
4 | 320 | 40 (7) | 0.30 | 0.26 | 2.3 | 2
5 | 420 | 80 (6) | 0.35 | 0.30 | 2.0 | 3
6 | 1,680 | 140 (14) | 0.34 | 0.29 | 2.0 | 3
7 | 2,380 | 640 (6) | 0.17 | 0.16 | 2.0 | 3
8 | 8,480 | 140 (8) | 0.35 | 0.30 | 1.0 | 4
9 | 11,760 | 140 (8) | 0.35 | 0.46 | 1.1 | 4
10 | 25,600 | 440 (8) | 0.88 | 0.58 | 0.8 | 3
11 | 1,280 | 220 (7) | 0.25 | 0.22 | 2.8 | 2

Mean ± 1 s.d. (with patient 1) | 0.43 ± 0.31 | 0.32 ± 0.18 | 2.7 ± 2.9 | 2
Mean ± 1 s.d. (without patient 1) | 0.46 ± 0.31 | 0.34 ± 0.17 | 1.9 ± 1.1 | 2

CD4+ cell turnover

SIR — The provocative papers by Ho et al.1 and Wei et al.2 both conclude that antiviral therapy of HIV infection results in a rapid turnover of CD4+ T cells, leading to the rapid production of about 2 × 10^8 CD4+ T cells each day. Three explanations could account for the rapid increase in CD4+ T cells in the first weeks after initiation of therapy: (1) new production from thymic progenitors; (2) proliferation of peripheral CD4+ T cells; or (3) mobilization of CD4+ T cells from infected lymphoid tissue. New production of CD4+ T cells in the thymus of adults with chronic HIV infection seems highly unlikely, so the competing hypotheses are cell proliferation versus cell mobilization.

Both papers conclude that there is a high rate of CD4+ T-cell proliferation which, in the absence of antiviral treatment, is balanced by an equally high rate of HIV-induced CD4+ T-cell death — the mobilization of CD4+ T cells is not considered. The data shown in Fig. 2b of ref. 1 are informative for deciding between proliferation versus mobilization of CD4+ T cells. The rate of CD4+ T-cell expansion might be expected to be independent of or positively correlated with pre-therapy CD4+ T-cell counts, because patients with the lowest starting CD4+ T-cell count should have fewer proliferating T cells as well as destruction of lymph-node architecture3. However, the patients with the lowest starting CD4+ T-cell count actually show the highest rate of CD4+ T-cell recovery (Fig. 2b of ref. 1).

An alternative possibility is that a higher viral load is correlated with more trapping of CD4+ T cells in lymphoid tissues, and that effective antiviral therapy liberates these cells into the peripheral circulation. Were this explanation to be true, then replotted Fig. 2b of ref. 1 as the exponential slope of CD4+ increase versus the baseline plasma viremia should show as significant correlation as the original figure. The recalculated figure is shown here (a). The correlation coefficient is +0.55, which is statistically identical to the original figure plotting the slope of CD4+ T-cell change versus starting CD4 number (r = 0.57). If the rate of virus turnover is plotted against the slope of CD4+ T-cell increase (b in the figure), a positive correlation is also seen, so both a high starting viral load and a large drop in viral load following therapy predict more rapid CD4+ T-cell recovery, and their effects cannot be distinguished from a low initial CD4+ T-cell count. It is thus equally likely that the starting viral load and the magnitude of the antiviral effect determine the ability of CD4+ T cells to reappear in the peripheral circulation, which is consistent with the mobilization hypothesis.

In addition, the 22-fold variation in the

had no prior AZT therapy. Serum HIV-1 RNA only from enveloped virions was measured with an assay previously described. After starting treatment, serum samples were taken every 2-3 days. Virus levels declined rapidly and reached a nadir (or inflection point) after 6-14 days of therapy. AZT prevents the infection of new cells, but not the production of virus from cells already infected. Thus we expect serum virus to decline according to v(t) = v(0)[ae^(-at)]/[(1-e^(-at)). Here v(t) denotes plasma virus at day 6 after treatment has started; v(0) is the initial viral load; a and d describe the decay of the virus-producing cells and free serum virus, respectively. We do not have enough data points to fit this function, but we approximate the compound decay by a simple exponential decline. It is important to note that data on serum HIV-1 RNA do not allow us to determine which of the two decay processes is rate-determining.
exponential slope of "CD4 lymphocyte turnover" (0.004-0.088) shown in Table 1 of ref. 1 is inconsistent with replacement of CD4 T cells solely by rapid cell division, as the exponential slope should be relatively constant (reflecting mainly the relatively minor variations in cell-cycle time). Examination of lymph nodes from both HIV-infected progressors a and non-progressors' provides evidence for B-cell proliferation in germinal centres, but little evidence for a pool of proliferating CD4 T cells. In conclusion, a large part of the increase in CD4 T cells seen after acute antiviral therapy is probably due to reduced trapping in lymphoid tissue and a short-term reappearance of cells in the peripheral circulation.

**Donald E. Mosler**

Department of Immunology-IMM7, The Scripps Research Institute, La Jolla, California 92037, USA


Sir — Wei et al. and Ho et al. report that administering inhibitors of viral replication to HIV-infected patients causes a considerable, transient increase in CD4+ cell counts in the blood. From this, the authors conclude that the production of CD4+ cells during HIV infection is very rapid and sufficient to replace about 5% of the CD4+ cell pool each day. Wei et al. then speculate that it may be possible to achieve "... a successful immunological reconstitution even in late-stage disease if effective control of viral replication can be sustained." Not surprisingly, this idea has elicited wide interest.

But the data on CD4+ cell dynamics are based solely on counting numbers of CD4+ cells in blood: in neither study did the authors provide direct evidence on the rate of production (division) of CD4+ cells. The tacit assumption in both studies is that finding an increase in CD4+ cells in the blood is indicative of an increased production of these cells. In making this assumption, however, the authors ignore the possibility that their data simply reflect an alteration in lymphocyte migration.

Like CD8+ cells and B cells, most CD4+ cells are long-lived cells which recirculate continuously between blood and lymph via the lymphoid tissues. But it is well known that lymphocyte migration is labile and can be radically altered by exposure to various infectious agents or to stress: rapid alterations in lymphocyte migration can apply to resting cells and be associated with little or no change in the rate of lymphocyte turnover. Because viral infections often impede lymphocyte migration through the lymphoid tissues (the phenomenon of "trapping") a,b, the transient increase in CD4+ cell counts in the blood of HIV patients given antiviral agents could be a reflection of enhanced lymphocyte recirculation (decreased trapping) due to the reduction in the viral load. If so, most of the CD4+ cells would be expected to display a resting rather than an activated phenotype. This is easily tested.

To reiterate, we take issue with the view that the data of refs 1 and 2 demonstrate that CD4+ cells have a high rate of self renewal. In our opinion the transient elevation of CD4+ cells seen in the blood could be an epiphenomenon: the reduction in the viral load induced by the antiviral agents reduces trapping of CD4+ cells and thereby facilitates recirculation of these cells.

**Jonathan Sprent**

David Tough

Department of Immunology, Scripps Research Institute, La Jolla, California 92037, USA


Sir — Ho et al. and Wei et al. report that levels of cell-free virus in the plasma of HIV-1-infected patients fell approximately 100-fold within 2-4 weeks of the start of antiviral therapy, and was accompanied by a rise (approximately 200 cells per μl) of CD4+ lymphocytes. The authors assumed that the rise in CD4+ cell number observed equaled the number of cells that were not infected and killed by HIV-1 as a consequence of the effective therapy, and calculated that HIV-1 infected and killed approximately 4 × 10^8 CD4+ peripheral blood lymphocytes each day. They also proposed that the measured rise in circulating CD4+ cells reflected an increase in the total number of CD4+ lymphocytes in all body compartments of an infected individual. Because only 2% of lymphocytes are present in the peripheral blood, the authors multiplied the CD4+ cell increase by 50 and calculated that an HIV-1 AIDS patient can replace approximately 2 × 10^9 cells each day. We believe that these analyses grossly overestimate the number of the CD4+ cells killed by the virus and the regenerative capacity of the immune system in AIDS patients.

We have monitored wild-type and mutant HIV-1 infection kinetics in a variety of tissue culture systems and have derived infection rate constants that allow two critical parameters of the virus life cycle to be calculated: (1) the number of infectious virus particles (n) produced during a single cycle of replication; and (2) the time (t) required to complete one cycle of infection. During a single cycle, each infected cell generated approximately 10^7 physical particles and 10^-10 infectious particles, depending on the particular isolate used.

The t calculated for several independent tissue-culture infections (3-4 days) is strikingly similar to the reported half-life (1.5-2 days) of cell-free virus particles (measured by RNA PCR) or HIV-1-producing cells in individuals responding to potent antiviral agents c,d. We have also determined the in vivo infection rate constants and n values from published reports of individuals undergoing a primary HIV-1 infection or AIDS patients receiving potent antiviral drugs e.g. The rate constant for the exponential increase of drug-resistant viral RNA in the plasma of AIDS patients was reported to be 0.27 (ref. 2). We calculated a very similar rate constant (approximately 0.3) for patients undergoing a primary HIV-1 infection f.g. Assuming that the length of a single cycle in vivo is also 3 days, the value of n under these conditions would be 2.5 (n = exp (kt) = exp (0.3 x 3)). Thus 2-3 infectious HIV-1 particles, on average, are released from each cell in the two types of in vivo infections.

An independent quantitative study, using an end-point dilution approach, reported that one infectious particle corresponded to about 6 × 10^9 physical particles in HIV-1-infected individuals h. Thus, in both tissue culture and in vivo infections, an infected cell produces approximately 10^7 physical particles and far fewer infectious virions.

The two recent Nature reports i,j estimated the number of newly infected/replaced CD4+ cells in the peripheral blood of AIDS patients to be 4 × 10^7 per day. As noted above, this number of infected cells should gen-

**NATURE • VOL 375 • 18 MAY 1995**
Antiviral therapy

Sir — Ho et al.1 state that “patients with lower initial CD4 cell counts had more prominent rises” (in CD4 count during treatment). This conclusion was substantiated with an analysis showing a correlation coefficient of -0.57 (P<0.01) for the association between initial CD4 count and slope of log CD4 count increase after therapy. This finding suggests there may be a greater propensity for CD4 lymphocyte regeneration at lower CD4 counts compared with higher counts. This could explain the paradox of why the CD4 count decline is on average more rapid early in HIV infection, when the CD4 count is high and cellular and cell-free virus are low, rather than later in the infection when CD4 counts are lower and virus levels are substantially higher2.

However, although indeed the number of new cells appearing in the blood per existing cell is greater at lower CD4 counts, the absolute number of new cells appearing in the blood at any given time may not necessarily be greater, for which the slope of unlogged CD4 count increase after therapy must be measured. When this is done the correlation coefficient for the association between initial CD4 count and slope of CD4 count increase is 0.08 (P = 0.75; Spearman rank correlation coefficient), suggesting that on average no more new cells appear in the blood in a given time after starting therapy in individuals with lower CD4 counts than in those with higher counts. When looked at in this light, the results3 do not appear to explain the paradox. The suggestion4 that the immune system is likely to play a major role in cell destruction — rather than the process being mainly due to direct cell killing by virus — is more consistent with the finding. The most possible explanation for why the rate of CD4 count decline gradually decreases as more severe immunodeficiency develops.

Ho et al. also suggest5 that analyses of the marked CD4 count rises seen after therapy “demonstrate convincingly that the CD4 lymphocyte depletion seen in AIDS is primarily a consequence of the destruction of these cells induced by HIV-1, not a lack of production”. Although this may be correct, it is not clear how the latter possibility has been excluded: if HIV-1 inhibited CD4 cell production, a rise in cell numbers might be expected when HIV levels are drastically reduced by therapy. It is also not clear that the newly increased CD4 count in the blood is indeed due to newly produced cells. As the viral load decreases acutely, the redistribution of CD4 cells from tissues to blood, perhaps with an accompanied decrease in CD8 count, is possible.

Andrew N. Phillips, Caroline A. Sabin
Amanda Mocroft
University Department of Public Health,
George Janossy
Department of Clinical Immunology,
Royal Free Hospital School of Medicine,
London NW3 2PF, UK

Other approaches

Sir — Some5,6 have inferred from two recent papers3,4 that CD4+ T-cell infection determines parameters of HIV disease, and that immunostimulatory therapies are doomed to fail5. But alternative interpretations remain.

The new work3,4 reports that the turnover of CD4+ lymphocytes in AIDS patients is about 8 cells per μl blood per day. Does this turnover reflect an accelerated rate of CD4+ T-cell production that strains the long-term proliferative capacity of the immune system? A definitive answer awaits accurate estimates of the turnover and half-life of both proliferating and peripheral CD4+ T-cells in healthy individuals, normative data for which the immunological community strangely lacks a robust appraisal. However, taking 75 days (range of 50–100 days, as adopted in ref. 4) as a best current estimate of peripheral blood mononuclear cell half-life in the circulation, and 1,100 CD4+ cells per μl peripheral blood as an accepted average5, we can calculate that 0.5 × 1,100/75, or about 7, CD4+ T-cells per μl are normally replaced in the circulation each day. This turnover number is surprisingly close to that calculated in AIDS patients, suggesting that the infection-specific rate of CD4+ T-cell production is close to normal and unlikely to exhaust proliferating populations, even after years of disease. If so, immunostimulatory strategies should not yet be discounted as viable therapeutic approaches against AIDS.

A most remarkable observation by both groups5,6 is that, in each patient, post-treatment titres of resistant (mutant) viral strains rapidly rise very nearly to pretreatment levels. Why are the antiviral responses of the immune system, despite its activation against HIV-1, unable to maintain viral replication at lower levels once the infection has been reduced by chemotherapy? Although viral replication immediately after the emergence of resistant strains outstrips the antiviral defences of the immune system, the rate of increase of virus production eventually slows, establishing an equilibrium between production and clearance of the virus.

One simple hypothesis would account for this phenomenon. If CD4+ T-cells...
infection depended, for instance, on cell-cell contact with a limited number of virus-presenting cells (for example, dendritic cells; ref. 6) in the lymphoreticular system, productive viral infections would begin to occur at a very high rate as drug-resistant strains spread through the population of antigen-presenting cells, each of which infects a large number of CD4+ T cells. Once most virus-presenting cells in a lymphoid organ are infected, the kinetics of virus production will dominantly reflect the rate of replacement of infected CD4+ T cells by uninfected cells at the loci of effective contact with virus-presenting cells, and will occur at a much slower rate. Under such a scheme, limiting the spread of HIV-1 infection within the virus-presenting cell population would represent a promising approach to the therapy of HIV disease.

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Paradox remains

SIR — The articles by Ho et al.1 and Wei et al.2 have been hailed as providing crucial new information that clarifies the enigma of HIV-mediated pathogenesis (see, for example, refs 3, 4). To the extent that they have estimated equilibrium rate constants and provided an explanation for rapid development of drug resistance, these studies1,2 do provide new and important information. But the central paradox of AIDS pathogenesis remains.

The new studies on the dynamics of HIV infection demonstrate that the underlying rate of virus production is still 4-20 times lower than the rate of cell turnover. Because each infected cell produces many virions, and a high proportion of them are known to be defective, there is about 100,000-fold more cell death than can be accounted for by the observed rate of virus production3. It is a murder scene with far more bodies than bullets.

Instead of addressing this discrepancy, the authors1,2 suggest that “virus must be underestimated”, or that many more infected cells must be hiding in deep lymphoid organs where they cannot be observed. Taken at face value, however, these data demonstrate that the CD4 lymphocyte population is highly activated, and that the fate of uninfected cells may be much more important than that of infected cells in AIDS pathogenesis. This view is consistent with observations that the rate of spontaneous apoptosis in the peripheral lymphocytes of HIV-infected individuals is both sharply elevated and at least 10-100-fold higher than the frequency of productively infected cells5.

Ho et al. suggest the analogy of a sink, with the tap and drain both equally wide open, which eventually empties because the “regenerative capacity of the immune system (the tap) is not infinite” and cannot quite keep up. However, the differential between the tap and drain is extremely small (20-200 × 10^6 cells per day) compared with the overall flow rate (2 × 10^6 cells per day), and must remain relatively fixed for an average of 10 years, as CD4 cell loss is roughly linear throughout most of the natural history of HIV infection5. Further, this model would predict no CD4 loss until virus production exceeded a critical threshold, and then an accelerating cell loss as the virus burden increases.

A more plausible explanation for these data is that a mechanism that finely regulates CD4 replacement makes a slight error, resulting in the failure to completely replace the cells (infected and uninfected alike) which are lost through programmes cell death, the natural consequence of immune activation. We have argued that this is exactly what would be expected if the immune system were exposed to a persistent co-stimulatory T-cell signal caused by the interaction of gp120 with CD4 (ref. 8). This extra signal causes the control mechanism to sense an elevated state of immune responsiveness and downregulate the recovery of “memory” cells to prevent growth of the immune system6. The result would be an inexorable but steady decay based on this difference in probability of survival.

Maddox4 wonders “why, after more than a decade of research, has it only now emerged that the response of the immune system to infection by HIV is hyperactivity rather than the opposite?”. The answer to this question is that those who would see AIDS as a more-or-less conventional viral infection have consistently refused to recognize the paradoxes that are clearly evident in the experimental data. The problem continues.

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10. Ho, D. D. et al. Nature 373, 123-126 (1995) state that HIV decay slopes (clearance rate constants) are independent of the initial viral loads, and that the slopes do not correlate with the initial CD4 lymphocyte counts. They conclude that the viral decay slope is not dependent on the stage of HIV-1 infection (their Fig. 1b).

But regression analysis of the same data shows that the correlation between HIV RNA (copies per ml) and the viral decay slopes is 0.17, with a P value of 0.47. When we stratify study subjects’ viral decay rates according to levels of HIV RNA with a cut-off point of 100 (<100 versus ≥100), however, the viral decay rate in the stratum with lower RNA is significantly different from that in the stratum with higher RNA (Kruskal-Wallis test: P = 0.048). The median of the viral decay rate in the lower RNA stratum was ~0.28, while the median in the higher RNA stratum was ~0.38. A univariate linear regression analysis shows that the dichotomous variable RNA (<100 versus ≥100) was significantly associated with the viral decay rate (P = 0.026). These analyses suggest that when RNA copies were at higher levels, the viral decay slopes would be higher. In other words, the viral decay slope is a function of many factors, including initial viral load and the stage of HIV infection.

Ho et al. also state that the slopes are inversely correlated with the baseline CD4 counts. Exponential modelling of CD4 increases reveals that the slopes are inversely correlated with the initial CD4 lymphocyte counts; the authors conclude that the CD4 lymphocyte depletion seen in AIDS is primarily a consequence of the destruction of these cells induced by HIV-1, not a lack of their production. The increase in CD4 lymphocyte counts following ABT-538 administration was also modelled linearly and the authors claimed that although their two sets of analyses (modelling both exponential and linear increases) do not yield identical numerical results, they are in close agreement and emphasize the same qualitative points about HIV-1 pathogenesis.

Based on our univariate linear regression analysis, the slope of increase generated by an exponential growth model is significantly and inversely associated with the baseline CD4 counts (P = 0.01). Nevertheless, the slope of increase associated by a linear production model was not significantly correlated with the baseline CD4 (P = 0.17). After adjusting for the viral loads, measured by HIV RNA, both the linear and exponential slopes seem to be correlated with the baseline CD4 counts, but in opposite directions. With the slope obtained from a linear production model, the slope was, although borderline, significantly and positively associated with the baseline CD4, after controlling RNA levels (P = 0.06). This implies that the slope
generated from a linear model could be a function of both the baseline CD4 level and viral loads. Further studies with larger sample sizes are needed to resolve these discrepancies.

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HIV an illusion

Sir — In an editorial in the 19 January issue of Nature, John Maddox invited "Duesberg and his associates" to comment on the "HIV-1 dynamics" papers published the previous week, indicating that these new results should prove an embarrassment to us. Although we do not think that a scientist should be embarrassed for pointing out inconsistencies and paradoxes in a hypothesis that has been only reportedly resolved 10 years later, we nonetheless prepared a fully referenced, approximately 2000-word critique of the Ho et al.1 and Wei et al.2 papers that we believed met the criteria of "not being longer than it needs to be, and pertaining to the papers at hand" that Maddox set out in his widely read challenge.

Unfortunately, he did not share our view and agreed to publish only a radically shortened version, and only after he had personally "gone over it with a fine-tooth comb" to remove our perceived misrepresentations of the issues. We found these new conditions so totally at variance with the spirit of free and fair scientific debate that we could not agree to them.

Readers of Nature who are interested in these questions, and feel that they do not need to be protected by Maddox from our ill-conceived logic, can find the complete text of our commentary in the monograph supplement to the most recent issue of Genetica.* Here we would point out only that the central claim of the Ho et al.1 and Wei et al.2 papers that 10^9 HIV virions per ml plasma can be detected in AIDS patients with various nucleic-acid amplification assays is misleading. The senior author of the Wei et al. paper has previously claimed that the PCR method they used overestimates by at least 60,000 times the real titre of infectious HIV: 100,000/60,000 is 1.7 infectious HIV per ml, hardly the "virological mayhem" alluded to by Wain-Hobson. Further, Ho and a different group of collaborators have just shown that more than 10,000 "plasma virions", detected by the branched-DNA amplification assay used in their Nature paper, correspond to less than one (!) infectious virus per ml. And infectious units, after all, are the only clinically relevant criteria for a viral pathogen.

Finally, in view of Wain-Hobson's statement that "the concordance of their [Wei and Ho's] data is remarkable", note that Loveday et al.3 report the use of a PCR-based assay and find only 200 HIV "viroin RNA's" per ml of serum of AIDS patients — 1,000 times less than Ho and Wei. So much for the "remarkable concordance".

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Peter Duesberg was offered space in Scientific Correspondence for 500 words of his own choice, but declined. — Editor, Scientific Correspondence.

Sir — The most recent AIDS press releases published as two "scientific articles" in your magazine1,2 indeed are the most clever and, if possible, the most deadly yet! Since March 1987, when Duesberg first published his critique of retrovirology3 and his proof that the "AIDS virus" is not sufficient to cause AIDS, the AIDS/HIV entrepreneurial galleys have railed against him both professionally and financially. Rather than test Duesberg's hypothesis or for that matter fund him to test his own hypothesis, you change your model, which has not saved a single life, to suit your own purposes, from HIV kills CD4 cells directly, to HIV kills cells indirectly, and now back to the latest direct kill model of Wei et al.1 and Ho et al.2.

Wei et al.1 and Ho et al.2 describe a viremia that on administration of certain drugs drops exponentially and raises CD4 counts exponentially, seemingly without a trace of "mayhem". What is this viremia of billions of RNA particles that can only be seen with an undocumented branch-PCR or PCR but not with a functional infectivity test? How do the authors know that these RNA particles are infectious and not defective? Is it reverse transcriptase activity? Is it some protein that is unique to the quasispecies HIV? The tests for HIV are not flawed. What is this free virus? How does one have free virus after immune response? Are not symptomatic people with AIDS HIV-positive, that is, don't they have antibodies against HIV? What is free virus?

The Wei et al. and Ho et al. models do not account for other probable immune activity and the uniqueness of each individual. Haynes and Fauci showed in 1978 that hydrocortisone selectively caused CD4 cells to hide in tissue. Indeed, what effect do multiple drugs have on CD4 cells? The notion of clearance of one billion CD4 cells per day versus one billion virions per day is not defined or addressed, nor can it be! No one else has access to either the unapproved drugs or the branch PCR technology! What is the benchmark rate for the turnover of CD4 cells in the general population?

To counter the 42 case studies of Wei et al.1 and Ho et al.2, we at HEAL (Health Education AIDS Liaison) can provide at least 42 people who are western-blot-positive for HIV, have low T4 cells, who are not using orthodox procedures, and have been healthy for years! On the other hand, we can also provide you with hundreds of "HIV"* people with high T4 cells, who were then hospitalized with opportunistic diseases! Not to mention the thousands of AIDS cases without HIV which were conveniently renamed idiotic pathic CD lymphocytopenia.

We at HEAL maintain that what is called AIDS is no more complicated than a recalcitrant drug-resistant drug disease syndrome which, with the exception of acute medical care, is out of the purview of orthodox medicine.

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Toxic shock

Sir — The recovery rate of CD4+ T cells reported by Wei et al.1 and Ho et al.2 in HIV patients following therapy is remarkably similar to that observed in another acute clinical condition. We have documented lymphocyte counts and proportions of CD4 cells double-staining for CD45 isoforms in three patients during episodes of toxic shock syndrome and one following toxic shock-like syndrome.

In these clinically defined conditions, bacterial superantigen initially induces massive T-cell and monocyte activation. There is subsequent apoptosis of the activated CD4+ cells, during which patients demonstrate a lymphopenia, followed by the return to normal of the lymphocyte count as patients recover. The mean rate of change in the counts in the first 2 weeks of recovery was 2.1, which compares with 2.0 (ref. 1) and 1.8 (ref. 2).

The similarity in rates of recovery suggest that this rate has some value as a common measure of maximal CD4 T-cell output to the circulation in adults. Slower rates may be observed, for instance, in patients following radiotherapy or chemotherapy. This rate decreases with time; it took one patient 14 months to reach a final CD4+ lymphocyte count of 1.4 x 10^5 cells cm^-2. The nature or mechanisms of the brake on this rapid prolifer-
Cyclosporin A

Sir — Ho et al.1 and Wei et al.2 draw attention to the dynamics of HIV replication in vivo, through close observation of the response of plasma viraemia, quantified by RNA PCR, to antiretroviral therapy. In all subjects, HIV RNA levels fell rapidly over the first 7 days of therapy at a constant rate. Reduction in viral load was associated with a prompt elevation in CD4 number, which appeared to be related to interruption of viral replication consequent to antiretroviral therapy. The rapid reduction of HIV viral load by RNA PCR and parallel CD4 increase has been observed with diverse drugs3.

We demonstrate a novel, distinct pattern of reduction in viral load in HIV infection that is slow, sustained and associated with a parallel reduction in CD4 number. We believe this observation has implications for the pathogenesis of HIV infection.

The patient, a 36-year-old HIV-1-infected homosexual male, developed psoriasis in 1991; diverse topical and systemic agents were prescribed, without clinical benefit. By June 1993, the patient was incapacitated by psoriatic arthropathy and generalized pustular psoriasis, and 250 mg per day cyclosporin A (CyA) was given as a last resort, in the full knowledge that HIV infection may be accelerated by concomitant immunosuppression. There was a dramatic clinical response: there was a reduction of viral load of 84% (0.87 × log10) over 30 weeks, which was sustained. But in contrast to the data presented in refs 1 and 2, CD4 number fell from 960 to 530 × 106/l over this period. The relationship of these changes to CyA therapy was demonstrated when the patient ceased therapy about 50–54 weeks later. An unrelated alcoholic hepatitis. Viral load and CD4 rose to baseline off therapy, and fell again when CyA was recommenced on week 70. No other therapy was given over this entire period.

CyA is known to inhibit T-cell activation; we believe these data provide evidence in vivo for the importance of T-cell activation in permitting HIV replication. CyA reduces CD4+ T-cell number, but also inhibits HIV replication by removing the host cell target. There has been speculation that there seems to be a ceiling in vivo for the absolute level of plasma viraemia; our observations suggest that the level of plasma viraemia may be determined by the level of availability of the activated target CD4+ cell. The use of glucocorticoids to reduce T-cell activation has recently been reported, but although these lead to an increase in CD4 cells, viral load is unaltered.

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Ho et al. reply — The most substantive point raised by the letters printed above concerns CD4 lymphocyte redistribution, rather than absolute decline, as an explanation for the rise in CD4 cell counts following treatment with potent antiretroviral agents. Although lymphocyte re-trafficking is a plausible explanation, as pointed out by Mosier, Sprent and Tough, Dimitrov and Martin, and Phillips et al., several observations collectively argue against this suggestion.

First, the elevations in CD4 cell counts were not transient, but were sustained as long as the antiviral effect was maintained, in some cases for more than 6 months. Second, in other studies, CD4 lymphocyte increases associated with viral suppression were accompanied by significant clinical improvement4. Third, in our recent unpublished studies, the surface-marker phenotypes of CD4 lymphocytes post-therapy differ substantially from those before treatment. In particular, our preliminary data reveal the expression of a number of activation markers on many of the CD4 lymphocytes after treatment, a finding that supports lymphocyte repopulation by cellular proliferation but argues against lymphocyte redistribution, because, as stated by Sprent and Tough, “most of the CD4+ cells would be expected to display a resting rather than an activated phenotype” if the latter were true.

In reanalysing our data, Mosier found a positive correlation between the exponential slope of the CD4 lymphocyte increase and the baseline plasma level or the viral turnover rate, whereas we showed an inverse correlation with the baseline CD4 lymphocyte count5. These findings are fundamentally the same, as all three parameters (viral load, viral turnover rate and CD4 count) reflect the baseline disease status of the patient; they do not necessarily support the lymphocyte redistribution hypothesis. To address properly his notion that antiviral treatment liberates CD4 lymphocytes from trapping in lymphoid tissues, Mosier should instead correlate the slope (perhaps the linear slope would be more appropriate) of the CD4 lymphocyte increase with the magnitude of the antiviral effect.

Bukrinsky et al. are incorrect to suggest that we4, Wain-Hobson6 or Coffin7 have stated or implied “that immunostimulatory therapies are doomed to fail”. In addition, we do not understand their logic of comparing our calculated CD4 lymphocyte turnover rates with previous estimates for normal peripheral blood mononuclear cells, which are a mixture of B cells, CD8 lymphocytes, monocytes and divergent populations of CD4 lymphocytes.

Dimitrov and Martin question our estimates of the CD4 lymphocyte turnover rate based on a mathematical calculation. They suggest that if each infected lymphocyte produces 103 virions, then the particle turnover rates are simply too low, perhaps by four orders of magnitude, given our estimates for infected cells. However, in their derivations, they did not take into consideration that many infected lymphocytes in vivo may be eliminated by immune responses before release of progeny virions. In essence, the “bullets” and “bodies” analogy of Ascher et al. suffers from the same point. Furthermore, in a recent follow-up study to determine more accurately the virion turnover rates, we have found that there are indeed more “bullets” than “bodies”.

Phillips et al. and Lai et al. correctly point out that the linear (rather than exponential) slope of the CD4 lymphocyte increase does not correlate with the baseline CD4 cell count. However, the use of the linear model is based on the assumption that the CD4 lymphocytes are coming from a source such as the thymus. Based on lymphocyte phenotype studies, we now have evidence that the increase in CD4 cell counts is largely due to post-thymic lymphocyte proliferation.

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198