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Evolution of Mutation Rate and Virulence among Human Retroviruses [and Discussion]

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Evolution of mutation rate and virulence among human retroviruses

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SUMMARY

High mutation rates are generally considered to be detrimental to the fitness of multicellular organisms because mutations untune finely tuned biological machinery. However, high mutation rates may be favoured by a need to evade an immune system that has been strongly stimulated to recognize those variants that reproduced earlier during the infection. HIV infections conform to this situation because they are characterized by large numbers of viruses that are continually breaking latency and large numbers that are actively replicating throughout a long period of infection. To be transmitted, HIVs are thus generally exposed to an immune system that has been activated to destroy them in response to prior viral replication in the individual. Increases in sexual contact should contribute to this predicament by favouring evolution toward relatively high rates of replication early during infection. Because rapid replication and high mutation rate probably contribute to rapid progression of infections to AIDS, the interplay of sexual activity, replication rate, and mutation rate helps explain why HIV-1 has only recently caused a lethal pandemic, even though molecular data suggest that it may have been present in humans for more than a century. This interplay also offers an explanation for geographic differences in progression to cancer found among infections due to the other major group of human retroviruses, human T-cell lymphotropic viruses (HTLV). Finally, it suggests ways in which we can use natural selection as a tool to control the AIDS pandemic and prevent similar pandemics from arising in the future.

1. INTRODUCTION

The generation and maintenance of heritable variation is central to evolutionary processes. Although genetic variation is generated ultimately by mutation, reductions in mutation rate are generally considered beneficial to sexually reproducing organisms because a low mutation rate appears sufficient to generate a great amount of variability. Genetic recombinations during meiosis are considered to be more beneficial than high mutation rates as a means for generating variation among offspring. This is because recombination puts together different instructions that have passed the test of time (and may help weed out inferior instructions), whereas mutations generate a very high proportion of variants with reduced fitness. The existence of proof-reading functions among DNA polymerases is evidence of the disadvantageousness of high mutation rates in multicellular organisms.

High mutation rates may be much more valuable for viruses, particularly those that infect animals with well-developed immune systems. Virologists have proposed that high mutation rates among viruses reflect adaptive mechanisms for generating variability (Holland 1993). Because replication from RNA templates is associated with high mutation rates, the coding of viral genomes using RNA has been interpreted as one mechanism through which high mutation rates are maintained (Holland 1993). The replication of DNA viruses, such as the hepatitis *B* virus, through RNA templates supports this view – RNA intermediates apparently function to introduce mutations (Holland 1993). But mutation rates among viruses vary greatly, even among RNA viruses. Virologists typically explain this variation in a proximate sense by referring to the different biochemical mechanisms through which mutations are introduced, but little attention has been directed toward ultimate reasons why different levels of mutation proneness may be favoured by natural selection.

Some of this variation in mutation rate may be attributable to genome size. As genome size increases, a high mutation rate becomes more costly to the organism because mutations are generally disadvantageous; the probability of advantageous mutations occurring in the absence of overriding disadvantageous ones would therefore be extremely small. But variation in genome size does not explain why different viruses of a given genome size have different mutation rates. To understand mutation rates, one must take into account both the genome size and the relative fitness of mutants (Eigen & Biebricher 1988).

In this paper, I focus on the fitness costs and benefits of mutations to develop a hypothesis for

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understanding why some viruses are more mutation-prone than others. This hypothesis represents a return to the problem of 'sex versus non-sex versus parasite' (Hamilton 1980), with a focus on the value of generating genetic variation among parasites. I then consider the variation in mutation rates among retroviruses in light of this hypothesis, suggest some tests of the hypothesis, and discuss the potential importance of this issue for controlling existing viruses and for guarding against emerging viruses.

My basic proposition is that differing selective pressures imposed by immune systems may cause the evolution of different mutation rates. High mutation rates can increase the fitness of a virus by making the mutant viruses 'look' different to the immune system. A virus with a different structure may escape destruction by an immune system that has 'learned' to recognize the parental viruses.

What aspects of a virus's exposure to the immune system should be associated with relatively great fitness benefits from mutation? I suggest that high mutation should be strongly favoured when viruses: (i) engage in both active replication at the onset of infection and delayed replication from latently infected cells within each infected host; (ii) generate persistent infections from which viral progeny can be transmitted to new hosts; and (iii) infect cell types that make the viruses vulnerable to destruction by the immune system during their reproduction in and escape from the body.

The specific argument proposes that actively replicating viruses stimulate an immune response that elevates the fitness benefits of being different from parental viruses. Any virus that reproduces after a strong immune response has been generated against parental viruses has a low chance of success if: (i) its replication exposes it to the activated immune system; and (ii) if the virus has not altered its structure to allow it to escape the immune attack. Mutations always carry the potential for untuning finely tuned biochemical machinery, but under these circumstances the costs of this untuning should be weighed against the benefits of looking different to the immune system. The balance of these fitness costs and benefits should affect the overall mutation rate to which any particular virus will evolve.

At the other end of the mutation continuum should be viruses that are minimally exposed to the specific immune responses during their reproduction in and escape from the body. Most viruses that cause acute infections should lie in the vast middle ground between these two ends of the mutation continuum. Viruses that infect without latency and in a way that exposes them to effective immunological control should benefit from looking different when they are reproducing after the immune response is generated. However, a high mutation rate that could facilitate this variation at that time would probably place them at a competitive disadvantage early during infection by untuning their replicative machinery. These viruses should therefore lie toward but not at the mutation-prone end of the continuum. Viruses that infect and are transmitted from tissues with little exposure to the immune system should get relatively little benefit from mutation and should therefore lie toward the mutation-averse side of the continuum.

This hypothesis presumes that the viral replication systems can evolve differences in mutation rates, and hence that heritable variation in mutation rate exists. Such variation has been documented: mutation rates of envelope genes in influenza *A* viruses, for example, vary by several-fold (Suárez *et al.* 1992).

The hypothesis presented above emphasizes the need to understand the evolution of replication rate. To understand the mutation-prone end of the continuum, we must understand why some viruses generate persistent infections associated with prolonged periods of transmission, and with exposure to strongly activated immune systems during transmission. To understand the mutation-averse end of the continuum, we must determine the factors that allow a virus to infect a host and be transmitted from a host with relatively little exposure to the immune system. My approach to the problem complements that of Bell (1993), who proposed that persistent infections might be responsible for long-term deterioration of hosts, that is, for senescence. The evolution of mutation rate is important to his proposition, because high mutation rates should tend to contribute to the eventual deterioration of the hosts.

2. HUMAN IMMUNODEFICIENCY VIRUSES

Human retroviruses include viruses that lie near either end of this mutation continuum. Human immunodeficiency virus type 1 (HIV-1) lies at the mutation-prone end. HIV infections are characterized by a combination of active replication of virions and latent infection. During the first few months of infection, the actively replicating viruses generate a high density of virus in the blood; thereafter, free virus is apparently continuously cleared from the bloodstream by an effective immune response (Fauci *et al.* 1991). Viral density again increases during the last two years of infection when the immune system disintegrates. Throughout this time, at least some viruses are actively replicating, primarily in the germinal centers of lymphoid organs where as many as one out of three target cells may be infected (Embretson *et al.* 1993; Piatak *et al.* 1993).

The dynamics of latency are not well understood, but some viruses are presumably breaking their latency fairly continuously during this time. Although genetic variation in tendencies to enter latency has not been well-studied in HIV, studies of SIV have demonstrated that transactivation is influenced by differences in promotor sequences (Anderson & Clements 1992). For almost all of these latent viruses, whether or not genetic differences influence tendencies toward latency, by the time latency is broken, the person's immune system will have been stimulated to defend against viruses that had previously begun active replication. The efficient clearance of virus from the bloodstream during the bulk of an HIV infection and the turnover of antigenic types during this time indicate that the immune system is exerting a

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strong selective pressure on viruses that are replicating during the years between the initial immune response and the eventual disintegration of the immune system.

A high mutation rate therefore probably increases the chances of diverging from the earlier viruses sufficiently to escape destruction by the immune system (Albert *et al.* 1990; Coffin 1990; Tremblay & Wainberg 1990). Indeed, direct measurements of mutations and reverse transcription show that HIV's reverse transcriptase tends to generate mutations at greater rates than the reverse transcriptases of most other retroviruses (Preston *et al.* 1988; Roberts *et al.* 1988; Takeuchi *et al.* 1988; Bakhanashvili & Hizi 1992*a*,*b*; Hübner *et al.* 1992; Ji & Loeb 1992; Monk *et al.* 1992; Varela–Echavarria *et al.* 1992).

The relevance to human infections of these high mutation rates has been questioned by Temin (1989*a*) because such high rates would make virtually all of the progeny viruses from an infected cell different from the parental virus, and many of the progeny non-functional. Indeed, HIV pays this cost: a substantial proportion of the HIV in infected cells appear to be so altered by mutation that they are incapable of completing their reproductive cycle (Bagasra *et al.* 1992). But the cost of losing some of the successful parental combinations must not be considered in isolation. Natural selection will weigh this cost against the benefits of divergence from the parental virus. One such benefit is the increased reproduction that results from a temporary escape from immune detection.

Different HIVs probably can recombine genetic instructions (Howell *et al.* 1991), but the potential of this recombination for generating genetic variation is relatively limited; recombination between viruses requires simultaneous infection of a single cell with two different virions. Given that only a small minority of target cells will be infected during most of the time that a person is infected, the probability of simultaneous infection is low. If HIV had a low mutation rate, even these joint infections would not involve much potential for generating variability through recombination. Among HIV then, mutation rate seems to be the primary generator of genetic variability over the short-term time-scale of viral generations.

Why has HIV evolved its two-fold strategy involving both active replication and latency? A consideration of the costs and benefits of replication rates suggests that increased rates of sexual partner change will tend to favour active replication (as opposed to latency), resulting in evolutionary increases in virulence (Ewald 1991, 1994). The distribution of severe and mild HIV infections accords with this view: in regions where sexual partner change appears to be low, the time between infection and AIDS tends to be long (Ewald 1994).

If the hypothesis presented above is correct, HIVs that have not been exposed to high rates of sexual transmission should have lower replication rates and lower mutation rates. A divergent group of HIV (called subtype O) that clusters with HIV-1 should prove particularly useful in assessing whether HIV-1 has recently generated a high mutation rate in response to high rates of sexual-partner change. The first isolate of this divergent group was called ANT-70 (De Leys *et al.* 1990). According to time scales derived from molecular clocks (Eigen & Nieselt–Struwe 1990), the ANT-70 branch diverged from the other HIV-1 branches centuries ago. Serological surveys in west and westcentral Africa (Gürtler *et al.* 1994; Nkengasong *et al.* 1994) indicate that about 2000 people in the Cameroon and Gabon are infected with viruses that belong to this divergent subtype. No evidence of infection has been found among people to the west of Cameroon (Nkengasong *et al.* 1994). If pandemic HIV-1 generated its mutation-proneness as a result of the high rates of sexual partner change that occurred in central and east Africa during this century (Ewald 1994), subtype O HIVs should have a lower mutation rate.

A similar comparison needs to be made with the other major group of human immunodeficiency viruses: HIV-2, which has been endemic in some geographic areas that have had relatively low rates of sexual partner change. Available evidence indicates that HIV-2s tend to have lower rates of replication and cause more mild infections than pandemic HIV-1s (Ewald 1994). In an *in vitro* assay for mutation rate, reverse transcriptase from HIV-2 generated mutants at a lower rate than reverse transcriptase from HIV-1 (Bakhanashvili & Hizi 1992*a*,*b*). HIV-2's reverse transcriptase might generate mutations more slowly simply because it works more slowly whenever it is synthesizing DNA strands (Hizi *et al.* 1991; Bakhanashvili & Hizi 1992*a*,*b*, 1993), or it might be less mutation-prone because it is less liable to connect mismatched building blocks during the construction of the new strand of DNA (e.g., see Goodman *et al.* 1993). HIV-2s that have been endemic in Senegal should offer a particularly interesting comparison because rates of sexual-partner change there appear to be unusually low, and the progression to AIDS particularly slow (Ewald 1994).

If further tests show that reverse transcription in such HIV-2s and the subtype O HIV-1s are not less mutation-prone, then we can reject the idea that the high mutation rate of pandemic HIV-1 resulted from exposure to high rates of sexual-partner change in central or east Africa. Such evidence would favour the null hypothesis that some other general aspect of lentivirus replication is responsible for the high mutation rate.

3. HUMAN T-CELL LYMPHOTROPIC VIRUSES

The other major group of human retroviruses are the human T-cell lymphotropic viruses (HTLVs), which have a similar genome size but belong to a different subfamily of retroviruses (Temin 1989*a*). HTLVs are geographically widespread and probably have been infecting humans for many millennia (Gessain *et al.* 1991; Goubau *et al.* 1992; Maloney *et al.* 1992). One type, HTLV-I, is globally distributed; prevalences are generally less than 1% but may be above 10% in certain endemic pockets. In about one out of 30

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infected people, HTLV-I eventually triggers adult T-cell leukemia/lymphoma (ATL) (Kondo *et al.* 1989; Blattner 1990, Tajima & Ito 1990). Evidence indicates that ATL results from proliferation of infected cells, which in turn is generated by the activity of HTLV inside them (Berneman *et al.* 1992; McGuire *et al.* 1993; Béraud *et al.* 1994; Yoshida 1994).

The substitution rates of HTLV-I are two or more orders of magnitude less than those of HIV-1 (Ratner *et al.* 1991). HTLV's lower rate is largely attributable to a lower reliance on reverse transcriptase for multiplication. Although free HTLV-I virions can infect cells (Fan *et al.* 1992), growth of viral populations inside people occurs mainly by division and fusion of infected cells; similarly, transmission between hosts occurs largely by vertical transfer of infected cells from mother to child (Sugiyama *et al.* 1986; Murphy & Blattner 1988; Yamaguchi 1994). Because this kind of replication and transmission reduces dependence on reverse transcription, it should contribute to a low mutation rate (Temin 1989*b*).

The hypothesis suggested above offers an explanation for HTLV's lower substitution rate. Relying less on production of virions and having relatively less active replication at the beginning of infection, HTLV is less exposed to the immune system. The benefits of generating variation are therefore relatively low. But why, in an ultimate sense, does HTLV rely less on production of virions at the price of reduced replication at the beginning of infection? Consideration of this question requires an analysis of transmission modes and the timing of transmission events.

HTLV-I can be needleborne, sexually transmitted, and transmitted from mother to baby largely through breast milk (Hino *et al.* 1985; Kajiyama *et al.* 1986; Kusuhara *et al.* 1987; Nagamine *et al.* 1991; Kawase *et al.* 1992; Stuver *et al.* 1993). Depending on the relative importances of these transmission modes, one would expect different selective pressures on rates of viral replication and mutation. Maternal transmission of HTLV should place a premium on replication with little stimulation of an immune response because maternally transmitted viruses must survive inside the person from the onset of infection (i.e., in and around the neonatal period) until opportunities for transmission arise (i.e., after the infected baby becomes sexually mature), and then must be transmissible when these opportunities arise. One of the best ways of doing so is by invoking a combination of latency and proviral replication through proliferation of infected cells. If no virions are produced, virions cannot be exposed to the humoral part of the immune system and presentation of viral fragments for control through cellular immunity will be reduced. Indeed, most HTLV infections are characterized by slow development of seroreactivity, extremely low densities of virions, and low to moderate densities of proviral genomes (Agius *et al.* 1988; Wattel *et al.* 1992).

The importance of the different routes of transmission has been most intensively studied in Japan. The geographic clustering of HTLV in Japan indicates that transmission occurs primarily from mother to offspring (Hinuma *et al.* 1982; Tajima *et al.* 1982; Yamaguchi *et al.* 1983; Tajima 1988). Even in cities, HTLV infection has been associated more strongly with the person's geographic origin within Japan than with risky sexual contact (Tajima 1988). This conclusion is supported by a study of HTLV-infected pregnant women: 62% of the women were vertically infected and 23% were sexually infected (Take *et al.* 1993).

This lower rate of sexual transmission probably results partly from attitudes towards sexual contact and birth control. Rates of unprotected sexual contact are relatively low in Japan. Because access to birth control pills is restricted, birth control depends largely on condoms (Trager 1982; Carey *et al.* 1992; Miller *et al.* 1992). A survey completed during the 1970s showed that condoms were used by over 80% of all women using birth control, and over 90% of women in their early twenties; only 3% of women surveyed said that they would use birth control pills (Trager 1982).

Sexual transmission takes place primarily between long-term sexual partners. The slow rise in prevalence among women as a function of age (Tajima *et al.* 1982; Tajima & Ito 1990) suggests that transmission of infections (or the development of detectable evidence of infection) often occurs after many years of sexual contact with an infected partner. This rise in prevalence probably overestimates the degree of sexual transmission because it is inflated by a cohort effect (Ueda *et al.* 1989, 1993). Among a small sample of infected pregnant women, an average of five years elapsed between onset of sexual relations with their husbands and the first seropositive blood sample (Take *et al.* 1993). Probabilities of transmission during sexual contact appear to be about an order of magnitude less for HTLV than for HIV (Blattner 1990).

Maternal transmission also seems to involve low infectivity per instance of contact. Mothers who nurse babies for longer than six months are about three times more likely to infect their babies than mothers who nurse for less than six months (Takahashi *et al.* 1991; Wiktor *et al.* 1993). Seroprevalence among babies stabilizes by about three years of age, suggesting low infectivity and/or slow development of infections within babies (Tajima 1990).

Studies of transmission probabilities indicate that the generally low viral densities reduce the probabilities of infecting a contacted individual: contact with susceptibles leads to infection more frequently as indicators of viral density and activation increase (Sugiyama *et al.* 1986; Hino *et al.* 1987; Sawada *et al.* 1989; Blattner 1990; Ho *et al.* 1991; Scarlatti *et al.* 1991; Stuver *et al.* 1993). Patterns of infection among children of infected parents also indicate that the low viral densities translate into fitness costs for the virus. Children born earlier during a marriage are less likely to be infected, apparently because of low transmissibility from husband to wives or the low transmissibility from mother to baby during the earlier years of a mother's infection (Wiktor *et al.* 1993; Take *et al.* 1994; Umemoto *et al.* 1994).

Taken together, this information supports the following generalizations: HTLV-I infection in Japan is characterized by a high degree of vertical transmission and relatively low potential for sexual transmission. The infrequent opportunities for transmission to susceptible hosts favour immune avoidance through low levels of replication and extremely low levels of virion production, which in turn result in low probabilities of transmission per instance of contact. These characteristics are associated with low mutation rates, which occur through reliance on proliferation of provirally infected cells rather than on reverse transcription.

4. MUTATION RATES AND THE EVOLUTION OF VIRULENCE

The precise reasons for the eventual disintegration of the immune system and the ensuing progression of HIV infection to AIDS are still unclear. Virtually all of the leading hypotheses, however, are based at least partly on high mutation rate. One hypothesis, for example, emphasizes the diversity of variants generated by mutation as the underlying cause of AIDS (Nowak *et al.* 1990). According to another hypothesis, rapidly replicating variants generated by the high mutation rate outcompete the more slowly replicating viruses, and eventually decimate the immune system, bringing on AIDS (Cheng–Mayer *et al.* 1988; Schneweiss *et al.* 1990; Tersmette & Miedma 1990; Gruters *et al.* 1991; Schellekens 1992; Connor *et al.* 1993).

If high mutation rates contribute to the lethality of HIV infections, understanding the evolution of high mutation rates should help us to identify slowly replicating, mutation-averse viruses that might evolve into more dangerous mutation-prone viruses. I suggest that HTLV is such a virus. Transmission of HTLV in populations with high rates of sexual partner change should favour variants that reproduce more rapidly after infecting an individual because the frequent opportunities for sexual transmission would present a lucrative alternative to the infrequent vertical transmission achieved through more cryptic infection. This selection would transform the endemic HTLV from a largely latent or slowly reproducing virus into a virus characterized by both latency and more active replication, like HIV. The hypothesis presented in this paper suggests that once this change in viral characteristics occurs, higher mutation rates will evolve. If high mutation rates, high sexual contact rates, long durations of infections, and infection of $CD4^+$ target cells are largely responsible for the virulence of HIV-1, then another AIDS-like virus could be inadvertently created by favouring the evolution of this collection of characteristics among HTLVs.

The geographic pattern of HTLV disease and sexual transmission suggests that the first steps of this scenario may have already occurred in the Caribbean. In contrast to the situation in Japan, HTLV-I in the Caribbean has the less patchy geographic distribution characteristic of sexual transmission, and it is especially prevalent among people who have many sexual partners (Clark *et al.* 1985; Murphy *et al.* 1989; Rodriguez *et al.* 1993). In Trinidad, for example, it is about six times as prevalent among male homosexuals as among the general population (Bartholomew *et al.* 1987). In contrast to the situation in Japan, condom use is low in most areas of the Caribbean (Schwartz *et al.* 1989; Halsey *et al.* 1992).

Because sexual transmission of retroviruses tends to occur more readily from men to women than from women to men (Padian *et al.* 1991; Stuver *et al.* 1993), sexually transmitted HTLV should be biased toward women. The age at which a female bias in infection rates begins to appear is therefore an indicator of the relative importance of sexually transmitted HTLV. The bias appears shortly after the age of 20 in Jamaica and Barbados, but after the age of 40 in Japan; it continues to rise with age in each of these areas (Tajima *et al.* 1982; Kajiyama *et al.* 1986; Riedel *et al.* 1989; Tajima & Ito 1990; Murphy *et al.* 1991). Only about 7% of the seropositive people in Jamaica are children (Murphy 1990). The analogous figure for Japan is about 17% (Kajiyama *et al.* 1986), this figure underestimates the relative importance of vertical transmission, however, because of a birth cohort effect, which appears to be responsible for much of the higher seropositivity among older age groups in Japan (Ueda *et al.* 1989, 1993) and may have been generated by a reduction in the duration of breast feeding in recent decades (Tajima 1990). Accordingly, the study of HTLV-infected pregnant women (Take *et al.* 1993; see above) suggests that most infections are vertically acquired in Japan. A cohort effect was not found in the Caribbean (Riedel *et al.* 1989).

If the evolutionary scenario proposed above is correct, the HTLVs in the Caribbean should be more harmful than those in Japan. Although conclusive tests of this prediction have not been conducted, the available data do allow a comparison of the rate at which HTLV infections progress to lethal cancers in the two areas. In Japan, people who eventually develop ATL tend to do so late in life, when about 60 years old on average (Tajima 1990; Shimoyama 1991). The absence of ATL among people who did not acquire their infection vertically suggests that ATL in Japan develops primarily and perhaps almost exclusively in infections acquired from parents (Sugiyama *et al.* 1986; Tajima & Ito 1990). The 60-year interval between birth and ATL therefore roughly reflects the time between infection and ATL. In the Caribbean, infected people begin to develop ATL earlier, typically during the early-to-mid-forties (Murphy & Blattner 1988; Murphy *et al.* 1989; Cleghorn *et al.* 1990). Accordingly, the yearly incidence of ATL per HTLV positive adult is about 50% greater in the Caribbean than in Japan (Cleghorn *et al.* 1990). The difference in median age of ATL patients also occurs among people of Japanese and African ethnicity living in the U.S. (the latter mostly of Caribbean extraction) (Levine *et al.* 1994).

These considerations indicate that both sexual partner rate and the virulence of HTLV infections are greater in the Caribbean than in Japan. Additional studies will be needed to determine whether this

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difference results from differences in the inherent virulence of the HTLVs or from other influences. HTLV researchers have considered a difference in inherent virulence to be improbable without addressing the concordance of such a difference with evolutionary predictions (Levine *et al.* 1994).

Measurements of the error-proneness of HTLV are important for this assessment. If HTLV's low mutation rate per round of replication is attributable entirely to its proviral replication, its reverse transcriptase might be highly error-prone like that of HIV. Direct comparisons of the mutation-proneness of these reverse transcriptases still need to be done. If the reverse transcriptase of HTLV is not less error prone, then the lower mutation rates of HTLV could be interpreted as a non-adaptive side-effect proviral replication. Alternatively, the fitness advantages of proviral replication could be due to both low mutation rate and low immune exposure. Either way, if the low mutation rates of HTLV are attributable solely to a reliance on proviral replication, high rates of sexual partner change could generate high mutation rates by selecting for increased reliance on reverse transcription. This change would be dangerous because high rates of replication and mutation would move HTLV one step closer to the combination of attributes that make HIV-1 so harmful and difficult to control. Alternatively, if HTLV's reduced exposure to an effectively activated immune system favoured a less error-prone reverse transcriptase, increased mutation rates may be more difficult to evolve, leaving a longer window of time before rates of replication and mutation would be coupled.

Data from other geographic areas are more fragmentary, but emerging trends are consistent with the predicted association between sexual partner rates and HTLV virulence (Ewald 1994). The uncertainties associated with the existing evidence emphasize the need for studies that assess rates of mutation, replication, and virulence for the endemic HTLVs of different regions and monitor these characteristics to detect any evolutionary changes. Perhaps the HTLVs in the Caribbean have not yet evolved into a more harmful state because they have not yet evolved increased mutation rates.

HTLV and HIV have similar transmission modes, cell tropisms, genome sizes, and encoded proteins. Although the most dangerous aspects of HTLV pathology concern cancerous growth of white blood cells rather than immune decimation, some ominous similarities exist. In a small minority of HTLV-I infections, for example, some kinds of white blood cells may eventually become decimated. This decimation is more severe as the density of HTLV in the blood increases (Yu *et al.* 1991) and can open the door for opportunistic infections with AIDS-causing organisms such as *Pneumocystis carinii* (Shearer & Clerici 1991).

Until additional data are obtained, it would be prudent to take preventative measures. If the hypothesis presented in this paper is incorrect, investments in reducing rates of sexual transmission would have the well-recognized effect of reducing retroviral prevalence. If the hypothesis is correct such investments will also generate an evolutionary effect, reducing the inherent harmfulness of pathogens like HIV and HTLV, or keeping such pathogens from evolving increased harmfulness in the first place.

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Discussion

C. A. MIMS (*Sheriff House, Ardingly, West Sussex, U.K.*). It has been fascinating to hear Dr Ewald's ideas about the role of viral mutations, viral reactivation from latency, and host behaviour on the evolution of virus diseases. Mutation rates, however, are known to be very great in RNA viruses compared with DNA viruses because of the high frequency of copying errors in RNA polymerases, the final frequency in the virus population depending on growth competition and selective transmission. This is an intrinsic characteristic of RNA viruses.

A second point. Although Dr Ewald suggests that viruses that establish latency and later reactivate are more likely to have high mutation rates, this seems not to be true. Human papillomaviruses, herpesviruses, and polyomaviruses are all DNA viruses and show far less genetic diversity (in so far as this reflects mutation rates) than do RNA viruses such as influenza or rhinoviruses. Indeed, the rate of change in human papillomavirus genomes is so slow that there is less sequence diversity between HPV 16 and HPV 18 than in the HIV virus from a single patient (Ho, L. *et al. J. Virol.* **67**, 6413 (1993); Ong, C.K. *et al. J. Virol.* **67**, 6424 (1993). If points like these were incorporated into Dr Ewald's hypotheses they would be strengthened.

P. W. EWALD. For the reasons that Professor Mims has mentioned, tests of these ideas need to account for the genomes' size and building blocks (DNA or RNA). My presentation focused on retroviruses for these reasons. It is noteworthy, however, that the relative use of DNA or RNA in the life cycle may evolve as a means for altering mutation rate. HTLV, for example, may have evolved a greater reliance on DNA provirus replication to reduce mutation rates whereas Hepatitis B virus may have incorporated an RNA intermediate in its replication cycle to increase mutation rate. The examples that Professor Mims mentions in the second part of his comment are consistent with the general hypothesis that I presented. Low mutation rates are expected among viruses that establish latency and can then be transmitted from the host with little exposure to the immune system. Herpes simplex, for example, does this; after it is activated from latency within neurons, viral progeny can then travel down axons and be transmitted from a blister with little exposure to the immune system. Papillomaviruses too are relatively unexposed to the immune system.

Although influenza viruses do not appear to be transmitted through activation from a latent state, I would expect them to suffer a heavy exposure to the immune system during transmission: those viruses reproducing during the latter part of the infection would benefit by looking different from the viruses that initiated the infection. I would therefore expect them to be toward the mutation-prone end but not as mutation prone as HIV. Studies indicate that they are slightly less mutation prone than HIV. I would expect rhinoviruses to be less mutation-prone than either HIV or influenza, because their exposure to an activated immune system during transmission should be lower, but I am not aware of any direct comparisons to allow a test of this prediction.

One problem with such predictions is that one does not know the fitness tradeoff between the costs and benefits of mutation rates when the mutation rate is near one per genome per replication cycle. It is possible that the extra benefit derived from immune escape after breaking of latency could be swamped by the costs when mutation rates fall into this range. In this case one might find a statistical pile-up of viruses that are exposed to activated immune systems during transmission, with little detectable effect of latency following active replication.

Given that genome size, nucleotide type and other unidentified factors will probably have an effect on mutation rates, I expect that the strongest tests of the general hypothesis will come from studies of closely related viruses that differ in key characteristics such as the breaking of latency in the face of an activated immune system that imposes strong variant-specific culling prior to transmission.

P. J. LACHMANN (*The Royal Society, London, U.K.*). I would like to add three further points to those already made.

1. 'Evolution' of HIV within a patient leading to more rapid growth rate and increased syncytial formation seems also to produce a virus that is less able to be transmitted, possibly because these virulent viruses rapidly lose GP120, so that mutational changes in one host are not necessarily reflected in isolates from the next host.
2. The rate of progression to AIDS is known to be due, at least in part, to both genetic and acquired host factors. Thus sexual promiscuity is related to the incidence of other sexually transmitted disease and thus to the presence of activated T cells in the genital tract. These may be the important factors leading to the accelerated progression of HIV in such subjects.
3. Antibodies to host membrane proteins are effective in lysing SIV grown in xenogenic cells and can presumably limit xenogenic spread of 'promiscuously budding' viruses. It is an interesting possibility that previous alloimmunization (e.g. to HLA) of a wife by her husband may limit infection by the sexual route in the monogamous couple.

The biology of HIV infection is very complex and there are alternative explanations for some of the phenomena you quote in favour of your theory that the rate of sexual partner change determines the mutation rate of the virus.

P. W. EWALD. I agree with Professor Lachman's statements, but none of them negates the hypothesis that I have proposed. We can expect the selective pressures within individuals to favour increased replication rate by a variety of mechanisms. The requirements for transmission to new hosts will tend to select against any that do not also have high capabilities of transmission. Increased syncytial formation appears to be associated with increased replication rate but decreased transmissibility.

The importance of host factors does not negate the

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possibility that variation in viral characteristics may be important. HIV-1 and HIV-2 differ in this regard, and available evidence suggests that similar variation occurs among HIV-1s. My point is that the evolutionary associations between replication rate, mutation rate, and social influences on transmission are expected. If we restrict our focus to host factors, we will not recognize and conduct the tests that are needed to determine whether these associations do in fact exist. I agree that alternative hypotheses exist, but we must identify alternative hypotheses before we can test them. The purpose of my presentation was to identify one alternative that needs to be tested.

A. L. Hughes (*Department of Biology, Pennsylvania State University, U.S.A.*). One of the most fundamental distinctions in evolutionary biology – indeed in all of biology – is the distinction between mutation and substitution. Mutation is an event that happens to a DNA molecule, whereas substitution is a process in a population. When Dr Ewald mentions differences among viruses of similar genome size with respect to 'mutation rate', what sort of evidence is there that these differences are in fact differences in mutation rate rather than in substitution rate?

P. W. Ewald. The difference between HIV-1 and HIV-2 is based on an *in vitro* assay measuring mispair extension frequencies. It therefore is a measure of mutation rate. The lower substitution rate of HTLV relative to HIV must reflect, at least in part, a lower mutation rate per cycle of DNA replication because HTLV DNA replication involves the cell's DNA polymerase, which has a high proof-reading capability. Replication of HIV more frequently involves error prone reverse transcription from an RNA genome. Mutation rate assays of HTLV's reverse transcriptase still need to be done to directly compare HTLV with HIV.

J. D. Gillett (*London School of Hygiene and Tropical Medicine, U.K.*). What proportion of the population of the monkey, *Cercopithecus aethiops*, is found positive for SIV-1 and SIV-2? I ask this because some 60 years ago the human population in a number of African territories began what we may call a love affair with the hypodermic syringe. Seeing these at that time new fangled instruments used for the improvement of health in various ways, quacks soon came on the scene offering, for suitable payment, injections to cope with every kind of indisposition, real or imagined. The syringes were 'rescued' from hospital and dispensary refuse bins and the like, while the contents varied but were usually diluted with water from the nearby river or swamp.

Now *C. aethiops* is a peri-domestic monkey in many parts of Africa, leaving the nearby forest to raid crop plantations of one sort or another. Men, whose sexual prowess was beginning to wane noticed that the nearby monkeys were, so to speak, always at it. The visiting quacks also noted this and it was not long before they cashed in on the situation and monkey blood, suitably diluted, was on offer for injection. Could this possibly have been the origin of HIV?

P. W. Ewald. Among wild populations of *Cercopithecus aethiops* infected with SIV-2, seroprevalence may range from about 10% to 50%. To my knowledge, no SIV that clusters with HIV-1 has ever been isolated from wild populations of *C. aethiops*. HIV-1 clusters with an SIV from chimpanzees (*Pan troglodytes*) and HIV-2 clusters with an SIV from sooty mangabeys (*Cercocebus atys*). If the invasive practices referred to played a role in the transfer of SIVs to humans they probably would have been transferring the virus from sooty mangabeys or chimps rather than *C. aethiops*. One problem with such syringe scenarios is that they cannot explain the spectrum of transfers of retroviruses between primate species. They do not, for example, explain the transfer of viruses between *Cercopithecus* species, *Cercocebus* species and mandrills (*Mandrillus sphinx*), nor transfers of HTLV between humans and other primate species that have occurred over the last few millennia. We therefore have evidence that some other route(s) of interspecies transfer is occurring, but no evidence for syringe transfers.

C. E. Parker (*Zoology Department, University of Oxford, U.K.*). I have two points to make.

First, both vertical and horizontal transmission occur in the Caribbean and in Japan, and both geographical regions show highly clustered, rather than uniformly distributed, prevalence of HTLV-I infection. It is quite erroneous to conclude that: (i) there is a shorter time interval to development of cancer (lymphoma/leukaemia) in the Caribbean; or (ii) to infer, from the misinterpretation, that this is a result of sexually acquired rather than vertically transmitted infection.

Indeed, in all locations where HTLV-I-related lymphoma occurs, this appears to be associated almost entirely with vertically transmitted infection. The requirement, in all geographic areas, for a long time interval for the development of leukaemia reflects multiple, host-dependent factors, as well as the requirement for HTLV-I-related lymphocyte transformation.

Second, in HTLV-I a very brisk immune response is elicited, both humoral, and cell-mediated, and activated cytotoxic T-cells can be detected in freshly isolated lymphocytes from many asymptomatic, HTLV-I-infected people, as well as in rare infected individuals who develop spastic paresis, the other disease associated with HTLV-I. These CTL are chronically activated, and predominantly directed against the tat transactivating protein, implying that chronic expression of HTLV-I tat frequently occurs *in vivo*. This implies that the host immune response, particularly the cytotoxic T-cell response, rather than the particular behavioural characteristics of the infected individual, is the major factor regulating the extent of viral replication.

P. W. Ewald. Both vertical and horizontal transmission do occur in the Caribbean and in Japan. My point is that, according to available evidence, the frequency of vertical relative to sexual transmission is greater in Japan than in Jamaica.

With regard to the time interval to development of cancer, I believe that Dr Parker has misunderstood my argument. The information in the literature shows that ATL tends to occur at younger ages in Jamaica than in Japan. If the frequency of vertical and horizontal transmission were the same in Japan and Jamaica, this difference would indicate that the time between infection and ATL was shorter in Jamaica. In Japan virtually all cases of ATL develop from infections that are acquired during the first few years of life. The median time between infection and ATL is therefore about 60 years in Japan. If the same were true in Jamaica, one would still have a shorter time interval to development of ATL. If, however, more sexually infected individuals develop ATL in Jamaica than in Japan, then we should find a higher proportion of ATL cases among women in Jamaica because sexual transmission occurs mainly from male to female. Although more thorough analyses are needed, published data suggest the latter trend: in Japan about 41% of the ATL patients were females (Tajima *et al.* 1988, Kondo *et al.* 1989), whereas in Jamaica about 57% were females (from table 1 in Murphy *et al.* 1989). If some of this greater ATL rate among Jamaican women is attributable to sexually transmitted HTLV, the time between infection and

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ATL would be even shorter in Jamaica than the median age of ATL onset (i.e. 40–45 years), resulting in an even greater difference between Japan and Jamaica in the interval between onset of infection and onset of ATL. These comparisons emphasize the need to directly assess whether the percentage of ATL cases resulting from neonatal infection is as high in Jamaica as it is in Japan.

I think that Dr Parker's second point exaggerates the intensity and effectiveness of the immune response against HTLV. The point concerns primarily the asymptomatic period: by the time ATL sets in, the viruses in those relatively few individuals are stuck in a sinking ship. The immune response to HTLV among asymptomatic HTLV infections is irregular and rather weak; for example, about half are positive for anti-tax antibody (Shioiri *et al*. *Int. J. Cancer* **53**, 1 (1993)) and about half have induction of HTLV-specific CTLs (Kannagi *et al*. *Leukemia* **8** (suppl.), s54 (1994)). Dr Parker's data from three asymptomatic patients (Parker *et al*. *Virology* **188**, 628 (1992)) are consistent with this generalization: one showed no CTL activation and the other two showed mild to moderate activation; antigen specificities were restricted to tax; and, in her words, the 'magnitude of the anti-tax responses in fresh blood were small'.

The degree of humoral immune response to tax and other viral proteins tends to be positively correlated with transmissibility (Hino *et al*. 1987; Sawada *et al*. 1989; Ho *et al*. 1991; Scarlatti *et al*. 1991; Shioiri *et al*. 1993; Stuver *et al*. 1993). The response therefore appears to be an indicator of viral activity but does not effectively suppress infectivity.

The chronic activation of CTL and its activation against tax do not represent evidence against an evolutionary effect of sexual behaviour on viral replication. According to the arguments I presented, a high mutation rate would be favoured only if the change in protein structure that it generates would allow escape from CTL control, but I know of no evidence suggesting that the irregular CTL response that tends to occur during asymptomatic infection would substantially inhibit sexual or maternal transmission of specific variants that generate this response. Dr Parker too has commented on the lack of any evidence for CTL escape mutants (Parker *et al*. 1992).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES