Stable virulence levels in the HIV epidemic of Switzerland over two decades

Viktor Müller[a,i], Bruno Ledergerber[b], Luc Perrin[c], Thomas Klimkait[d], Hansjakob Furrer[e], Amalio Telenti[f], Enos Bernasconi[g], Pietro Vernazza[h], Huldrych F. Günthard[b], Sebastian Bonhoeffer[i] and the Swiss HIV Cohort Study

Objective: To determine whether the virulence of HIV-1 has been changing since its introduction into Switzerland.

Design: A prospective cohort study of HIV-1 infected individuals with well-characterized pre-therapy disease history.

Methods: To minimize the effect of recently imported viruses and ethnicity-associated host factors, the analysis was restricted to the white, north-west-European majority population of the cohort. Virulence was characterized by the decline slope of the CD4 cell count ($n = 817$ patients), the decline slope of the CD4 : CD8 ratio ($n = 815$ patients) and the viral setpoint ($n = 549$ patients) in untreated patients with sufficient data points. Linear regression models were used to detect correlations between the date of diagnosis (ranging between 1984 and 2003) and the virulence markers, controlling for gender, exposure category, age and CD4 cell count at entry.

Results: We found no correlation between any of the virulence markers and the date of diagnosis. Inspection of short-term trends confirmed that virulence has fluctuated around a stable level over time.

Conclusions: The lack of long-term time trends in the virulence markers indicates that HIV-1 is not evolving towards increasing or decreasing virulence at a perceptible rate. Both highly virulent and attenuated strains have apparently been unable to spread at the population level. This result suggests that either the evolution of virulence may be slow or inhibited due to evolutionary constraints, or HIV-1 may have already evolved to optimal virulence in the human host.

AIDS 2006, **20**:889–894

Keywords: virulence, virus evolution, temporal trends, epidemiology, HIV-1

Introduction

Human immunodeficiency virus type 1 (HIV-1) is thought to have originated by the cross-species transmission of the simian immunodeficiency virus of chimpanzees about 70 years ago [1,2]. Considering its recent origin, the virus may still be evolving to adapt to its new host species. Whether the virulence of HIV-1, that is its

From the [a]Bioinformatics Group, Department of Plant Taxonomy and Ecology, Eötvös Loránd University, Budapest, Hungary, the [b]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, Zürich, the [c]Laboratory of Clinical Virology, University Hospital Geneva, Geneva, the [d]Institute of Medical Microbiology, University of Basel, Basel, the [e]Division of Infectious Diseases, University Hospital Berne, Berne, the [f]Institute of Microbiology, University of Lausanne, Lausanne, the [g]Division of Infectious Diseases, Hospital of Lugano, Lugano, and the [h]Division of Infectious Diseases, Kantonsspital St. Gallen, St. Gallen, [i]Theoretical Biology, ETH Zürich, Zürich, Switzerland.

Correspondence to Dr Viktor Müller, Bioinformatics Group, ELTE Department of Plant Taxonomy and Ecology, Pázmány P. s. 1/C, 1117 Budapest, Hungary.

E-mail: viktor.mueller@env.ethz.ch

Received: 28 July 2005; revised: 14 November 2005; accepted: 12 January 2006.

ISSN 0269-9370 © 2006 Lippincott Williams & Wilkins

ability to cause disease, is evolving towards higher or lower levels, is highly relevant for the future of the AIDS pandemic. The prerequisites for evolution, variability and heritability, seem to be fulfilled: different subtypes of the virus may induce different rates of disease progression [3]; the SI/NSI (R5/X4) switch of the viral genotype can accelerate progression [4]; a *nef*-deficient virus strain has been associated with a long-term non-progressor phenotype [5]; and studies of patients with known transmission history indicate that the severity of disease in the infecting and in the infected patient is correlated [6,7]. Evolutionary theory indicates that the optimal level of virulence of a pathogen is a complex function of the detailed biology of within-host dynamics and between-host transmission [8]. The initial level of virulence in a new host, however, is a matter of chance, and may be either below or above the optimal level for the pathogen. Subsequent evolution can thus drive the pathogen towards both increasing and decreasing virulence, and HIV-1 may thus be evolving in either direction. We performed a statistical analysis on various measures of virulence in the Swiss HIV Cohort Study (SHCS) to determine the presence or absence of temporal trends that could hint at underlying viral evolution.

Methods

Study population and the calculation of virulence markers

The SHCS is a nationwide prospective study based on voluntary participation of persons infected with HIV-1. The rationale, organization and baseline characteristics of the study have been described elsewhere in detail [9], and a continuously updated description can be found at www.shcs.ch. We defined three markers of virulence in antiretroviral-naive patients: the linear slope of the decline of the CD4 cell count, the linear slope of the decline of the CD4 : CD8 cell ratio, and the mean log virus load (setpoint) of each patient. Pilot analyses indicated that ethnicity (black versus white) has a significant effect on the virulence markers. To reliably detect local evolution, we therefore restricted our analysis to the white, north-west-European majority population of the cohort (the results on ethnicity-associated effects will be published elsewhere). The CD4 slope is a direct measure of the rate of disease progression [10]; the CD4 : CD8 ratio has the advantage of reducing the effect of fluctuations in the lymphocyte counts; and the viral setpoint has been shown to correlate with disease progression [11]. Serial measurements of CD4 and CD8 cell counts and plasma RNA determinations for all registered patients were obtained from the SHCS database. Only data points preceding the first initiation of antiretroviral treatment in each patient were included in the analysis. The infection of each patient was dated to the earliest date of confirmed HIV-1 infection in the patient. To eliminate the effect of transient changes (peak viremia and drop in the CD4 cell count) associated with primary infection, we discarded data points obtained during the first 200 days after the earliest date of confirmed infection in each patient. Late-stage infection was another potential confounding factor, because drastic depletion of the CD4 cell count prevents further linear decline, and the virus load may also rise above the setpoint at this stage. We therefore discarded also the data points after the first CD4 cell count below 100 cells/μl. The viral setpoint was then calculated in patients who had at least three remaining RNA measurements spanning at least 100 days, as the mean $\log_{10}$ virus load per ml. For the calculation of the CD4 and CD4 : CD8 slopes we introduced the additional selection criterion of having at least one CD4 cell count of 500 cells/μl or above. The CD4 slope and the slope of the CD4 : CD8 ratio were then estimated by linear regression in patients who had at least five remaining measurements of the CD4 cell count or of the CD4 : CD8 ratio, respectively, spanning at least 1 year. By comparing the residual sums of squares, we have verified that linear fitting was better than exponential for both the CD4 slope and the slope of the CD4 : CD8 ratio in the majority of patients. All analyses of the cellular slopes and the setpoint were restricted to patients who had their original first CD4 cell count or RNA measurement obtained within 1 year of the earliest date of confirmed infection.

Statistical analysis

Regression analyses (general linear models) were used to study the relationship between virulence markers and potential explanatory variables. The primary explanatory variable of interest was date of confirmed infection and other variables were included for adjustment purposes. Categorical factors included gender and exposure category (riskgroup); continuous predictors included the earliest date of confirmed infection (converted to Julian date with a scale of days), age at the date of confirmed infection and the initial CD4+ cell count, defined as the first CD4 cell count obtained at least 200 days after the earliest date of confirmed infection. In the analysis of temporal trends exposure category was restricted to intravenous drug-users (IDUs) and heterosexuals to enable reliable simultaneous control for exposure category and gender. The effect of the men having sex with men (MSM) exposure category was tested in a separate analysis restricted to male patients. Statistical analyses were performed with the Statistica software (StatSoft, Inc. Tulsa, Oklahoma, USA).

Moving averages

The three virulence markers were adjusted for gender, exposure category, age at entry and initial CD4+ cell count. Effects were estimated in regression analyses restricted to male and female patients, respectively. Least squares (LS) means of virulence markers were calculated for all gender/riskgroup subpopulations with all factors held at their overall population means. Subpopulation-specific modifiers for each marker were defined as the

difference of the subpopulation LS mean from the median LS mean among the five subpopulations. Individual patient values were then adjusted for age and CD4 cell count at entry according to the effects estimated in the appropriate gender-restricted analysis, and by the subpopulation-specific modifier. To exclude apparent local trends produced by outliers, we omitted patients with virulence markers more than two standard deviations removed from the mean of adjusted values. The selection procedure yielded three separate lists for the three virulence markers. The lists were sorted into ascending order according to the date of confirmed infection. The average of 50 patients was calculated at a time, with the window of averaging moving along the lists of patients. The first point in each graph represents the average of the first 50 patients in the respective list; the second point represents the average of the patients second to fifty-first in the list, etc. Each average was dated on the horizontal time axis to the mean date of confirmed infection among the respective 50 patients.

Results

After the selection procedure, the CD4 slope was calculated for 817 patients. [The effect of sequential exclusion criteria was as follows: of the 13 308 patients registered in the cohort, 2095 had five or more pre-therapy CD4 measurements after the discarding of early and late data points. A total of 1536 patients had data spanning more than 365 days and at least one count $\geq$ 500 cells/μl. A total of 1345 patients had their first count recorded within 1 year of the earliest date of confirmed infection, and 834 patients had registered white ethnicity and north-west-European nationality. Of these, 817 belonged to the three major exposure categories.] The median number of CD4 cell counts used in the calculation of the slope was 9, the median baseline CD4 cell count was 640 cells/μl, and median age was 30 years at entry. The median CD4 slope was −0.150/day, amounting to an annual loss of about 55 cells/μl. Within the group, 31.6% of the patients were female. The representation of the major exposure categories was as follows: IDUs 36.5%, MSM 36.7% and heterosexuals 26.8%. The earliest date of confirmed infection ranged between 1984 and 2002. The slope of the CD4 : CD8 ratio could be calculated on essentially the same population ($n = 815$), and had a median of -1.54×10^{-4}/day.

The viral setpoint was calculated for 549 patients. [A total of 1257 patients had three or more data points; all of them satisfied the criterion of spanning at least 100 days. A total of 784 patients had their first RNA load recorded within 1 year of the earliest date of confirmed infection, and 564 patients had registered white ethnicity and

Table 1. Multiple linear regression model relating markers of virulence to date of confirmed infection and other covariates[a].

	CD4 slope (per day) ($n = 517$)			CD4 : CD8 slope (per day) ($n = 515$)			Setpoint (log10 RNA copies/ml) ($n = 341$)		
	Effect ($\times 10^4$)	P	95% CI ($\times 10^4$)	Effect ($\times 10^7$)	P	95% CI ($\times 10^7$)	Effect ($\times 10^3$)	P	95% CI ($\times 10^3$)
Initial CD4+ cell count (/μl)	−2.35	0.000	−3.01, −1.69	−1.02	0.004	−1.71, −0.334	−1.25	0.000	−1.62, −0.883
Date of confirmed infection (Julian date in days)	−0.0492	0.442	−0.175, 0.0766	0.0168	0.802	−0.115, 0.148	0.0818	0.234	−0.00532, 0.217
Age at confirmed infection (in days)	−0.0146	0.687	−0.0859, 0.0567	−0.0635	0.095	−0.138, 0.0111	0.00199	0.892	−0.0309, 0.0269
Female	70.1	0.489	−129, 269	−14.4	0.892	−223, 194	−4.32	0.937	−111, 103
Heterosexuals vs. IDUs	−42.8	0.683	−249, 163	−121	0.271	−336, 94.6	16.8	0.770	−96.4, 130
Gender × riskgroup	26.0	0.793	−168, 220	54.8	0.597	−149, 258	35.5	0.506	−69.4, 140

[a]The analysis was restricted to the heterosexual and intravenous drug-user (IDU) exposure categories. CI, Confidence interval. The range of dates was 1984–2002 for the CD4 and CD4 : CD8 slopes, and 1994–2003 for the viral setpoint. Effects represent unstandardized regression coefficients.

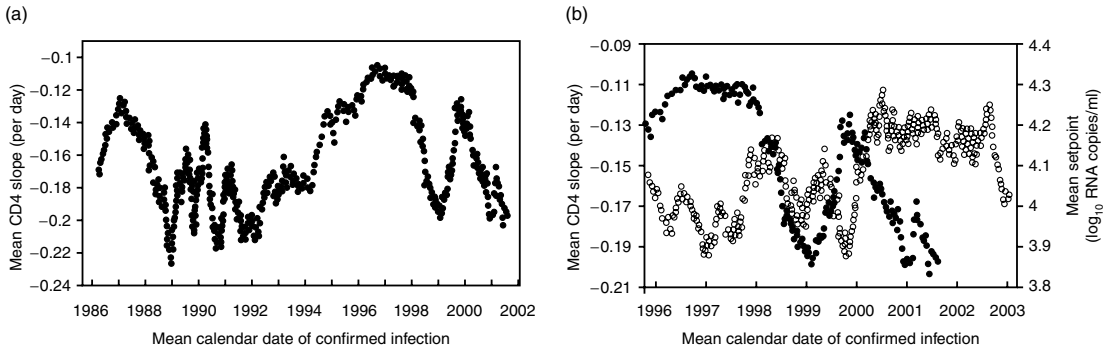

Fig. 1. Moving averages of the CD4 slope and the viral setpoint adjusted for gender, exposure category, age and CD4 cell count at entry. (a) The moving average of the CD4 slope reveals that the lack of a long-term trend in this virulence marker conceals considerable fluctuations occurring on the time scale of a few years. (b) The moving average of the viral setpoint (open circles) roughly mirrors the moving average of the CD4 slope (solid circles) in the time window where both measurements are available, which confirms the association of the two virulence markers. The data series of setpoints extends beyond that of the CD4 slopes, because the calculation of the former required fewer data points and was therefore possible for patients with more recent diagnosis. Each point represents the averaged value of 50 patients and is dated to their mean date of confirmed infection. The window of averaging moved along the list of patients sorted according to the date of confirmed infection: the first point represents the average of the first 50 patients, the second point represents the average calculated over the second to the fifty-first patient, etc. Data trimming was used to eliminate the effect of outliers.

north-west-European nationality. Of these, 549 belonged to the three major exposure categories.] The median number of RNA measurements used for the calculation of the setpoint was 5, the median age at entry was 35 years, and the median of the setpoint was 4.24 $\log_{10}$ - RNA copies/ml. Within the group 26.2% of the patients were female. The representation of the major exposure categories was as follows: IDUs 28.8%, MSM 37.9% and heterosexuals 33.3%. The date of confirmed infection ranged between 1994 and 2003. All three virulence markers could be calculated in 257 patients.

We found no correlation between the date of confirmed infection and any of the virulence markers in the main analysis restricted to IDUs and heterosexuals (Table 1). Gender had no significant effect in any of the analyses. In the pairwise comparison of risk groups, significant differences were found only in the analysis of white north-west-European males: IDUs displayed slower decline of the CD4 : CD8 ratio than MSM (LS means: -1.57×10^{-4}/day versus -2.06×10^{-4}/day, $P = 0.017$), and MSM had a significantly higher setpoint than either IDUs or heterosexual patients (LS means: 4.27 versus 4.02 and 4.00 $\log_{10}$ RNA copies/ml; $P = 0.018$ and 0.013). Date of infection had no significant effect in this analysis, either. Older age at entry was correlated with steeper CD4 and CD4 : CD8 slopes in white, north-west-European men, but not in the analysis including both genders; age displayed no effect on the setpoint. Consistent with earlier results [12], we found a negative correlation between the initial CD4+ cell count and the CD4 and CD4 : CD8 slopes; that is, a higher initial count predicted faster decline. This association corroborates the use of the initial cell count as control variable for disease stage, rather than as an additional marker of virulence. Higher initial CD4+ cell count was also correlated with lower setpoint. The CD4 slope and the setpoint displayed a highly significant negative correlation in our dataset ($n = 257$; $P < 10^{-8}$).

To visualize short-term trends in the virulence markers, we plotted a moving average of the virulence markers, adjusted for control variables, over time (Fig. 1). Using unadjusted instead of adjusted values had little effect on the graphs (not shown). Each point in the graphs represents the average of 50 patients and is dated to their mean date of confirmed infection. The moving average of the CD4 slope reveals that the lack of a long-term trend in this virulence marker conceals considerable fluctuations occurring on the time scale of a few years (Fig. 1a). The decline slope of the CD4 : CD8 ratio closely follows the behavior of the CD4 slope (not shown). The moving average of the viral setpoint depicts roughly a mirror image of the moving average of the CD4 slope in the time window where both measurements are available (Fig. 1b). Most short-term fluctuations of the averaged CD4 slope are mirrored by changes of the averaged setpoint in the opposite direction. The results from the three virulence markers are thus consistent with each other, and it seems plausible to extrapolate that the viral setpoint, coupled to the CD4 slope, has also undergone fluctuations of alternating directions during the longer time scale of the CD4 measurements. We therefore conclude that there is no evidence for long-term trends in the rate of disease progression in untreated HIV-1-infections in Switzerland in the past two decades, and thus no indication for directional evolution of virulence.

Discussion

In the light of the enormous evolutionary potential of retroviruses, the apparent lack of long-term directional evolution of virulence in the Swiss HIV-1 epidemic is remarkable. The lack of robust time trends in the virulence markers is consistent with some [13–15], but not all [12,16–18] earlier studies. In this context, it is worth noting that although the long-term average of the virulence of HIV-1 infections has been stable in the two decades of the Swiss HIV-1 epidemic, considerable fluctuations have occurred on the time scale of months to a few years. This observation recommends caution in the interpretation of similar analyses: studies looking at shorter time intervals may detect short-term transient trends as significant.

Some confounding factors may have affected our analysis and may have contributed to the observed fluctuations. First, host genetic factors have been shown to influence the outcome of HIV-1 infection [19], and the spread of the virus in a heterogeneous and structured population can generate fluctuations in the observed disease severity. Second, the earliest date of confirmed infection probably has a varying time lag in comparison with the true date of infection in the patients, which introduces variation in the disease stage at entry and uncertainty in the timing of infections. To minimize the effect of variation in the disease stage, we discarded the data points potentially associated with late-stage disease, and we also explicitly accounted for disease stage by controlling for the initial CD4 cell count. Uncertainties in the timing of infections may have contributed to short-term fluctuations, but they average out in the long run, and therefore cannot generate, nor hide long-term trends. Third, our data selection procedure required three viral measurements and five CD4 cell counts in untreated individuals to qualify for the estimation of the setpoint or the cellular decline slopes, respectively. These criteria selectively remove fast progressors, who may not have sufficiently long untreated disease history to accumulate the required number of measurements. However, evolutionary changes in the virulence of the virus would be expected to shift the whole distribution of virulence markers in the population, and should therefore be detectable also in a dataset depleted of fast progressors.

Remarkably, the introduction of highly active antiretroviral therapy (HAART) around 1995 had no discernible effect on the virulence markers (Fig. 1), although it may have altered the optimal virulence of HIV-1 by changing the cost of virulence and the conditions of transmission. HAART may have had a further direct effect by increasing the transmission of drug-resistant viruses that may have impaired fitness and virulence. Changes in treatment guidelines may have affected the inclusion of patients into our analysis: the last pre-therapy CD4 cell count of patients displayed a transient rise around 1996–1997, indicating earlier treatment initiation. The lack of observable changes in the trends of virulence after 1995 indicates, however, that such direct and indirect effects were probably weak. CD4 cell count at entry was not correlated either with its date of recording or with the earliest date of confirmed infection.

Finally, it is probable that HIV-1 has been introduced to Switzerland multiple times. Observed trends in viral properties may therefore not reflect strictly local evolution. However, newly introduced variants can spread at the expense of earlier strains only if they are better adapted for transmission, and therefore virulence is expected to be approaching the optimal level even in the case of multiple introductions.

The lack of long-term trends over two decades is consistent with the possibility that the virus may have already attained its optimal virulence in humans. This explanation is supported by a quantitative mathematical model, which estimates that the transmissibility of the virus over the lifetime of the host is maximized close to the viral load observed in current infections (Fraser *et al.*: presented at the 12th International Workshop on HIV Dynamics and Evolution, Cleveland, USA, 2005). Using evolutionary simulations, Fraser *et al.* also demonstrated that HIV-1 may have indeed attained optimal virulence in the human population by the mid-1980s. Alternatively, the lack of observable directional evolution may indicate strong evolutionary constraints that could arise, for example, from the dominance of within-host evolution, which may be 'short-sighted' in the long run [20,21]. This possibility is reinforced by the recent finding that a large fraction of transmission events may occur during acute HIV infection [22], which decreases the strength of evolution acting on virulence manifested during chronic infection. The fluctuations in the virulence markers that we observed on the time scale of months to a few years would also be consistent with drift occurring due to strong within-patient selection and between-patient transmission bottlenecks [23]. Such constraints may slow down or inhibit evolution even if the virulence of HIV-1 has not yet attained its optimum: our analysis cannot distinguish between the two scenarios. Finally, a recent study estimated higher ex-vivo fitness for historical compared to recent viral isolates from Belgium, which was interpreted as a potential indicator of viral attenuation over time [24]. Our results suggest that either this decrease of viral fitness has not occurred in Switzerland, or that changes in ex-vivo fitness are not necessarily linked to changes in virulence as manifested in untreated patients. The lack of long-term trends in the Swiss HIV-1 epidemic indicates no attenuation of the virus on the time scale of decades.

Acknowledgments

We thank Rob J. de Boer, Angela McLean, Frank Miedema and Christophe Fraser for valuable comments.

Moreover, we would like to thank all patients participating in the Swiss HIV Cohort Study, all doctors and study nurses involved and the datacenter.

Sponsorship: V. M. was supported by the Hungarian National Research Fund (OTKA grant No. D45948), and S.B. gratefully acknowledges support by the Swiss National Science Foundation. This study has been financed in the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation.

References

1. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, *et al*. **Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*.** *Nature* 1999; **397**:436–441.
2. Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, *et al*. **Timing the ancestor of the HIV-1 pandemic strains.** *Science* 2000; **288**:1789–1796.
3. Kanki PJ, Hamel DJ, Sankale JL, Hsieh C, Thior I, Barin F, *et al*. **Human immunodeficiency virus type 1 subtypes differ in disease progression.** *J Infect Dis* 1999; **179**:68–73.
4. Tersmette M, Gruters RA, de Wolf F, de Goede RE, Lange JM, Schellekens PT, *et al*. **Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates.** *J Virol* 1989; **63**:2118–2125.
5. Deacon NJ, Tsykin A, Solomon A, Smith K, Ludford-Menting M, Hooker DJ, *et al*. **Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients.** *Science* 1995; **270**:988–991.
6. Ashton LJ, Learmont J, Luo K, Wylie B, Stewart G, Kaldor JM. **HIV infection in recipients of blood products from donors with known duration of infection.** *Lancet* 1994; **344**:718–720.
7. Ward JW, Bush TJ, Perkins HA, Lieb LE, Allen JR, Goldfinger D, *et al*. **The natural history of transfusion-associated infection with human immunodeficiency virus/Factors influencing the rate of progression to disease.** *N Engl J Med* 1989; **321**: 947–952.
8. Anderson RM, May RM. **Coevolution of hosts and parasites.** *Parasitology* 1982; **85 (Pt 2)**:411–426.
9. Ledergerber B, von Overbeck J, Egger M, Luthy R. **The Swiss HIV Cohort Study: rationale, organization and selected baseline characteristics.** *Soz Praventivmed* 1994; **39**:387–394.
10. Phillips AN, Lee CA, Elford J, Janossy G, Timms A, Bofill M, *et al*. **Serial CD4 lymphocyte counts and development of AIDS.** *Lancet* 1991; **337**:389–392.
11. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. **Prognosis in HIV-1 infection predicted by the quantity of virus in plasma.** *Science* 1996; **272**:1167–1170.
12. Vanhems P, Lambert J, Guerra M, Hirschel B, Allard R. **Association between the rate of CD4+ T cell decrease and the year of human immunodeficiency virus (HIV) type 1 seroconversion among persons enrolled in the Swiss HIV cohort study.** *J Infect Dis* 1999; **180**:1803–1808.
13. O'Brien TR, Hoover DR, Rosenberg PS, Chen B, Detels R, Kingsley LA, *et al*. **Evaluation of secular trends in CD4+ lymphocyte loss among human immunodeficiency virus type 1 (HIV-1)-infected men with known dates of seroconversion.** *Am J Epidemiol* 1995; **142**:636–642.
14. CASCADE Collaboration. **Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis. Collaborative Group on AIDS Incubation and HIV Survival including the CASCADE EU Concerted Action. Concerted Action on Sero-Conversion to AIDS and Death in Europe**. *Lancet* 2000; 355:1131–1137.
15. CASCADE Collaboration. **Differences in CD4 cell counts at seroconversion and decline among 5739 HIV-1-infected individuals with well-estimated dates of seroconversion**. *J Acquir Immune Defic Syndr* 2003; 34:76–83.
16. Keet IP, Veugelers PJ, Koot M, de Weerd MH, Roos MT, Miedema F, *et al*. **Temporal trends of the natural history of HIV-1 infection following seroconversion between 1984 and 1993.** *AIDS* 1996; **10**:1601–1602.
17. Sinicco A, Fora R, Raiteri R, Sciandra M, Bechis G, Calvo MM, *et al*. **Is the clinical course of HIV-1 changing? Cohort study.** *BMJ* 1997; **314**:1232–1237.
18. Dorrucci M, Phillips AN, Longo B, Rezza G. **Changes over time in post-seroconversion CD4 cell counts in the Italian HIV-Seroconversion Study: 1985–2002.** *AIDS* 2005; **19**:331–335.
19. Telenti A. **Adaptation, co-evolution, and human susceptibility to HIV-1 infection.** *Infect Genet Evol* 2005; **5**:327–334.
20. Levin BR, Bull JJ. **Short-sighted evolution and the virulence of pathogenic microorganisms.** *Trends Microbiol* 1994; **2**: 76–81.
21. Bonhoeffer S, Nowak MA. **Intra-host versus inter-host selection: viral strategies of immune function impairment.** *Proc Natl Acad Sci USA* 1994; **91**:8062–8066.
22. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, *et al*. **Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda.** *J Infect Dis* 2005; **191**:1403–1409.
23. Rambaut A, Posada D, Crandall KA, Holmes EC. **The causes and consequences of HIV evolution.** *Nat Rev Genet* 2004; **5**: 52–61.
24. Arien KK, Troyer RM, Gali Y, Colebunders RL, Arts EJ, Vanham G. **Replicative fitness of historical and recent HIV-1 isolates suggests HIV-1 attenuation over time.** *AIDS* 2005; **19**:1555–1564.

Appendix

The members of the Swiss HIV Cohort Study are M. Battegay, E. Bernasconi, J. Böni, H. Bucher, Ph. Bürgisser, S. Cattacin, M. Cavassini, R. Dubs, M. Egger, L. Elzi, P. Erb, M. Fischer, M. Flepp, A. Fontana, P. Francioli (President of the SHCS, Centre Hospitalier Universitaire Vaudois, CH-1011-Lausanne), H. Furrer (Chairman of the Clinical and Laboratory Committee), M. Gorgievski, H. Günthard, B. Hirschel, I. Hösli, Ch. Kahlert, L. Kaiser, U. Karrer, O. Keiser, C. Kind, Th. Klimkait, B. Ledergerber, B. Martinez, N. Müller, D. Nadal, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, J.-C. Piffaretti, M. Rickenbach (Head of Data Center), C. Rudin (Chairman of the Mother & Child Substudy), P. Schmid, J. Schüpbach, R. Speck, P. Taffé, P. Tarr, A. Telenti, A. Trkola, P. Vernazza (Chairman of the Scientific Board), R. Weber, S. Yerly.