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Viral levels in newborn African infants undergoing primary HIV-1 infection

We examined weekly changes in viral levels in seven untreated infants infected with HIV at birth. Viral levels spiked immediately but reverted quickly to plateau levels typical of infant HIV infection within 2 weeks of first detected viraemia. We speculated that the depletion of naive, susceptible cells is responsible for the rapid decrease in spike levels and that the rapid replacement of lymphocytes in infants causes the high plateau viral levels (10^5 copies/ml) to be sustained.

Infants typically have very high HIV-1 levels compared with adults [1–3]. In our previous study, we found that African infants undergoing perinatal infection had high HIV-1 levels that continued as a plateau during the first year of life [3]. However, that study obtained samples only after one month of age and could have missed a perinatal infection HIV-1 spike if it occurred in the first month. Other investigators have suggested that a viral spike can occur soon after primary infection in infants [4,5]. Although these studies followed infants longitudinally, they presented the results grouped by age rather than for individual subjects. Furthermore, in those studies, the first month visits were non-scheduled, which could have introduced bias into the patterns observed if the reasons for visiting affected viral levels. Changes in HIV levels during primary infection have not been described in individual infants.

To evaluate the profile of primary viraemia in infants, we studied HIV-exposed infants, scheduled weekly during their first month of life. After obtaining maternal consent, cord blood samples from newborns in Blantyre, Malawi were tested for HIV-1 antibody (HIV-1 enzyme immunoassay; Genetics Systems, Seattle, WA, USA). Reactive HIV-1 antibody results reflected the presence of passively acquired antibody from an HIV-infected mother. Mothers of vaginally delivered infants whose cord blood samples were HIV-1 antibody positive were asked to bring their infants to our clinic at 1, 2, 3, 4, 6 and 12 weeks of age. At each visit, filter paper samples were obtained by heel stick. Additional samples obtained on days 1 or 2 of life were available from some babies who remained at the hospital after delivery because they or their mothers required an extended stay. The last available sample from each infant was tested for HIV by polymerase chain reaction (PCR). Filter paper samples from PCR-positive infants were then tested for viral levels by a

second-generation quantitative isothermal nucleic acid silica-bound amplification assay (NucliSens HIV-1 RNA Q-T kit; Organon Teknika, Durham, NC, USA) previously found to be reliable in filter paper samples [3,6]. To exclude in-utero infections, cord blood samples were also PCR tested and, if positive, these infants were excluded from the study. Viral testing was performed by a laboratory in Canada that also participated in the Virology Quality Assurance Program of the AIDS Clinical Trial Group. All infants were breast-fed and therefore could have become infected by early breast-feeding. No infants were treated with antiretroviral agents.

Of 89 infants born to 87 HIV-1-infected women (two sets of twins), 50 had samples obtained at 28 days of life or older and were tested by PCR for HIV-1. One infant was cord blood PCR-positive (in-utero infection), and two infants first tested positive at 90 and 366 days, respectively, indicating transmission that probably occurred via breast milk. Seven subjects were PCR-negative in cord blood samples but positive in the first 28 days of life (1, 7, 14, 14, 14, 24 and 28 days), suggesting infection at or near the time of delivery. Evidence of a spike in viral replication was clearly apparent, with the highest level being $10^{7.6}$ copies/ml (39 million copies) one week after viraemia was first detected (Fig. 1). Declines to typical plateau levels of approximately 10^5 copies/ml quickly followed. One infant had a slightly variant pattern, with a PCR-negative cord blood sample but a positive sample (1000 copies/ml) on day 1. During his first month of life, viral levels gradually increased to a slight peak at day 20 before falling to plateau levels.

Infants thus follow the pattern of primary viraemia observed in adults, with a spike followed by declines to a plateau [7]. The levels in the post-spike follow-up period between 30 and 90 days were stable in a range usually observed for plateau levels during the first year [3]. Plateau levels in infants (typically 10^5 copies/ml) were higher than in adults (10^4 copies/ml) or young children ($10^{4.5}$ copies/ml) [8].

We agree with speculation that the depletion of a subset of susceptible cells that are HIV-naive at the onset of infection contributes importantly to the decline in HIV-1 levels [9,10]. If so, the higher plateau in infants might reflect the more rapid lymphocyte replication observed in infants compared with adults

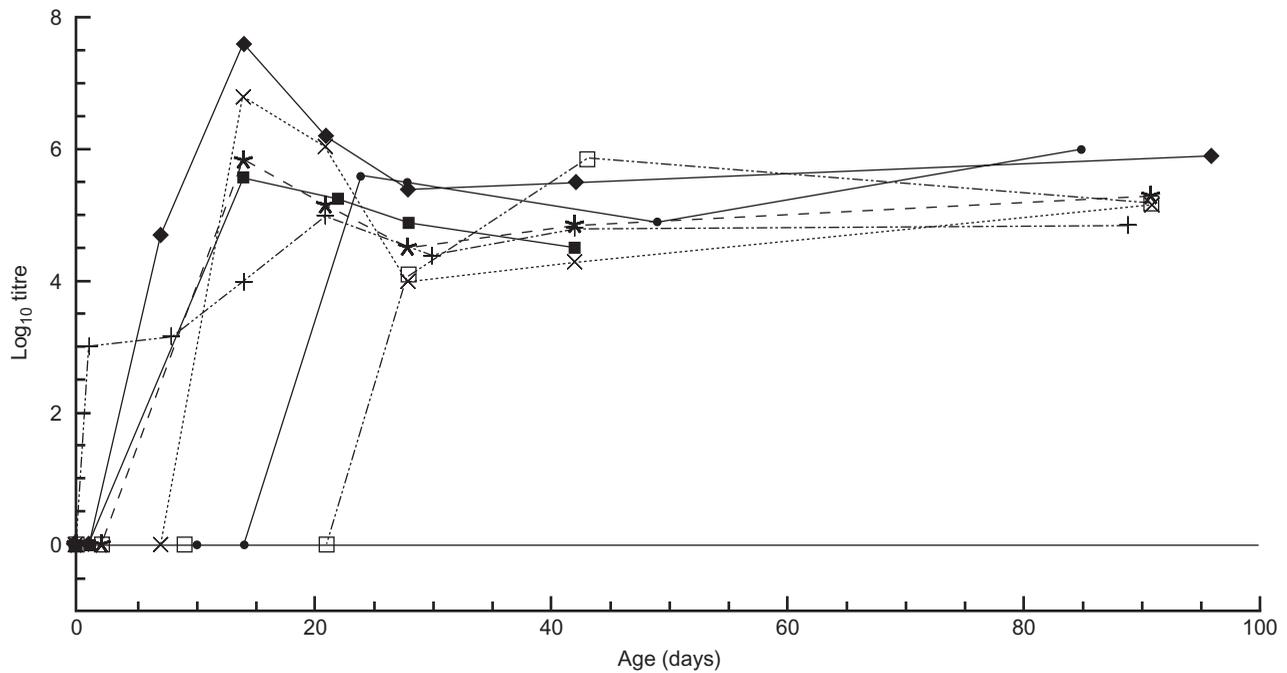


Fig. 1. HIV-1 viral levels in six infants closely followed through the first month of life. By 30 days, levels were high but stable in all infants.

[11], a process that would replenish naive lymphocytes subject to infection. In adults, the decline from the spike levels has been suggested to be caused by an effective immunological response [10,12], but infants are thought to have an immature immune system. A third hypothesis is that the involution of an infected thymus also contributes to the plateau levels in infants [13], but the timing and rapidity of the change do not support this hypothesis.

The prevention of infection is clearly the optimal HIV-1 control strategy. However, given the poor prognosis associated with high viral levels in infants [4,5,14], finding ways to reduce the plateau levels could benefit infants who become infected. Short-course antiretroviral therapy administered immediately after delivery for days to a few weeks might be a practical approach for the prevention or reduction of HIV-1 plateau levels in resource-poor areas. In these longitudinally followed infants, plateau levels were established within 30–90 days, suggesting that clinical trials can use a relatively short-term follow-up of treated infants to assess the effectiveness of such therapies on plateau viral levels.

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Mutations of the 3' untranslated region of the SDF1 gene in apes and monkeys: potential impact on sensitivity to AIDS induced by lentiviruses

The comparison of the stromal cell-derived factor-1 (SDF1) gene 3' untranslated region (3'UTR) of four great ape and four monkey species with their human counterparts shows that the human SDF1-3'A mutation is present in primate species that are the most susceptible to lentivirus-induced AIDS and is absent in species that are particularly resistant to lentivirus-induced AIDS. The results enlighten the possible relationship between SDF1-3'UTR polymorphism and sensitivity to AIDS.

It was demonstrated that sensitivity to HIV infection and HIV disease progression are associated with genetic markers located in chemokine genes (SDF1 and regulated upon activation: normal T cell expressed/secreted; RANTES) and chemokine-receptor genes (CCR5, CCR2, CX₃CR1) [1–5]. A G–A transition in the 3'UTR of the human SDF1 gene (SDF1-3'A) has been described at position 801 [1]. Homozygosity for this transition was first associated with a delay in disease progression [1]. It was proposed that the SDF1-3'A allele was associated with an increase in SDF1 secretion, which could be capable of competing with HIV on CXCR4, and limiting progression towards AIDS [1]. However, this hypothesis was not confirmed by later observations. In particular, Arya and colleagues [6] showed that the SDF1-3'A mutation did not affect SDF1 RNA synthesis nor its translation. Moreover, other studies [7,8] demonstrated that SDF1-3'A homozygous individuals showed an accelerated progression of HIV disease. Finally, it was discovered that the SDF1-3'A mutation alone might not influence the progression of disease induced by HIV, but could be linked to other mutations capable of modifying sensitivity to HIV [6]. The observation of the opposing effects of the SDF1-3'A mutation on HIV infection parallels the situation observed with the RANTES promoter polymorphism, which has contrasting effects on HIV disease progression [2,9].

As proposed by Ramamoorti and Banerjea [10], the 3'UTR of the SDF1 genes of non-human primates could offer the possibility of checking this hypothesis because they could share with man genetic polymorph-

ism and because their sensitivity to lentivirus infections (either natural or experimental) varies from species to species [11–16]. Lentivirus infection in non-human primates does not always lead to immunodeficiency syndromes [11,12]. The molecular mechanism of resistance to AIDS progression has not yet been fully elucidated. We characterized the 3'UTR of SDF1 genes of four primate species not studied so far: 11 chimpanzees (*Pan troglodytes*), 13 gorillas (*Gorilla gorilla*), two gibbons (*Hylobates lar*) and one orangutan (*Pongo pygmaeus*). These four species are the primates phylogenetically closest to humans. We also characterized the sequences of 10 crab-eating macaques (*Macaca fascicularis*), five rhesus monkeys (*Macaca mulatta*), eight marmosets (*Calithrix jaccus*) and three baboons (*Papio papio*).

Animal genomic DNA samples, purified from peripheral blood, were amplified by polymerase chain reaction (PCR) with SDF1 forward and backward primers (5'–AGCTTTGGTCCTGAGAGTCC–3' and 5'–CAGTCAACCTGGGCAAAGCC–3') [1]. PCR products were size purified by agarose gel electrophoresis and sequenced on a 373 Applied Biosystems sequencer (Applied Biosystems, Courtaboeuf, France). When sequences revealed a possible heterozygous genotype, 20 cloned PCR products were sequenced to determine both allelic sequences.

Among the four *Hominidae* (gorilla, chimpanzee, gibbon, orangutan), the SDF1-3'A mutation (position 801) was observed only in the gorilla (Fig. 1a). The results demonstrated that at position 801, A–G or G–A transitions arose more than once during primate evolution (Fig. 1b). Other differences between man and ape sequences were concentrated between positions 776 and 802 (Fig. 1a). Outside this limited region, only the orangutan sequence differed from the human sequence by two point mutations (positions 911 and 975). Rhesus macaque, baboon and crab-eating macaque sequences differed from the human reference at positions already observed by Ramamoorti and Banerjea [10] (positions 778, 801, 802 and 838, see Fig. 1a). Apart from these mutations, we found numerous differences between human and Old World monkey

a

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	6666777899000222333466899000000000	111244557
	245616832512934503890180901234567	167903075
HUMSDF1B	CTGGCCCTAGGGCCAGGAGGCCCCACATCCT-----CTGCAAGCC	
HUMSDF1BA.....	
GOGOCG.A.....	
PATRT.G.....	
POPYGA.T.....	
HYLAT.....	
MAMUA...AA...T.....CCACATCCT...GG...	
MAFA-1A...AA...T.....GGG...	
MAFA-2A...AA...T.....CCACATCCT...GG...	
PAPA-1A...AA...C.A.T.....GG...	
PAPA-2A...AA...A.T.....GG...	
PAPA-3A...AA...A.T.....GG...	
CAJA	TCATT.T...A.TTGA.C.A.T...G-----TCAG...AA	
Mamu*	???.A...AA.....????????????????????????????	
Mara*	???.A...AA.....????????????????????????????	
Papa*	???.A...AA.....A.????????????????????????????	
Caja*	???.A...AA.....????????????????????????????	

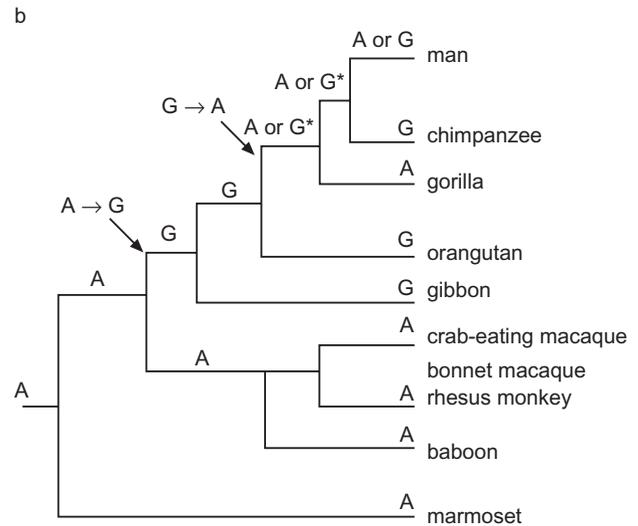


Fig. 1. Sequences analysis of 3' untranslated region of the SDF1 gene.

(a) Comparison of sequences.

HUMSDF1B, Human sequence of SDF1B, counting from ATG initiation codon [17]; GOGO, *Gorilla gorilla*; PATR, *Pan troglodytes*; POPY, *Pongo pygmaeus*; HYLA, *Hylobates lar*; MAMU, *Macaca mulatta*; MAFA, *Macaca fascicularis*; PAPA, *Papio anubis*; CAJA, *Callithrix jacchus*; Mara, *Macaca radiata*.

Names of species are followed by number when more than one allele was characterized.

*Sequences obtained by Ramamoorti and Banerjea [10] in *Macaca mulatta*, *Macaca radiata*, *Papio anubis*, and *Callithrix jacchus* are represented. ??? are positions not shown in Ramamoorti and Banerjea [10].

(b) Parsimonious evolution at position 801.

*At position 801, the common ancestor of the human, gorilla and chimpanzee and of the human and chimpanzee most probably has two alleles, one being 801 A, the other 801 G. As frequently observed [18], only one allele was fixed in the gorilla and chimpanzee whereas the human kept both alleles. The evolutionary tree is deduced from currently admitted phylogenesis of primates [19].

sequences in a region not shown by Ramamoorti and Banerjea [10]. All these mutations are distributed randomly along the analysed region. The five rhesus monkeys and three out of the 10 crab-eating macaques presented a 9 base pair (bp) insertion between positions 907 and 908, which corresponds to a repeat. The presence or absence of the 9 bp insertion defines two crab-eating macaque alleles that also differ at position 943 (A–G transition). When compared with the human sequence, marmoset, the species the most phylogenetically distant to man in our study, exhibited the highest number of differences.

Although SDF1-3'UTR primate sequences do not only vary at position 801, we searched for a correlation between the SDF1-3'A mutation and sensitivity to lentiviruses-induced AIDS. We observed the presence of an SDF1-3'A mutation in primate species who are the most susceptible to HIV- or SIV-induced AIDS (*M. mulatta*, *M. fascicularis* and *Papio anubis*) [13–15]. On the contrary, species who are particularly resistant to lentivirus-induced AIDS (*P. troglodytes* and *H. lar*) [12,16] do not have the SDF1-3'A mutation. As for apes, only gorillas present the SDF1-3'A mutation. Unfortunately, in the gorilla, the consequence of

natural or experimental infection by HIV or other lentiviruses has not been reported so far. Moreover, to confirm the possible correlation between sensitivity to disease and the SDF1-3'A mutation, it would be interesting to characterize the 3'UTR of SDF1 of sooty mangabey (*Cercocebus atys*), which is well known to be a healthy carrier of SIV [14]. The only data available reported that gorilla peripheral blood mononuclear cells are susceptible to HIV-1 and HIV-2 infection *in vitro* [20]. On the contrary, cherry crowned mangabey peripheral blood mononuclear cells showed replication of HIV-2 but not HIV-1 *in vitro* [20]. However, the sensitivity to in-vitro infection of lymphocytes is not related to the in-vivo sensitivity of animals to AIDS, as demonstrated by the sensitivity of the chimpanzee to in-vitro infection, which contrasts with the resistance of most chimpanzees to AIDS induced by HIV [12]. The present study shows that the human polymorphism has to be studied in the light of primate evolution.

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No evidence that vaccination with a polysaccharide pneumococcal vaccine protects drug users against all-cause pneumonia

Individuals infected with HIV are at high risk of pneumonia and invasive disease caused by *Streptococcus pneumoniae* [1]. To date, the effect of vaccination with a polysaccharide pneumococcal vaccine on this group is unclear. Breiman *et al.* [2] suggested a protective effect of vaccination on the incidence of invasive disease, whereas a recent study in Uganda [3] showed an increased risk of invasive disease and no protection against all-cause pneumonia among vaccinees.

Since November 1994, pneumovax (Pasteur Merieux MSD, Brussels, Belgium) has been offered to drug users in the Amsterdam Cohort Studies (ACS) who have a history of pneumonia, an asthmatic constitution, or HIV-positive serostatus, because these conditions (besides intravenous drug use) increase the risk of pneumonia [4]. In the ACS, immunological, virological, and clinical data are collected at standardized cohort visits every 4 months. Physicians decide whether an anamnestic event is a pneumonia based on symptoms, diagnostics, and treatment.

We studied the effect of pneumovax on the incidence of all-cause pneumonia in a group of 48 drug users who seroconverted to HIV during ACS follow-up. Subjects were part of a clinical follow-up project in which immunological, virological and clinical data, in addition to ACS data, were collected in hospitals in Amsterdam. In the clinical follow-up, data were collected retrospectively and prospectively until 1 January 1998. To be included in our study and be eligible for vaccination, individuals had to seroconvert to HIV after ACS entry and continue follow-up after 1 November 1994. The study period ran from ACS entry until death or 1 January 1998.

Our study population consisted of 33 men and 15 women, whose mean age at ACS entry was 29.4 years (SD 6); 92% were of west European origin. Of the 48 subjects, 12 refused vaccination and two had medical contraindications; for two subjects, the reason for not receiving a vaccination was unknown. The remainder of 32 subjects received pneumovax at a mean age of 36.2 years (SD 6). Of these, 31 individuals were vaccinated after seroconversion to HIV; one individual

was vaccinated before that date. Five subjects died during follow-up. After July 1996, when highly active antiretroviral therapy became the standard of care, seven participants received this therapy: at least one protease inhibitor or non-nucleoside reverse transcriptase inhibitor, combined with nucleoside reverse transcriptase inhibitors.

To compare the baseline characteristics of vaccinees with non-vaccinees, we considered vaccinees eligible for vaccination at the date of vaccination. Those who did not receive vaccination were considered to be eligible for vaccination on 1 November 1994 if they had seroconverted before that date ($n = 36$), or at their first HIV-positive visit if they seroconverted after 1 November 1994 ($n = 12$). At the date they were eligible for vaccination, vaccinees ($n = 32$) and non-vaccinees ($n = 16$) were comparable in age, years since seroconversion, age at seroconversion, CD4 cell count, episode(s) of pneumonia before seroconversion, the use of antiretroviral therapy (according to the intention-to-treat principle), and the number of drug injections since the last ACS visit. Therefore we assumed the two groups to be at the same risk of pneumonia. Poisson regression was used to estimate the incidence of pneumonia and to calculate the relative risks (RR) with 95% confidence intervals (CI). Evaluated as potential risk factors were vaccination, sex, years since seroconversion (HIV negative; ≤ 2 ; > 2 and ≥ 5 ; > 5), age at seroconversion (≤ 29 ; > 29 and ≤ 35 ; > 35), HIV serostatus with CD4 cell counts (HIV-negative; HIV-positive, CD4 count > 200 cells/mm³; HIV-positive, CD4 count ≤ 200 cells/mm³), and pro-

phylaxis against *Pneumocystis carinii* pneumonia (according to the intention-to-treat principle). All risk factors were treated as time-dependent variables except for sex and age at seroconversion.

During the study period, 65 episodes of pneumonia were documented (incidence 0.15 per person-year; 95% CI 0.12; 0.20), three with a sputum culture positive for *S. pneumoniae* and five positive for another identifiable pathogen. In 57 cases, the causative pathogen was unknown. In univariate analysis, vaccinees had a non-significantly higher risk of pneumonia than the non-vaccinees (RR 1.36; 95% CI 0.76; 2.46). Compared with HIV-negative individuals, the risk of pneumonia was significantly higher among HIV-positive individuals (RR 3.00; 95% CI 1.34; 6.71) and among HIV-positive individuals with a CD4 cell count of 200 cells/mm³ or less (RR 3.69; 95% CI 1.40; 9.69) (Table 1). In comparison with HIV-negative individuals, those who had seroconverted more than 2 years previously had a significantly increased risk of pneumonia (RR 3.82; 95% CI 1.46; 10.00). In multivariate analysis, HIV-positive serostatus (RR 3.00; 95% CI 1.32; 6.79) and HIV-positive serostatus with a CD4 cell count of 200 cells/mm³ or less (RR 3.68; 95% CI 1.36; 9.95) remained significant risk factors. However, the RR for pneumonia among vaccinees decreased to 1.01 (95% CI 0.53; 1.91). To avoid recall-biased data and misclassification during ACS visits we repeated the analysis, including only pneumonia documented in hospital charts ($n = 21$), and found an unadjusted RR of 1.99 (95% CI 0.77; 5.12) for vaccinees (data not shown). HIV-positive serostatus with a CD4 cell count of 200

Table 1. Uni- and multivariate associations between risk factors and all-cause pneumonia in a group of 48 HIV seroconverters from the Amsterdam Cohort Studies.

Risk factor	All-cause pneumonia (N = 65)			Univariate		Multivariate	
	Pneumonia	Person-years	Incidence rate per 100 PY	RR	95% CI	RR	95% CI
Pneumovax = 0	51	352.23	14.48	1		1	
Pneumovax = 1	14	70.88	19.75	1.36	0.76; 2.46	1.01	0.53; 1.91
Male	44	284.61	15.46	1			
Female	21	138.50	15.16	0.98	0.58; 1.65		
Time since seroconversion in years							
HIV negative	5	88.12	5.67	1			
≤ 2	18	123.88	14.53	2.56	0.95; 6.90		
> 2 and ≤ 5	24	110.81	21.66	3.82	1.46; 10.00		
> 5	18	100.30	17.95	3.16	1.17; 8.52		
Age at seroconversion							
≤ 29	20	157.90	12.67	1			
> 29 and ≤ 35	21	115.10	18.24	1.44	0.78; 2.66		
> 35	24	150.11	15.99	1.26	0.70; 2.29		
HIV negative	7	109.30	6.40	1		1	
HIV positive CD4 cell count > 200	39	202.97	19.21	3.00	1.34; 6.71	3.00	1.32; 6.79
HIV positive CD4 cell count ≤ 200	10	42.33	23.63	3.69	1.40; 9.69	3.68	1.36; 9.95
Prophylaxis = 0	55	360.63	15.25	1			
Prophylaxis = 1	10	62.48	16.00	1.05	0.53; 2.06		

CI, Confidence interval; RR, relative risk; Prophylaxis: trimethoprim-sulphamethoxazole or pentamidine; PY: person-years

cells/mm³ or less was significantly associated with pneumonia in univariate analysis (RR 3.87; 95% CI 1.09; 13.73). In multivariate analysis (RR for vaccinees 1.47; 95% CI 0.48; 4.55), no significant risk factors were found, possibly because of the small numbers. To correct for dependency among the visits of each subject, the model was re-analysed using generalised estimation equations, and the results were comparable. In the multivariate models, no statistically significant interaction terms were found.

This study confirms that HIV-positive serostatus and a deteriorating immune system are associated with a significantly higher risk of all-cause pneumonia [4]. Our results suggest that vaccination with pneumovax has no protective effect against all-cause pneumonia and should not be given to HIV-positive drug users for that reason. However, the power in this observational study was limited, and additional studies need to be conducted in order to evaluate pneumovax further, and to provide evidence-based recommendations on the use of pneumococcal vaccine in HIV-positive patients in the era of highly active antiretroviral therapy.

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Discordant CD4 T lymphocyte responses to antiretroviral therapy for HIV infection are associated with ex-vivo rates of apoptosis

Our purpose was to determine if changes in CD4 cell counts in HIV-infected patients with good viral suppression on stable antiretroviral regimens could be predicted by ex-vivo rates of apoptosis of peripheral blood mononuclear cells (PBMC). Patients were grouped by lowest pre-treatment and highest on-treatment CD4 cell counts and classified as complete immune responders, partial responders, or non-responders. Whole blood was collected from a subgroup of patients and controls, and rates of the ex-vivo apoptosis of PBMC were assessed. Non-responders exhibited significantly increased apoptosis, whereas good immune responses were associated with decreased apoptosis. Persistently accelerated apoptosis may contribute to persisting immune deficiency independent of the viral load.

A progressive decrease in the number and function of CD4 T lymphocytes is the primary mechanism that leads to secondary infections in AIDS, and the goal of immune reconstitution is the reversal of this process.

Much of the depletion and dysfunction of CD4 cells involves uninfected cells. One potential mechanism is accelerated apoptosis [1]. Landmark studies have demonstrated that CD4 cell counts promptly increase when antiretroviral therapy suppresses HIV replication [2]. However, discordant immune responses to antiretroviral therapy occur, including poor immune responses that result in disease progression despite marked reductions in viral load [3]. The purpose of this study was to determine if immune responses in HIV patients with complete viral suppression on antiretroviral therapy could be predicted by ex-vivo rates of apoptosis.

Records were reviewed for approximately 800 HIV-infected patients. A total of 122 patients on stable antiretroviral regimens with HIV-RNA levels of less than 500 copies/ml for over 6 months were identified. Patients were grouped according to absolute CD4 lymphocyte counts before the initiation of antiretroviral therapy: group 1, CD4 cell count < 100/mm³; group 2, 100–199/mm³; group 3, 200–399/mm³; group 4,

400–699/mm³; and group 5, 700/mm³ or greater. This grouping was used rather than the three-group Centers for Disease Control and Prevention classification (normal immune status, CD4 cell count > 500/mm³; moderate immune suppression, CD4 cell count 200–500/mm³; and severe immune suppression, CD4 cell count < 200/mm³) to increase discrimination and detect more modest immune recovery. After antiretroviral therapy patients were classified as follows. Complete immune responders were defined as patients whose CD4 lymphocyte count increased to 700/mm³ or greater on antiretroviral therapy and partial responders as patients with a CD4 cell count increase of more than 50% over their lowest pre-treatment value and improved by at least one immune grouping according to the above criteria. All others were considered as non-responders.

Heparin anti-coagulated blood was collected from six HIV-negative controls and 20 HIV-infected patients with complete viral suppression. PBMC were isolated and cultured at 37°C in 5% carbon dioxide. After 72 h cells were stained with acridine orange and ethidium bromide, and then examined by fluorescent microscopy to observe the morphological changes of apoptosis and assess viability.

There were 38 complete responders (31.1%), 68 (55.7%) partial responders, and 16 (13.1%) non-responders out of the 122 patients with HIV-RNA levels of less than 500 copies/ml identified by chart review. Patients for whom ex-vivo apoptosis was evaluated were representative of this cohort, with four (20%) complete responders, 12 (60%) partial responders, and four (20%) non-responders (see Table 1). All but one patient had their viral load determined by an ultra-sensitive assay with a detection cut-off of 50 copies/ml, and only four patients had a detectable viral load at this

level; one complete responder, two partial responders, and one non-responder.

Immune non-responders exhibited significantly accelerated apoptosis compared with all other control and patient groups by analysis of variance ($P < 0.01$). Partial responders were also significantly different from controls. A highly significant correlation was found between immune response and PBMC apoptosis (Spearman $R = -0.69$; $P = 0.001$). Multiple regression analysis demonstrated statistically significant contributions from both male sex ($P = 0.01$) and from lymphocyte apoptosis ($P = 0.001$), but obviously the small number of female patients limited this analysis.

t-Test analysis comparing male and female patients showed no statistically significant difference in rates of apoptosis ($P = 0.6$). No other relationships were significant, including the lowest pre-treatment CD4 cell counts, pre-treatment viral load, peak viral load, previous opportunistic infections, or particular specific antiretroviral agents, but again, the number of patients were small. Data were also analysed using the three immune categories defined by the Centers for Disease Control and Prevention and there was still a highly significant correlation between immune response and apoptosis.

Our data demonstrate a strong relationship between the immune response to antiretroviral therapy and the ex-vivo rates of apoptosis of PBMC for patients on antiretroviral therapy with complete viral suppression. Even when controlling for age, duration of antiretroviral therapy, duration of viral suppression, peak viral load, baseline CD4 cell counts, antiretroviral medications, opportunistic infections or other co-infections, and other concomitant medical illnesses, this relationship is highly significant ($P = 0.001$).

Table 1. Rates of peripheral blood mononuclear cell apoptosis and clinical characteristics by immune response category.

	Controls (N = 6)	Non-responders (N = 4)	Partial responders (N = 12)	Complete responders (N = 4)
Age	43 ± 12.4	36 ± 11.3	47 ± 9.8	47 ± 6.6
Percentage male	100	100	100	25
Percentage of PBMC apoptosis	7.3 ± 6.2	42.5 ± 8.0 ^a	23.7 ± 10.0 ^a	16.8 ± 4.4
Baseline CD4 cell count (cells/mm ³)		238 ± 182	138 ± 78	236 ± 108
Baseline CD4 cell percentage		17.3 ± 13.9	11.8 ± 6.5	15.0 ± 5.2
Peak HIV-RNA level (copies/ml) ^b		155 847 ± 177 836	40 476 ± 48 214	104 505 ± 110 104
Peak HIV-RNA level (copies/ml) ^{ab}		232 875 ± 166 288	76 211 ± 53 544	120 859 ± 150 470
Duration suppression (months) ^a		7 ± 1	20 ± 10	17 ± 5
Viral load at time of sample collection ^a		135 ± 171	84 ± 88	149 ± 200
Percentage of NNRTI		25	17	50
Percentage of protease inhibitors		100	92	50
Percentage of opportunistic infections		50	50	0
Percentage of hepatitis C infections		50	33	0

NNRTI, Non-nucleoside reverse transcriptase inhibitors; PBMC, peripheral blood mononuclear cells.

^aDifference compared with control significant by analysis of variance. $P < 0.01$.

^bPeak viral loads of patients with measurements of antiretroviral therapy.

The significance of T cell apoptosis in HIV immune pathogenesis has been debated. Our study suggests that apoptosis is an important mechanism of T cell depletion in HIV infection, independent of viral replication. Karmochkine and colleagues [4] showed that ex-vivo rates of apoptosis for PBMC correlated with the viral load and correlated negatively with the CD4 cell count. Thirty per cent of the variability in apoptosis rates after activation were predicted by the viral load and 40% by the CD4 cell count, suggesting that viral replication is not the only process leading to T cell depletion.

Data on the effects of antiretroviral therapy on T cell apoptosis are limited. Chavan *et al.* [5] showed a decreased rate of lymphocyte apoptosis after the initiation of antiretroviral therapy. Regamey *et al.* [6] observed reduced apoptosis and increased expression for patients on antiretroviral therapy compared with patients not on therapy. Other studies, however, produced conflicting results. A recent kinetic study [7] showed decreased survival of both CD4 and CD8 T cell subsets, but highly active antiretroviral therapy had no effect on this. A more recent study by the same group [8], however, yielded different results, with improved T cell survival and a decreased rate of ex-vivo apoptosis, as well as increased T cell production resulting from effective antiretroviral therapy.

To our knowledge this is the first study showing persistent apoptosis in a subgroup of patients with complete viral suppression in association with poor immune recovery. Immune alterations independent of active viral replication may be responsible. Recent data suggest that immune responses to antiretroviral therapy depend on residual or restored thymic function. Improved CD4 cell counts in patients, despite virological treatment failure, are associated with greater thymic function, whereas poor T cell responses despite the suppression of HIV are seen with decreased thymic function [9]. Discordant immune responses may also be caused by the differential effects of particular antiretroviral agents on T cell apoptosis independent of viral suppression. For example, protease inhibitors have been shown to decrease rates of apoptosis of uninfected T cells [10]. Viral replication is never completely suppressed with highly active antiretroviral therapy, even

when patients have undetectable plasma HIV-RNA levels. Therefore, varying degrees of low-level viral replication or replication in certain cellular compartments may continue to drive T cell apoptosis.

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Time trend in incidence of HIV seroconversion among homosexual men repeatedly tested in Madrid, 1988–2000

An open cohort of 2670 homosexual men repeatedly tested for HIV at a Madrid clinic has registered 8050 person-years (PY) of follow-up and 157 seroconversions from 1988 to 2000. After declining from 1988 (4.71 per 100 PY) to 1995 (1.06 per 100 PY), the incidence rate began a

significant upward trend, reaching a figure of 2.16 per 100 PY in 2000. These findings ought to alert surveillance systems and prevention programmes.

The early 1990s witnessed advances in the control of

HIV transmission among homosexual men in western European countries [1]. HIV seroprevalence among homosexual and bisexual men voluntarily tested at a Madrid clinic fell from 30% in 1990 to 12% in 1994 [2]. Recently, increases in the incidence of sexually transmitted diseases (STD) and sexual risk practices have been detected in homosexual men [3,4], but there is no clear evidence of an increase in HIV transmission [5].

We have analysed the time-trend in HIV seroconversion among homosexual men repeatedly tested for HIV at Madrid's largest STD/HIV diagnostic clinic in the period 1988–2000. This clinic provides free medical attention to all patients on demand, including anonymous voluntary HIV testing and counselling. During the first visit, each patient was assigned a code, which allowed for a record to be kept of the results, and was thereafter maintained for all successive visits to the clinic. Regular examinations were offered to all seronegative patients reporting risk practices. HIV diagnosis relies on enzyme-linked immunosorbent assay testing and Western blot confirmation. The results of all HIV tests performed at the clinic were prospectively computerized, along with the relevant date, patient code, sex, age and HIV risk exposures.

An open cohort was formed, including all homosexual men who had no record of drug injection, had undergone a first negative HIV test, and had repeated the test at least once at the clinic between 1988 and December 2000. Individuals with less than 90 days between their first and last tests ($n = 14$) or with periods of over 5 years without repeat testing ($n = 92$) were excluded. Annual HIV incidence rates were calculated by taking the PY of follow-up per calendar year as denominators. The numerators were obtained under the hypothesis of the uniform distribution of HIV seroconversions over the period between the last negative and first positive tests. For example, an individual who had tested negative for HIV in April 1990 and positive in May 1991 contributed as a case for 8/13 in 1990 and for 5/13 in 1991 [6].

The 2670 patients making up the cohort registered a total of 8050 PY of follow-up and 157 seroconversions from 1988 to 2000. The average number of HIV tests per patient was 3.7 and the median time between two successive tests was 13.7 months. Among seroconverters the median time between the last negative and first positive tests was 13.1 months (range 3–59 months). The mean age at first visit was 29.4 ± 8.2 years and held steady throughout the period. After declining from 1988 (4.71 per 100 PY) to 1995 (1.06 per 100 PY), the incidence rate has registered a progressive increase, reaching a figure of 2.16 per 100 PY in 2000 (Table 1). Poisson regression analysis showed a slight but statistically significant upward trend from 1995 to 2000. Such a trend became more pronounced after

Table 1. HIV incidence among homosexual men repeatedly tested in Madrid, 1988–2000.

Calendar year	PY of follow-up	No. of seroconversions	Incidence per 100 PY
1988	116	5.5	4.71
1989	217	9.8	4.52
1990	326	12.5	3.83
1991	438	11.2	2.54
1992	596	15.7	2.63
1993	800	13.5	1.69
1994	919	18.6	2.02
1995	977	10.3	1.06
1996	952	11.0	1.16
1997	904	15.1	1.67
1998	815	13.9	1.71
1999	664	13.1	1.97
2000	324	7.0	2.16

PY, Person-years.

adjusting for age (< 30 and ≥ 30 years) and the time from the beginning of follow-up (< 24 and ≥ 24 months), giving a rate ratio for the annual increase of 1.41 (95% confidence interval 1.07–1.85).

Caution is called for in the interpretation of these results because of the selection biases inherent in the studies. However, the clinic that served as the setting for this study is well established among Madrid's gay community by reason of its location, ease of access and offer of free anonymous HIV testing. During the study years, no noteworthy changes took place in the features and functioning of the clinic. In addition, only test results obtained at the clinic itself were considered, unlike other studies that include patients' self-reported results [7].

The incidence of HIV, ascertained through follow-up studies, is the most sensitive measure of recent shifts in HIV transmission, inasmuch as it analyses infections on the basis of the date on which they occur. Despite this, few studies have analysed the corresponding trend over time. HIV diagnosis reporting systems and seroprevalence surveys can take several years to detect changes in the incidence of HIV.

We detected a moderate increase in the incidence of HIV among homosexual men repeatedly tested in Madrid over the period 1995–2000, thus reversing the downward trend of previous years. This new increase in HIV transmission is in the line with the increase in the incidence of gonorrhoea and the frequency of sexual risk practices recently found among homosexual men in other European countries [3–5]. A report has recently described an increase in HIV incidence among repeated testers in San Francisco [7]. These findings ought to alert surveillance systems to confirm and

analyse this new situation. In the meantime, prevention has been intensified to avoid any relaxation of safe-sex practices, by seeking more attractive ways to disseminate the message, so as to reawaken the interest of the homosexual population in general and the youngest segment in particular.

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Increasing incidence of HIV infections among young gay and bisexual men in Vancouver

Since the beginning of the HIV epidemic in north America, the majority of HIV infections have occurred among men who engage in sexual relations with other men. As the HIV epidemic enters its third decade, gay and bisexual men continue to have among the highest rates of HIV infection. Previous studies [1,2] have highlighted the decline in the incidence of HIV and risk behaviour among gay and bisexual men. However, several studies [3,4] have suggested that young gay and bisexual men continue to engage in unprotected sexual behaviours and are at continued risk of HIV infection. Recent reports in the media [5–9] and research literature [10,11] have indicated an increase in the incidence of HIV among gay and bisexual individuals in many of the world's major cities. The purpose of this study was to determine trends in HIV incidence using data from a prospective cohort of young gay and bisexual men.

The Vanguard Project is a prospective study of gay and bisexual men aged 15–30 years, living in the greater Vancouver region. These men were recruited through outreach, clinics, and physicians' offices. To be eligible for this longitudinal study, the participants must have not previously tested positive for HIV and must have self-identified as gay or bisexual or have had sex with other men. Vanguard participants have completed a self-administered questionnaire and undergone HIV antibody testing on an annual basis since May 1995.

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The incidence of HIV was calculated per annum as the number of new infections divided by the total person-time under observation for each calendar year from study inception to December 2000. Person-time was calculated as the interval between enrolment and the most recent follow-up visit for individuals who did not seroconvert. For individuals who became HIV positive, person-time was calculated as the interval between enrolment and the first visit at which an HIV-positive test result was detected. Ninety-five per cent confidence intervals (CI) for the incidence estimates were calculated on the basis of Poisson distribution.

As of 31 December 2000, 668 men had completed at least one questionnaire and two HIV tests. Table 1 provides a summary of HIV incidence in the cohort by year since its inception in 1995. As shown here 26 HIV infections were prospectively observed over the follow-up period (mean follow-up 2.87 years), resulting in an overall incidence rate of 1.4 per 100 person-years (PY; 95% CI 0.8–1.9). Among participants who reported injection drug use, the incidence rate was higher (3.9/100 PY) than among those participants who did not report the use of injection drugs (1.0/100 PY). A significant increase in new HIV infections was observed between 1995–1999 and 2000 within the entire cohort. This remained true when participants who had injected drugs were excluded from the analysis ($P < 0.05$). Among men who had never injected drugs, the rate of new infections increased from 0.6 per 100 PY in 1995–99 (95% CI 0.2–1.0) to 3.7 per 100 PY in the year 2000 (95% CI 1.0–6.5).

Table 1. Incidence of HIV infection among gay and bisexual men^a enrolled in the Vanguard Project, by calendar year and category.

Year	All participants (n = 668)		Non-injection drug users (n = 590)		Injection drug users (n = 76)	
	New infections	Rate (95% CI)	New infections	Rate (95% CI)	New infections	Rate (95% CI)
1995	1	1.9 (0.0–5.7)	1	2.1 (0.0–6.1)	0	–
1996	4	1.3 (0.0–2.6)	3	1.1 (0.0–2.3)	1	4.1 (0.0–12.1)
1997	4	0.9 (0.0–1.8)	1	0.2 (0.0–0.7)	3	9.4 (0.0–20.1)
1998	5	1.1 (0.1–2.0)	4	0.9 (0.0–1.9)	0	–
1999	1	0.2 (0.0–0.7)	1	0.3 (0.0–0.8)	0	–
2000	11	5.0 (2.1–8.0)	7	3.7 (1.0–6.5)	3	9.6 (0.0–20.6)
1995–99	15	0.9 (0.4–1.3)	10	0.6 (0.2–1.0)	4	2.7 (0.1–5.3)
All years	26	1.4 (0.8–1.9)	17	1.0 (0.5–1.4)	7	3.9 (1.0–6.8)

CI, Confidence interval.

^aData regarding injection drug use were unavailable for two seroconverters, who were only identified through anonymous database linkage.

The observed increase in incidence in our cohort corroborates previous reports from other major cities [6–12]. Furthermore, the upturn in the incidence of HIV is consistent with increased rates of rectal gonorrhoea and sexual risk behaviour reported among gay and bisexual men [13]. Overall, such studies suggest the need for continued vigilant surveillance and further investigation of the determinants of seroconversion, in order to assist with the efforts to stabilize or decrease seroincidence in this population of men.

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