Research Letters

AIDS 2005, **19**:727–738

Risk factors for late HIV diagnosis in French Guiana

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Risk factors for delayed HIV diagnosis in French Guiana were studied in 1952 patients between 1992 and 2003. At the time of diagnosis, 30% of patients had less than 200 CD4 lymphocytes/mm^3; age, male sex, and foreign nationality were independently associated with a low CD4 cell count. The availability of highly active antiretroviral therapy was not associated with an earlier HIV diagnosis. Promoting HIV information and testing should be done in several languages to reach minorities.

With 48 new AIDS cases per 100 000 inhabitants per year and 1.6% of pregnant women infected with HIV, French Guiana is the French overseas department in which the HIV epidemic is most worrying [1]. Transmission occurs through heterosexual sex in over 90% of cases. Approximately two-thirds of HIV patients are foreign citizens [2]. The standards of healthcare are close to those of metropolitan France. Free and anonymous HIV testing is available for all. However, many immigrants have little contact with the healthcare and information systems. In French Guiana, 40% of patients diagnosed with AIDS ignored their HIV status at the time of diagnosis [3]. An early diagnosis of HIV infection is important in order to reduce the risk of HIV transmission to other partners and to reduce morbidity and mortality rates [4]. Our objective was to determine the risk factors for a late diagnosis of HIV infection in order to optimize HIV screening strategies.

Between 1 January 1992 and 31 December 2003, 1952 HIV-positive patients were enrolled in the French Hospital Database for HIV. Patients were classified into three groups according to their CD4 cell counts at the time of diagnosis of the HIV infection: less than 200 cells/mm^3 ($n = 594$, 30%), 200 or more but less than 500 cells/mm^3 ($n = 561$, 29%), and 500 or more CD4 lymphocytes/mm^3 ($n = 797$, 41%). An ordinal logit model with the CD4 cell category at the time of diagnosis as a dependent variable was used to determine which independent variables were related to the outcome.

There was no relationship between the transmission mode and late HIV diagnosis (chi square 10.8, $P = 0.55$). After controlling for confounding using ordinal logit regression, age, male sex, and foreign nationality were independently associated with a low CD4 cell count at the time of diagnosis (Table 1). The availability of highly active antiretroviral therapy (HAART) did not seem to be associated with an earlier HIV diagnosis. In the past 12 years there was no improvement in the early detection of HIV infections (data not shown).

Using a Cox regression model adjusting for age, sex, nationality, and antiretroviral treatment, the hazard ratio of dying was 4.5 [95% confidence interval (CI) 3.2–6.2] for patients with CD4 cell counts at the time of diagnosis of less than 200 cells/mm^3 compared with those with more than 500 CD4 cells/mm^3 ($P < 0.0001$), the hazard ratio of dying was 1.4 (95% CI 0.98–2) for patients with CD4 cell counts less than 500 and 200 cells/mm^3 or greater at the time of HIV diagnosis compared with those with more than 500 CD4 cells/mm^3 ($P = 0.06$). Similarly, adjusting for age, sex, nationality, and antiretroviral treatment, the hazard ratio of developing AIDS was 3.4 (95% CI 2.4–4.8) for patients with CD4 cell counts at the time of diagnosis of less than 200 cells/mm^3 compared with those with more than 500 CD4 cells/mm^3 ($P < 0.0001$), the hazard ratio of dying was 1.4 (95% CI 1.04–1.9) for patients with CD4 cell counts less than 500 and 200 cells/mm^3 or greater at the time of HIV diagnosis compared with those with more than 500 CD4 cells/mm^3 ($P = 0.02$).

Despite the availability of HAART, an early diagnosis of HIV is still important to avoid immunosuppression, severe morbidity and death, and to prevent the unwilling infection of sex partners. Age, sex, and nationality influence the CD4 cell count at the time of diagnosis, possibly because they are related to health-seeking behaviours. The influence of age here seemed to be linear rather than bimodal (younger and older age groups having been found to be more at risk of a late diagnosis elsewhere than intermediate ages [5,6]). This supports findings suggesting that HIV awareness in younger age groups is greater than in older age groups in French Guiana [1]. Older men in French Guiana have longer sex lives during which they cumulate the risk of getting infected. Their higher socioeconomic status often facilitates access to sex partners. They are also more often reluctant to use condoms, and are usually not as forthcoming regarding HIV testing as younger men [7]. Many HIV infections are diagnosed during pregnancy screening, which leads to earlier diagnoses in women in their reproductive age compared with men. The influence of male sex on late HIV testing has been consistently observed on several continents [5,6,8–12]. The HIV transmission mode was not associated with any

Table 1. Risk factors for late HIV diagnosis among 1952 HIV-positive individuals in French Guiana between 1992 and 2003.

	Univariate odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)	*P*
France ($n = 369$)	1	1	
Haiti ($n = 707$)	1.3 (1.2–1.4)	1.9 (1.5–2.4)	< 0.0001
Guyana ($n = 122$)	1.4 (1.3–1.6)	1.5 (1.02–2.03)	0.03
Africa ($n = 16$)	2.4 (1.9–3.1)	3.35 (1.3–8.5)	0.01
Brazil ($n = 96$)	1.45 (1.3–1.65)	2.1 (1.4–3.3)	< 0.0001
Age < 20 years ($n = 95$)	0.36 (0.24–0.55)	0.42 (0.25–0.7)	0.001
Age 20–30 years ($n = 471$)	0.43 (0.35–0.55)	0.5 (0.4–0.7)	< 0.0001
Age 31–40 years ($n = 695$)	1	1	
Age 41–60 years ($n = 590$)	1.4 (1.14–1.7)	1.3 (1.06–1.7)	0.01
Age 60–max ($n = 101$)	1.42 (0.97–2.07)	1.8 (1.1–2.8)	0.01
Men ($n = 938$)	2.07 (1.76–2.45)	1.8 (1.5–2.2)	< 0.0001
HAART availability ($n = 1072$)	0.97 (0.82–1.14)	1 (0.99–1.00)	0.6

HAART, Highly active antiretroviral therapy; CI, confidence interval.
[a]Obtained using an ordinal logit regression model.

particular trend here, whereas it has been repeatedly observed elsewhere, including in metropolitan France [9], that heterosexual transmission was more likely to be associated with late diagnosis [8,10,13]. This may be due to the fact that transmission in French Guiana occurs predominantly through heterosexual sex and that homosexuality in the area is mostly hidden.

Legal and illegal foreign nationals were globally more likely to have a later HIV diagnosis than French citizens for several possible reasons. First, a simple explanation is that they may have recently arrived in French Guiana (maybe seeking medical care), which could have delayed diagnosis. A majority of foreign nationals does not speak French and is not reached by information campaigns that are mostly conducted in French. For illegal citizens the fear of being arrested by the police and expelled from French Guiana may also reduce their presence in hospitals or dispensaries, which they perceive as too risky. Finally, widespread cultural beliefs in witchcraft or voodoo and illiteracy often make the viral explanation of AIDS quite difficult to believe, therefore making HIV testing illogical for those who hold such beliefs.

As observed elsewhere [10], the advent of HAART and its spectacular impact on the evolution of HIV has not had any noticeable impact on the CD4 cell counts at the time of diagnosis, suggesting that behaviours regarding HIV testing were not affected by the prospect of effective antiretroviral treatments. There is indeed a need to strengthen HIV information and screening in order to diagnose HIV infections earlier.

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Received: 30 November 2004; revised: 19 January 2005; accepted: 31 January 2005.

References

1. Delfraissy JF. *Treatment of persons infected with HIV* [in French]. Paris: Médecine-Sciences Flammarion; 2002.
2. Huguet D. *Regional Health Programme on HIV-AIDS in French Guiana* [in French]. In: Direction de la Santé et du Développement Social; 2003.
3. Institut National de Veille Sanitaire (INVS). **First results of the new HIV infection surveillance system and AIDS situation on September 30th 2003** [in French]. *Bull Epidemiol Hebdomadaire* 2004; **25**:102–108.
4. Nunez M, Asencio R, Valencia ME, Leal M, Gonzalez-Lahoz J, Soriano V. **Rate, causes, and clinical implications of presenting with low CD4+ cell counts in the era of highly active antiretroviral therapy.** *AIDS Res Hum Retroviruses* 2003; **19**:363–368.
5. Castilla J, Noguer I, Belza MJ, del Amo J, Sanchez F, Guerra L. **Are we diagnosing people infected with HIV in time?** [in Spanish]. *Aten Primaria* 2002; **29**:20–25.
6. Porter K, Wall PG, Evans BG. **Factors associated with lack of awareness of HIV infection before diagnosis of AIDS.** *BMJ* 1993; **307**:20–23.
7. Giraud M. *Analysis of sexual behaviour in the French Antilles and French Guiana*. Paris: Agence Nationale de Recherche sur le Sida (ANRS); 1995.
8. Giard M, Gambotti L, Besson H, Fabry J, Vanhems P. **Factors associated with the late management of HIV-infected patients: literature review** [in French]. *Sante Publique* 2004; **16**:147–156.
9. Couturier E, Schwoebel V, Michon C, Hubert JB, Delmas MC, Morlat P, *et al*. **Determinants of delayed diagnosis of HIV infection in France, 1993–1995.** *AIDS* 1998; **12**:795–800.
10. Wong KH, Lee SS, Low KH, Wan WY. **Temporal trend and factors associated with late HIV diagnosis in Hong Kong, a low HIV prevalence locality.** *AIDS Patient Care STDS* 2003; **17**:461–469.
11. McDonald AM, Li Y, Dore GJ, Ree H, Kaldor JM. **Late HIV presentation among AIDS cases in Australia, 1992–2001.** *Aust NZ J Public Health* 2003; **27**:608–613.
12. Girardi E, Sampaolesi A, Gentile M, Nurra G, Ippolito G. **Increasing proportion of late diagnosis of HIV infection among patients with AIDS in Italy following introduction of combination antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2000; **25**:71–76.

13. Johnson DF, Sorvillo FJ, Wohl AR, Bunch G, Harawa NT, Carruth A, *et al*. **Frequent failed early HIV detection in a high prevalence area: implications for prevention.** *AIDS Patient Care STD* 2003; **17**:277–282.

Change in atherosclerosis progression in HIV infected patients: ANRS Aquitaine Cohort, 1999–2004

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This study reported the changes in carotid intima-media thickness (IMT) during a 36-month period in 233 HIV-infected patients. Median IMT increased in the first 12 months and then decreased by month 36. The prevalence of treatment with lipid-lowering agents and protease inhibitor-free highly active antiretroviral therapy regimens increased, whereas smoking prevalence decreased. The progression of atherosclerosis in HIV-infected patients can be controlled. The impact of individual measures to reduce the cardiovascular risk should be evaluated further.

Early after highly active antiretroviral therapy (HAART) became available for HIV-infected patients, an increased cardiovascular risk was hypothesized on the basis of several case reports [1], and because of the increased prevalence of an atherogenic biological profile [2]. This trend was confirmed thereafter by large prospective studies [3,4]. Atherosclerosis in HIV-infected patients was also investigated through carotid intima-media thickness (IMT) measured by B-mode ultrasonography. The studies conducted among HAART-treated patients showed an increased IMT, mainly associated with conventional cardiovascular risk factors such as older age, male sex, tobacco use, elevated body mass index (BMI) or total cholesterol [5–8]. Intervention measures were then proposed to aim at reducing the cardiovascular risk of HIV-infected patients, such as a switch of antiretroviral regimen from protease inhibitors (PI) to non-nucleoside reverse transcriptase inhibitors [9] or the use of lipid-lowering drugs [10]. Therefore, since 2000, national and international guidelines have included recommendations for limiting the metabolic side-effects of HAART [11]. One could expect to observe changing patterns of case management and thus a reduction in the cardiovascular risk in HIV-infected patients. In the present study, we report on the atherosclerosis risk measured through the IMT 36-month changes in relation to the evolution of care practices in a French cohort of HIV-infected patients.

Patients included in the present report were those from the SUPRA Study described elsewhere [7], with information available at three scheduled visits: month 0 (baseline), month 12 (12 months after) and month 36. Briefly, patients of the Aquitaine Cohort were included successively if they were seen in one of the five participating centres in Bordeaux (France) from September 1999 to April 2000. If the patient agreed to participate in this study, IMT evaluation was performed at the three planned clinic visits. In addition to information routinely collected within the Aquitaine Cohort, more precise data about the cardiovascular risk factors were systematically recorded.

Carotid IMT was measured by B-mode ultrasonography, on the common part of the left and right carotid arteries. IMT calculation was performed semi-automatically by an IMT analysis software (version 5.0) in a 'Workstation Imaging station' (IôDP, Paris, France).

A total of 233 patients were included in the study with a median age of 44 years at baseline [interquartile range (IQR) 39; 50], 57 (25%) were women, 138 (59%) were current smokers and 74 (32%) were at the AIDS stage. At month 0, 200 patients (86%) were treated by a combination of at least three antiretroviral drugs, including 127 with a PI-containing regimen; five patients were treated by fibrates, none by statins. During the first 12 months, eight new patients were treated by statins and eight by fibrates, 42 had their antiretroviral regimen changed to stop the PI and 10 patients stopped smoking. A total of 63 patients thus benefited from at least one of these three preventive measures for cardiovascular risk. During the same period, serum triglycerides, LDL and total cholesterol remained stable: $P = 0.24$, 0.75 and 0.78, respectively (Table 1). Conversely, the median IMT increased from 0.55 to 0.57 mm ($P < 0.0001$). In the following 24-month period, 52 more patients switched from a PI-containing to a PI-free regimen, 30 new patients were treated with lipid-lowering drugs and 14 stopped smoking, resulting in a 36-month prevalence of 40 patients (17%) treated with fibrates or statins, 141 (60%) with a PI-free antiretroviral regimen and 114 (49%) who did not smoke. During the same 2-year period, there was a significant decrease in total cholesterol ($P < 10^{-4}$), LDL-cholesterol ($P = 0.05$) and median IMT from 0.57 to 0.53 mm ($P < 10^{-4}$). The proportion of patients performing physical activity at least once per week was stable, approximately 30%, throughout follow-up. However, among them, the proportion of those who practised more than once per week tended to increase: 64% at month 0, 69% at month 12 and 72% at month 36. The median BMI at baseline (22.6, IQR 20.6; 24.6) remained stable during follow-up ($P = 0.09$ and $P = 0.14$). When analysing the association at the individual level between these interventions aimed at reducing the cardiovascular risk and IMT evolution, the only significant association was with smoking cessation ($P = 0.06$). Using a linear mixed model for repeated measures taking into account all three interventions, we estimated a decreasing IMT

Table 1. Change in prevalence of patients treated by lipid-lowering drugs (statins or fibrates), protease inhibitor-free antiretroviral regimens, non-smokers, serum lipid levels and carotid intima media thickness during 36 months in 233 HIV-infected patients. SUPRA Study. ANRS Aquitaine Cohort, 1999–2003.

	Month 0 *N* (%)	Month 12 *N* (%)	New patients from months 0–12 *N* (%)		Month 36 *N* (%)	New patients from months 12–36 *N* (%)
Patients treated by lipid-lowering drugs	5 (2.1)	20 (8.6)	16 (6.9)		40 (17.2)	30 (12.9)
Patients treated with PI-free regimen	106 (45.5)	139 (59.7)	42 (18.0)		141 (60.5)	52 (22.3)
Non-smokers (current)	95 (40.8)	103 (44.2)	10 (4.3)		114 (48.9)	14 (6.0)

	Month 0	Month 12	Difference from months 0–12		Month 36	Difference from months 12–36	
	Median (IQR)	Median (IQR)	Mean (SD)	*P**	Median (IQR)	Mean (SD)	*P**
Total cholesterol	5.30 (4.48; 6.10)	5.37 (4.56; 6.18)	0.08 (1.07)	0.24	5.10 (4.28; 5.80)	−0.35 (1.26)	$< 10^{-4}$
Triglycerides	1.66 (1.06; 2.64)	1.61 (1.02; 2.57)	0.03 (1.46)	0.75	1.60 (1.07; 2.45)	−0.09 (1.46)	0.33
LDL-cholesterol	3.21 (2.49; 3.91)	3.29 (2.57; 4.07)	0.02 (0.84)	0.78	3.08 (2.41; 3.82)	−0.15 (1.01)	0.05
IMT	0.55 (0.51; 0.61)	0.57 (0.52; 0.65)	0.02 (0.08)	$< 10^{-4}$	0.53 (0.49; 0.60)	−0.04 (0.08)	$< 10^{-4}$

IMT, Intima media thickness; IQR, interquartile range; PI, protease inhibitor.
*Student's *t*-test for paired data.

slope between month 12 and month 36 of −0.045 [95% confidence interval (CI) −0.056; −0.033] in those without intervention compared with −0.086 (95% CI −0.13; −0.043) in those who stopped smoking ($P = 0.06$). However, the significance of this association tended to decrease when adjusting for age, sex, BMI and total cholesterol ($P = 0.11$). During follow-up, cardiovascular events were documented in two patients: both men, 39 and 51 years old, treated by HAART with PI, both presenting with lipid disorders; one of them smoked 15 pack-years. IMT values were 0.55 and 0.57 mm at the time of coronary angioplasty, which was performed in the context of angina pectoris.

One limitation of this study was the representativeness of the study sample compared with the population of HIV-infected patients in France and other industrialized countries. One might expect that patients' behaviour and clinical management were better in this group compared with the rest of the Aquitaine cohort, because of early information on the study objectives at the time of enrolment and full access of the attending physician to baseline IMT results and cardiovascular risk assessment. Study participants may thus represent patients to whom special attention was paid for their cardiovascular risk. Also, IMT is a surrogate marker of long-term atherosclerosis and not of acute coronary events. Other factors such as oxidative stress, the inflammatory process and cytokine production are involved in plaque rupture occurring in unstable plaques leading to clinical events. Finally, our results come from a small sample with no randomization of preventative measures. The potential impact of interventions aimed at cardiovascular risk prevention thus need to be confirmed in larger study populations and with clinical outcomes (myocardial infarction, stroke) as in the D:A:D collaboration [4]. Nevertheless, should our results be confirmed, the potential impact of smoking cessation in particular should encourage its active promotion by clinicians providing HIV care.

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Sponsorship: This study was partly supported by the French charity ECS 'Ensemble Contre le Sida' (9e appel d'offres). ACUSON Inc., Bristol-Myers Squibb, Roche and Glaxo-Welcome (Paris, France) provided additional financial support to the project. The Aquitaine Cohort within which the study was conducted is supported by the French National AIDS Research Agency (ANRS) through the Coordinated Action no. 7 'Cohorts'.

Received: 10 December 2004; accepted: 11 January 2005.

References

1. Henry K, Melroe H, Huebsch J, Hermundson J, Levine C, Swensen L, Daley J. **Severe premature coronary artery disease with protease inhibitors.** *Lancet* 1998; **351**:1328.
2. Periard D, Telenti A, Sudre P, Cheseaux JJ, Halfon P, Reymond MJ, *et al*. **Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study.** *Circulation* 1999; **100**:700–705.
3. Mary-Krause M, Cotteb L, Simon A, Partisani M, Costagliola D. **Increased risk of myocardial infarction with duration of protease inhibitor therapy in HIV-infected men.** *AIDS* 2003; **17**:2479–2486.
4. Friis-Møller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr WM, Reiss P, *et al*. **Combination antiretroviral therapy and the risk of myocardial infarction.** *N Engl J Med* 2003; **349**:1993–2003.
5. Maggi P, Serio G, Epifani G, Fiorentino G, Saracino A, Fico C, *et al*. **Premature lesions of the carotid vessels in HIV-1-infected patients treated with protease inhibitors.** *AIDS* 2000; **14**:F123–F128.
6. Depairon M, Chessex S, Sudre P, Rodondi N, Doser N, Chave JP, *et al*. **Premature atherosclerosis in HIV-infected individuals - focus on protease inhibitor therapy.** *AIDS* 2001; **15**:329–334.
7. Mercié P, Thiébaut R, Lavignolle V, Pellegrin JL, Yvorra-Vives MC, Morlat P, *et al*. **Evaluation of cardiovascular risk factors in HIV-1 infected patients using carotid intima-media thickness measurement.** *Ann Med* 2002; **34**:55–63.
8. Hsue PY, Lo JC, Franklin A, Bolger AF, Marti JN, Deeks SG, Waters DD. **Progression of atherosclerosis as assessed by carotid intima-media thickness in patients with HIV infection.** *Circulation* 2004; **109**:1603–1608.
9. Martinez E, Conget I, Lozano L, Casamitjana R, Gatell JM. **Reversion of metabolic abnormalities after switching from HIV-1 protease inhibitors to nevirapine.** *AIDS* 1999; **13**:805–810.
10. Bonnet F, Balestre E, Thiébaut R, Mercie P, Dupon M, Morlat P, *et al*. **Fibrates or statins and lipid plasma levels in 245 patients treated with highly active antiretroviral therapy. Aquitaine Cohort, France, 1999–2001.** *HIV Med* 2004; **5**:133–139.
11. British, HIV. Association (BHIVA). **Guidelines for the treatment of HIV-infected adults with antiretroviral therapy.** *HIV Med* 2001; **2**:276–313.

Panel of prototypical infectious molecular HIV-1 clones containing multiple nucleoside reverse transcriptase inhibitor resistance mutations

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We have created a panel of recombinant HIV-1 infectious clones containing common patterns of reverse transcriptase (RT) mutations responsible for resistance to each of the currently available nucleoside reverse transcriptase inhibitors (NRTI), and we have submitted the panel to the National Institutes of Health AIDS Research and Reference Reagent Programme. Testing the activity of new antiretroviral compounds against this panel of drug-resistant clones will determine their relative activity against many clinically relevant NRTI-resistant viruses.

Although the large number of drug-resistant HIV-1 mutations makes the development of new non-cross-resistant antiretroviral inhibitors challenging, HIV-1 strains from heavily treated individuals often develop common, co-linear combinations of these mutations [1]. We have created a panel of recombinant infectious molecular clones containing combinations of mutations that confer resistance to multiple nucleoside reverse transcriptase inhibitors (NRTI). We hypothesize that NRTI that maintain full activity against the clones in this panel will also be active against the much larger number of NRTI-resistant variants currently found in individuals failing therapy.

In previous studies, we identified common combinations of NRTI-resistance mutations by determining the extent of co-variation between all pairs of NRTI-resistance mutations [2], by determining the frequency of specific NRTI-resistance mutations in a clinical database [3], and by using statistical clustering approaches [4]. We used the data from these studies and from recent clinical trials [5–7] to select cryopreserved plasma samples containing previously sequenced HIV-1 isolates with specific patterns of NRTI-resistance mutations.

HIV-1 RNA was extracted using the QIAamp Viral RNA Kit (QIAGEN Inc., Valencia, CA, USA) from 500 μl of cryopreserved plasma and amplified by reverse transcriptase–polymerase chain reaction to create an amplicon encompassing RT codons 23–312 (871 bp). Amplified RT fragments were ligated into an RT-deleted pNL4-3 vector (pNLPFB digested with *Msc1* and *PflM1*) [8] and transformed with competent *Escherichia coli* Top10 cells (Invitrogen, Carlsbad, CA, USA). These recombinant clones were transfected into C8166 cells using Lipofectin (Invitrogen). When syncytia were observed, the C8166 cells were co-cultured with SupT1 cells to create high-titer virus stocks as measured by p24 enzyme-linked immunosorbent assay (Perkin Elmer, Boston, MA, USA) and endpoint dilution. The mutations present in each of the clones were then confirmed by sequencing. Virus stocks were then submitted for HIV-1 drug susceptibility testing using the PhenoSense assay (ViroLogic, South San Francisco, CA, USA). In 2002 and 2004, the clones and their associated virus stocks were submitted to the National Institutes of Health AIDS Research and Reference Reagent Programme (Rockville, MD, USA; www.aidsreagent.org, catalog #7384-7395) for use without restriction.

The mutations in the 12 clones and their associated drug susceptibilities are shown in Table 1. Eight clones have reduced susceptibility to each of the six NRTI tested; four have reduced susceptibility to three to five inhibitors. Susceptibilities were not available for emtricitabine, which has a drug-resistance profile identical to lamivudine. Median reductions in susceptibility were greater than 300-fold to lamivudine, 161-fold to zidovudine, 7.6-fold to abacavir, 4.4-fold to stavudine, 3.1-fold to tenofovir, and 2.7-fold to didanosine.

Table 1. Nucleoside reverse transcriptase inhibitor resistance mutations and drug susceptibility results of the 12 infectious molecular reverse transcriptase clones.[a]

Clone	GenBank	NIH	p24	TAM									151-Associated mutations								Fold-decreased susceptibility[b]					
				41	67	70	210	215	219	184	69	65	75	77	116	151	74	115	44	118	ZDV	d4T	TDF	ABC	ddI	3TC
1	AY351774	6463-13	5.2	L	N		W	Y		V										I	**24**	**4.1**	**1.5**	**7.4**	**2.1**	≫
2	AY351750	7303-3	5.4	L	N		W	Y			D								D	I	≫	**6.7**	**5.9**	**8.4**	**2.3**	**7.5**
3	AY351769	4755-5	5.5	L	N		W	Y		V	D								D	I	**61**	**3.9**	**2.3**	**7.7**	**2.4**	≫
4	AY351719	7324-4	4.8		N	R		F	E												**464**	**2.3**	**5.2**	3.8	1.5	3.6
5	AY351744	7295-1	5.1		N	R		F	Q	V	N										**9.9**	**1.9**	1.1	**6.1**	**1.9**	≫
6	AY351717	7324-1	4.6	L	N	R		F	E		N										**923**	**2.6**	**8.1**	**4.2**	**1.7**	4.1
7	AY351729	52534-2	5.7	L			W	Y		V	ins						V				**719**	**9**	**4.2**	**22**	**3.3**	≫
8	AY351767	1617-1	5.5			G				V	K		I	L	Y	M		Y			**261**	**11**	**2.4**	≫	**23**	≫
9	AY351770	35764-2	6.2										I	L	Y	M					**55**	**11**	**1.9**	**7.1**	**11**	**5.4**
10	AY351736	56252-1	4.5			R						R	I	L	Y	M					≫	**20**	**11**	≫	**28**	**89**
11	AY829262	71361-1	5.4									R									0.7	**1.9**	**3.8**	**3.6**	**3.0**	**10**
12	AY829263	8415-2	5.3							V		R									0.3	1.2	1.1	**9.1**	**3.0**	≫

ABC, Abacavir; ddI, didanosine; d4T, stavudine; NIH, National Institutes of Health; p24, logarithm of the p24 antigen concentration (pg/ml) of the initial virus stock; TAM, thymidine analog mutation; TDF, tenofovir; 3TC, lamivudine; ZDV, zidovudine.

[a]The complete sequences, list of mutations, and list of drug susceptibility results (including for the non-nucleoside reverse transcriptase inhibitors) for each clone can be found on the NIH AIDS Research and Reference Reagent Programme website (http://www.aidsreagent.org) or on the Stanford Drug Resistance Database (http://hivdb.stanford.edu).

[b]Zalcitibine results are not shown because this drug is rarely used and its resistance profile is similar to that of ddI. Although susceptibilities to emtricitabine, the most recently approved nucleoside reverse transcriptase inhibitor, were not determined, its resistance profile is considered identical to that of 3TC. Results in bold are above the PhenoSense assay clinical cut-off. ≫ indicates that the fold resistance (reduction in drug susceptibility) was greater than the upper limit of assay detection, which is 300-fold for 3TC and approximately 1000-fold for ZDV. Non-nucleoside reverse transcriptase inhibitor resistance mutations at position 103 (K103N) were present in clones 2, and 10, and at position 190 (G190C and G190A) in clones 7 and 11. The 'ins' at position 69 for clone 7 indicates the presence of a double amino acid (SS) insertion following a T69S substitution at position 69.

The dynamic range in susceptibility to stavudine, didanosine, and tenofovir is much lower than that to zidovudine, lamivudine, and even abacavir. With the PhenoSense assay, reductions in susceptibility of greater than 1.5-fold to stavudine, didanosine, and tenofovir occur only in isolates with drug-resistance mutations [9,10], and reductions of three to fourfold are associated with markedly decreased antiretroviral activity *in vivo* [11–13].

The panel contains HIV-1 isolates with four previously described mechanisms of multiple NRTI resistance [14,15]: (i) multiple thymidine analog mutations (TAM) + M184V ± T69D/N (clones 1–6); (ii) multiple TAM + T69 insertion (clone 7); (iii) Q151M-mediated multi-NRTI resistance ± K65R ± M184V (clones 8–10); and (iv) K65R ± M184V (clones 11–12). Because the first pattern is the most common, it is represented by the greatest number of clones. Three are characterized by the common mutation triad M41L, L210W, and T215Y, and four by D67N, K70R, T215F, and K219Q/E. These four mechanisms of multi-NRTI resistance are partly overlapping – T69 insertions nearly always occur with multiple TAM, and K65R often occurs with Q151M. However, Q151M and particularly K65R rarely occur in combination with TAM [2,16].

New compounds that demonstrate in-vitro antiretroviral activity are usually tested on a range of drug-resistant clinical isolates. However, because no standard sets of clinical drug-resistant isolates have been identified, it is usually not possible to determine the activity of a new compound relative to other experimental compounds and approved drugs. Testing the activity of new compounds against this panel will allow researchers from different laboratories to standardize their results against a set of reference viruses. Infectious molecular clones provide an advantage over primary HIV-1 isolates because primary isolates often contain mixtures of wild-type and mutant viruses that may evolve independently during in-vitro passage and because molecular clones are amenable to biochemical and biophysical studies. The panel we have described will be continuously updated as new NRTI are developed and new patterns of RT mutations associated with multi-NRTI resistance are observed.

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Sponsorship: E.J., M.J.G., K.M.D, M.A.W., and R.W.S. were partly supported by a grant from the NIAID (National Institutes of Health) AI-46148-03.

Received: 7 December 2004; accepted: 16 December 2004.

References

1. Gonzales MJ, Johnson E, Dupnik KM, Imamichi T, Shafer RW. **Colinearity of reverse transcriptase inhibitor resistance mutations detected by population-based sequencing.** *J Acquir Immune Defic Syndr* 2003; **34**:398–402.
2. Gonzales MJ, Wu TD, Taylor J, Belitskaya I, Kantor R, Israelski D, *et al*. **Extended spectrum of HIV-1 reverse transcriptase mutations in patients receiving multiple nucleoside analog inhibitors.** *AIDS* 2003; **17**:791–799.
3. Rhee SY, Liu T, Ravela J, Gonzales MJ, Shafer RW. **Distribution of human immunodeficiency virus type 1 protease and reverse transcriptase mutation patterns in 4,183 persons undergoing genotypic resistance testing.** *Antimicrob Agents Chemother* 2004; **48**:3122–3126.
4. Gonzales MJ, Belitskaya I, Dupnik KM, Rhee SY, Shafer RW. **Protease and reverse transcriptase mutation patterns in HIV type 1 isolates from heavily treated persons: comparison of isolates from Northern California with isolates from other regions.** *AIDS Res Hum Retroviruses* 2003; **19**:909–915.
5. Gallant J, Rodriguez AE, Weinberg W. **Early non-response to tenofovir DF (TDF) + abacavir (ABC) and lamivudine (3TC) in a randomized trial compared to efavirenz (EFV) + ABC and 3TC: ESS30009.** In: *43rd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Chicago, IL, 2003 [Abstract 1722a].
6. Landman R, Peytavin G, Descamps D, Brun Vezinet F, Benech H, Benalisherif A, *et al*. **Low genetic barrier to resistance is a possible cause of early virologic failures in once-daily regimen of abacavir, lamiduvine, and tenofovir: the Tonus Study.** In: *11th Conference on Retroviruses and Opportunistic Infections*. San Francisco, CA, 2004 [Abstract 52].
7. Roge BT, Katzenstein TL, Obel N, Nielsen H, Kirk O, Pedersen C, *et al*. **K65R with and without S68: a new resistance profile in vivo detected in most patients failing abacavir, didanosine and stavudine.** *Antivir Ther* 2003; **8**:173–182.
8. Imamichi T, Berg SC, Imamichi H, Lopez JC, Metcalf JA, Falloon J, Lane HC. *Relative replication fitness of a high-level 3′-azido-3′-deoxythymidine-resistant variant of human immunodeficiency virus type 1 possessing an amino acid deletion at codon 67 and a novel substitution (Thr(Gly) at codon 69. J Virol* 2000; **74**:10958–10964.
9. Parkin NT, Hellmann NS, Whitcomb JM, Kiss L, Chappey C, Petropoulos CJ. **Natural variation of drug susceptibility in wild-type HIV-1.** *Antimicrob Agents Chemother* 2004; **48**: 437–443.
10. Whitcomb JM, Parkin NT, Chappey C, Hellmann NS, Petropoulos CJ. **Broad nucleoside reverse-transcriptase inhibitor cross-resistance in human immunodeficiency virus type 1 clinical isolates.** *J Infect Dis* 2003; **188**:992–1000.
11. Miller MD, Margot N, Lu B, Zhong L, Chen SS, Cheng A, Wulfsohn M. **Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients.** *J Infect Dis* 2004; **189**:837–846.
12. Molina JM, Marcelin AG, Pavie J, Merle C, Troccaz M, Leleu G, Calvez V. **Didanosine (ddI) in treatment experienced HIV-infected patients: results from a randomized double-blind study (AI454-176 Jaguar).** In: *43rd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Chicago, IL, 2003.[Abstract H-447].
13. Shulman NS, Hughes MD, Winters MA, Shafer RW, Zolopa AR, Hellmann NS, *et al*. **Subtle decreases in stavudine phenotypic susceptibility predict poor virologic response to stavudine monotherapy in zidovudine-experienced patients.** *J Acquir Immune Defic Syndr* 2002; **31**:121–127.
14. Gallant JE, Gerondelis PZ, Wainberg MA, Shulman NS, Haubrich RH, St Clair M, *et al*. **Nucleoside and nucleotide analogue reverse transcriptase inhibitors: a clinical review of antiretroviral resistance.** *Antivir Ther* 2003; **8**:489–506.
15. Shafer RW. **Genotypic testing for human immunodeficiency virus type 1 drug resistance.** *Clin Microbiol Rev* 2002; **15**:247–277.
16. Faruki H, Sebastian J, Scott J, Stamp J, Lanier E. **Changes in prevalence of NRTI resistance associated mutations among clinical isolates from 1999–2003.** *Antivir Ther* 2004; **9**:S91.

A conserved HLA B13-restricted cytotoxic T lymphocyte epitope in Nef is a dominant epitope in HLA B13-positive HIV-1-infected patients

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We report on the first HLA B13-restricted minimal cytotoxic T lymphocyte (CTL) epitope RQDILDLWI (RI9, amino acids 106–114 in HIV-1 Nef). In most patients the frequency of RI9-specific CTL exceeded the number of CTL against other epitopes, indicating that RI9 is a dominant epitope in HLA B13-positive patients. Targeting this conserved Nef epitope may be an important factor for the published association of HLA B13 with a favourable course of HIV-1 infection.

A potential strategy to overcome the obstacles of HIV-1 diversity for vaccine development is the design of multi-epitope vaccines containing conserved cytotoxic T lymphocyte (CTL) epitopes [1]. However, for many HLA alleles, no or only a few HIV-1-specific CTL epitopes have yet been defined. Analysing the CTL response in three HLA B13-positive HIV-1-infected patients using overlapping Nef peptides in a γ-IFN enzyme-linked immunospot assay (ELISPOT) we could delineate the epitope RQDILDLWI (RI9; amino acids 106–114) as the first minimal HLA B13-restricted CTL epitope published so far. The omission both of the N-terminal arginine and of the C-terminal isoleucine abrogated recognition. The three patients were good controllers without therapy: patient 1 was infected for 18 years with a CD4 cell count of 580 cells/μl and a viral load of 400 copies/ml. Patient 2 was infected for over 8 years with CD4 cell counts of over 600 cells/μl and low viral loads (110–6800 copies/ml). Patient 3 infected for 4 years was transiently treated by highly active antiretroviral therapy (HAART) with a CD4 cell count of 310 cells/μl and a viral load of 5200 copies/ml. Eight months later he interrupted therapy preserving his CD4 cell count above 400 cells/μl and low viral loads fluctuating between 850 and 9700 copies/ml. Analysis of the recognition of peptide pulsed autologous and HLA-matched B-cell lines (B-LCL) by RI9-specific CTL lines in a γ-IFN ELISPOT assay demonstrated the presentation of RI9 by HLA B13. As HLA B13 has a strong linkage with CW6, we were able to determine the HLA B13 restriction only by excluding the recognition of B-LCL sharing only CW6 and not HLA B13. This confirmed previous reports describing an HLA B13 restriction of the peptide SQRRQDILDLWIYHTQ-GYFPDWQNY containing the epitope RQDILDLWI [2,3]. Further investigations showed the recognition of RI9 also in patients with a previous diagnosis of AIDS (patient 4, cytomegalovirus retinitis; and patient 5, oral thrush). Both these patients were on HAART for 5 and 3 years with a suppressed viral load (< 50 copies/ml) and CD4 cell counts greater than 400 cells/μl. Except for patient 2 the frequency of RI9-specific CTL strongly exceeded the number of CTL directed against other peptides corresponding to known CTL epitopes restricted by the patient's HLA alleles, indicating that this epitope is a major immunodominant CTL epitope in HLA B13-positive patients. Although the target cell sensitizing activity of the RI9 peptide is relatively weak with the loss of recognition already at 10 ng/ml, the frequency of RI9-specific CTL reached high values ranging from 2320 to 5009/10^6 peripheral blood mononuclear cells (PBMC) in the three controllers. Four of the five patients were HLA A2 positive, but none showed recognition of the HLA A2-restricted epitope ILKEPVHGV, and only patient 1 recognized the HLA A2 epitope SLYNTVATL; however, with a much lower frequency of SLYNTVATL-specific CTL (333 spot forming units/10^6 PBMC) in comparison with the RI9-specific CTL (5009 spot forming units/10^6 PBMC).

The RI9 epitope is highly conserved, with only a few amino acid substitutions reported in the Los Alamos database [4]. Essential functions of Nef are coded within this epitope because the N-terminal arginine is essential for the dimerization of Nef, and the glutamine at the P2 position is involved in recruitment of PAK-2 [5–7]. The most frequent variants are a Q to K substitution at position P2, a D to E substitution at position P3 and an I to V substitution at position P9. Testing with variant peptides revealed that all these mutations were recognized (example shown in Fig. 1); however, both the D to E substitution at position P3 and even more the Q to K substitution at position P2 decreased recognition with the loss of recognition in peptide titration experiments at 100 ng/ml. There were differences between the patients regarding the recognition of the D to E substitution at the P3 position that were better recognized by patients 1 and 2 than by the other patients. An artificial exchange of the

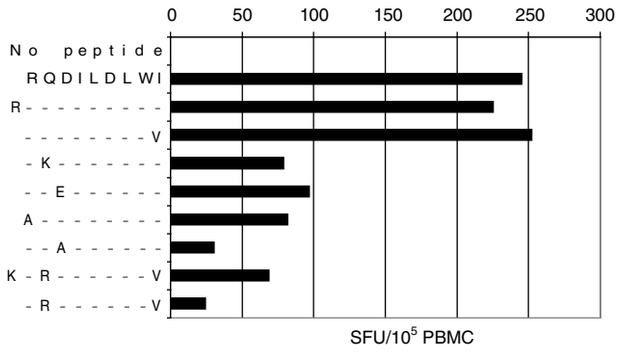

Fig. 1. Recognition of viral variants of the RQDILDLWI epitope. Peripheral blood mononuclear cells (PBMC) from patient 3 (10^5 PBMC/well in duplicates) were incubated overnight with variant peptides at a concentration of 10 μg/ml in a standard γ-IFN ELISPOT assay. Results are given in spot forming units (SFU)/10^5 PBMC.

arginine at position P1 by an alanine, which is not found in the Los Alamos database, induced a strong decrease, but not a total loss of recognition, indicating that an arginine at position P1 is important, but not absolutely essential for binding to the HLA B13 molecule (Fig. 1). Substitution of the aspartate at the P3 position by an alanine decreased but did not fully abrogate recognition by PBMC (Fig. 1). The most frequently observed variation is an isoleucine to valine substitution at the C-terminal P9 position. Peptide titration experiments showed an equal recognition of both the RQDILDLWI and the RQDILDLWV peptides, suggesting that both valine and isoleucine can serve equally well as C-terminal binding anchors for HLA B13. On the basis of this analysis we assume that the optimal peptide is a 9-mer requiring an arginine at position P1 and a hydrophobic amino acid such as an isoleucine or a valine at position P9. Autologous Nef sequences could be analysed after polymerase chain reaction amplification of Nef genes from PBMC or from plasma, respectively. Despite high frequencies of RI9-specific CTL, viral sequences corresponding to the RI9 epitope were conserved in the three controllers (patients 1, 2 and 3) except for a conservative isoleucine to valine substitution at the C-terminal amino acid (114), which was well recognized by CTL from all three patients. In contrast, in both patients with a previous diagnosis of AIDS (patients 4 and 5) we observed a Q to R mutation at the P2 position (amino acid 107) of the epitope that strongly reduced recognition, indicating that this mutation confers CTL escape. Although progression may occur despite recognition of the RQDILDLWI epitope, recognizing this epitope seems to be advantageous for patients, as in three controllers with low viral loads this epitope was a dominant target of the CTL response. There have been reports that HLA B13 is associated with a more favourable course of HIV-1 infection [8]. We speculate that this association could be caused by the recognition of this dominant Nef epitope. Therefore, we suggest that this HLA B13 epitope should be considered for the design of HIV-1-specific polytope vaccines.

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Sponsorship: This work was supported by the Deutsche Forschungsgemeinschaft (SFB 466, T. Harrer, Project B6), the German Competence Network on HIV/AIDS (Project F2, T. Harrer), the ELAN programme of the University Hospital Erlangen (T. Harrer, 98.08.32.1), the AIDS Reagent Project of the Medical Research Council (Herts, UK) and the European Community EVA Programme.

Received: 7 November 2004; revised: 14 December 2004; accepted: 5 January 2005.

References

1. Wee EG, Patel S, McMichael AJ, Hanke T. **A DNA/MVA-based candidate human immunodeficiency virus vaccine for Kenya induces multi-specific T cell responses in rhesus macaques.** *J Gen Virol* 2002; **83**:75–80.
2. Oxenius A, Price DA, Easterbrook PJ, O'Callaghan AD, Kelleher AD, Whelan JA, *et al*. **Early highly active antiretroviral therapy for acute HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes.** *Proc Natl Acad Sci U S A* 2000; **97**:2287–3382.
3. Jassoy C, Harrer T, Rosenthal T, Navia BA, Worth J, Johnson RP, *et al*. **Human immunodeficiency virus type 1-specific cytotoxic T lymphocytes release gamma interferon, tumor necrosis factor alpha (TNF-alpha), and TNF-beta when they encounter their target antigens.** *J Virol* 1993; **67**:2844–2852.
4. Korber B, Brander C, Haynes B, Koup R, Kuiken C, Moore JP, *et al*. *HIV molecular immunology*. Los Alamos, New Mexico: Los Alamos National Laboratory, Theoretical Biology and Biophysics; 2002.
5. Foster JL, Molina RP, Luo T, Arora VK, Huang Y, Ho DD, *et al*. **Genetic and functional diversity of human immunodeficiency virus type 1 subtype B Nef primary isolates.** *J Virol* 2001; **75**:1672–1680.
6. Hua J, Blair W, Truant R, Cullen BR. **Identification of regions in HIV-1 Nef required for efficient downregulation of cell surface CD4.** *Virology* 1997; **231**:231–238.
7. Sawai ET, Baur AS, Peterlin BM, Levy JA, Cheng-Mayer C. **A conserved domain and membrane targeting of Nef from HIV and SIV are required for association with a cellular serine kinase activity.** *J Biol Chem* 1995; **270**:15307–15314.
8. Tang J, Tang S, Lobashevsky E, Myracle AD, Fideli U, Aldrovandi G, *et al*. **Favorable and unfavorable HLA class I alleles and haplotypes in Zambians predominantly infected with clade C human immunodeficiency virus type 1.** *J Virol* 2002; **76**:8276–8284.

Lower incidence and severity of cytomegalovirus-associated immune recovery uveitis in HIV-infected patients with delayed highly active antiretroviral therapy

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An analysis of clinical records after the introduction of highly active antiretroviral therapy (HAART) in Mexico showed a lower prevalence and severity of immune recovery uveitis when HIV-infected patients started HAART after completing cytomegalovirus retinitis treatment than when treatments were concomitant. The findings suggest a protective role of delaying HAART until cytomegalovirus retinitis is controlled.

Highly active antiretroviral therapy (HAART) has greatly reduced the incidence of opportunistic cytomegalovirus retinitis, the leading cause of blindness in acquired immunodeficiency syndrome patients [1,2]. However, a number of patients under HAART have developed immune recovery uveitis (IRU) [3,4], characterized by mild vitritis, macular oedema, papillitis, retinal neovascularization, and epiretinal membranae. IRU leads to a

substantial decrease in visual capacity or blindness, and is important in regions where HIV infection is usually diagnosed at late stages [5].

When HAART was instituted as the standard treatment for HIV infection in Mexico there were patients in our clinics who had already been treated for HIV-associated cytomegalovirus retinitis, and who underwent a switch from former antiretroviral therapies to HAART. Later, when the use of HAART became common, HIV patients diagnosed with cytomegalovirus retinitis received HAART concomitant with cytomegalovirus treatment. Clinical observations suggested that severe inflammatory ocular lesions associated with immune reconstitution were more frequent in this latter group. Therefore, we determined statistically whether a difference in the frequency of HAART-associated IRU existed between these patient groups. This difference would suggest a possible role of the time of HAART initiation relative to cytomegalovirus treatment as a risk factor for IRU.

A retrospective study was carried out with clinical records from 1996 to 2003, corresponding to a total of 542 patients with HIV infection from the Infectious Diseases Unit for Immunocompromised Patients at the National Institute for Respiratory Diseases, and from the referral center in ophthalmology of Médica Sur (Mexico City). Originally, all patients had a confirmed HIV infection, and a clinically diagnosed infection with cytomegalovirus. All patients received intravenous ganciclovir or valganciclovir as cytomegalovirus retinitis treatment. Two patients received intravenous ganciclovir plus foscavir. None used cidofovir or fomivirsen. All patients received the standard of care for HIV infection in use at the time of treatment.

Patients who initiated HIV and cytomegalovirus treatments before the availability of HAART in Mexico received antiretroviral treatment without a protease inhibitor, usually monotherapy, parallel to cytomegalovirus treatment. When these patients shifted to HAART 2 to 13 months later (average 6.3 months later), cytomegalovirus infection had already been controlled. They all showed a healed retinitis by the time of IRU detection. These patients constituted group 1 (see Table 1). Group 2 was constituted by patients who received only HAART as an HIV control treatment, consisting of at least three antiretroviral agents, including a protease inhibitor or one non-nucleoside reverse transcriptase inhibitor. In this group, HAART and anti-cytomegalovirus therapy were initiated together, and therefore cytomegalovirus infection was still not controlled when immune reconstitution began (see Table 1). As data came from a retrospective survey of clinical records informed consent was not considered.

The diagnosis of IRU was made according to the description of Karavellas and coworkers [6]. Patients with mild inflammation showed two or more cells [7] *in vitreo* without macular oedema or epiretinal membranes. Severe inflammation was diagnosed if there were two or more cells *in vitreo*, macular oedema, epiretinal membranes and papillitis. All patients underwent a fluoroangiography study, and received treatment according to the intensity of intraocular inflammation. Descriptive and non-parametric statistics were used to compare the incidence and severity of ocular lesions. The CD4 T-cell counts, age, and time of IRU onset were compared using the Student's *t*-test.

Cytomegalovirus retinitis was diagnosed in 104 out of 542 patients. Those surviving at least 12 months after the diagnosis of cytomegalovirus retinitis and showing a substantial increase in the CD4 T-lymphocyte count after HAART (43 patients) were selected for the study groups. As shown in Table 1, even though the groups did not differ in basal parameters or in immune reconstitution (indicated by CD4 cell count), the total number of IRU cases and severe IRU cases was significantly higher in group 2. The time of onset of IRU was no different between the groups.

Our data reveal a lower incidence of uveitis after immune recovery in patients who received HAART after a less active antiretroviral treatment and a successful treatment against cytomegalovirus. This difference between the groups cannot be attributed to differences in basal immune deficiency or immune reconstitution after HAART. It may be relevant to the prevention and understanding of inflammatory conditions associated with immune reconstitution. The prevention of IRU is important because its treatment is currently restricted to the periocular and systemic administration of steroidal and non-steroidal anti-inflammatory drugs [8], and the efficacy of concomitant anti-HIV and anti-opportunistic infection therapies is insufficiently known [9]. Therefore, considering our results, we propose that delaying the initiation of HAART until the retina is healed by anti-cytomegalovirus medications might protect from IRU. This delay could be considered if it does not increase the morbidity of other opportunistic infections, which must be evaluated in each patient. The debate on the correct moment during HIV infection to start HAART, currently decided on the CD4 T-lymphocyte count and plasma viral load [10], should therefore consider immune reconstitution inflammatory syndrome prevention.

Even though IRU is known to be related to an anticytomegalovirus lymphocyte response [11], its dependence on the presence of antigen is not clear. Our results agree with the possibility that residual cytomegalovirus antigen or an active infection are required in IRU pathogenesis. Patients who switched

Table 1. Characteristics of HIV-positive patients with diagnosed cytomegalovirus retinitis before and after highly active antiretroviral therapy.

Measurement	Group 1	Group 2	Statistics
No. patients			
Male	18	22	
Female	1	2	
Mean age (range)	35.4 (24–0)	35.1 (21–9)	$P = 0.37$
Basal cytomegalovirus retinitis cases			
Unilateral	14	15	
Bilateral	5	9	
Basal no. eyes with cytomegalovirus retinitis according to extension (% group)			
Zone I[a]	6 (25)	12 (36.4)	$P^{MH} = 0.37$, OR 1.71
Zones II–II[b]	18 (75)	21 (63.6)	95% CI 0.47–6.47
Mean basal lesion extension (range)[c]	20.9% (5–40%)	16.8% (5–50%)	$P = 0.18$
Cytomegalovirus retinitis before treatment			
Active	0 eyes	29 eyes	
Healed	24 eyes	4 eyes	
Mean CD4 T-cell count before HAART (range)	40/mm^3 (2–112)	37/mm^3 (8–85)	$P = 0.38$
Mean CD4 T cell count 6 months after HAART	259/mm^3 (75–952)	391/mm^3 (159–1236)	$P = 0.143$
IRU cases (% of group total)	6 (31.5)	17 (70.8)	OR 5.26, $P^{MH} = 0.0113$, 95% CI 1.21–23.96
Mild IRU cases (%)	4 (21)	2 (8.3)	OR 15.00, $P^{MH} = 0.01$
Severe IRU cases (%)	2 (10.5)	15 (62.5)	95% CI 1.07–239
Mean time of IRU onset after HAART (range)	14.2 months (1–29)	9.8 months (2–38)	$P = 0.19$

CI, Confidence interval; HAART, highly active antiretroviral therapy; IRU, immune recovery uveitis; OR, odds ratio; P^{MH}, P value in Mantel–Haenzel test.
[a]Zone 1 comprises 3000 μm around the optic nerve or 1500 μm around the centre of the fovea.
[b]Zones II and III lay outside zone I.
[c]Percentage of total retina extension, determined in each eye.
Group 1 received HAART after non-HAART HIV treatment and after successful anticytomegalovirus treatment. Group 2 received HAART as a first antiretroviral therapy and anticytomegalovirus treatment simultaneously.

to HAART when cytomegalovirus was clinically under control showed significantly less risk of developing IRU, despite a substantial boost in CD4 T-cell counts. The delay in the initiation of HAART relative to cytomegalovirus therapy in group 1 could have reduced the risk of developing IRU by controlling cytomegalovirus replication before immune reconstitution. Accordingly, the lower incidence of IRU found in HIV patients under HAART receiving ganciclovir implants as anticytomegalovirus treatment [12] could be related to a greater suppresion of viral replication as a result of the better tissue availability of ganciclovir. Our data therefore suggest the possibility of lowering the risk of developing IRU by controlling cytomegalovirus infection before initiating HAART, whenever this delay does not represent a risk for the patient.

Acknowledgements

The authors would like to thank Dr Aurora Tapia and Natalia Hernández for their assistance with the statistical tests.

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Received: 19 January 2005; accepted: 9 February 2005.

References

1. Holbrook JT, Jabs DA, Weinberg DV, Lewis RA, Davis MD, Friedberg D. **Visual loss in patients with cytomegalovirus retinitis and acquired immunodeficiency syndrome before widespread availability of highly active antiretroviral therapy.** *Arch Ophthalmol* 2003; **121**:99–107.
2. Macdonald JC, Torriani FJ, Morse LS, Karavellas MP, Reed JB, Freeman WR. **Lack of reactivation of cytomegalovirus (CMV) retinitis after stopping CMV maintenance therapy in AIDS patients with sustained elevations in CD4 T cells in response to highly active antiretroviral therapy.** *J Infect Dis* 1998; **177**:1182–1187.
3. Karavellas MP, Plummer DJ, Macdonald JC, Torriani FJ, Shufelt CL, Azen SP, *et al*. **Incidence of immune recovery vitritis in cytomegalovirus retinitis patients following institution of successful highly active antiretroviral therapy.** *J Infect Dis* 1999; **179**:697–700.
4. Karavellas MP, Azen SP, Macdonald JC, Shufelt CL, Lowder CY, Plummer DJ, *et al*. **Immune recovery vitritis and uveitis in AIDS: clinical predictors, sequelae, and treatment outcomes.** *Retina* 2001; **21**:1–9.
5. French MA, Price P, Stone SF. **Immune restoration disease after antiretroviral therapy.** *AIDS* 2004; **18**:1615–1627.
6. Karavellas MP, Lowder CY, Macdonald C, Avila CP Jr, Freeman WR. **Immune recovery vitritis associated with inactive cytomegalovirus retinitis: a new syndrome.** *Arch Ophthalmol* 1998; **116**:169–175.
7. Nussenblatt RB, Palestine AG, Chan CC, Roberge F. **Standardization of vitreal inflammatory activity in intermediate and posterior uveitis.** *Ophthalmology* 1985; **92**:467–471.

8. Robinson MR, Reed G, Csaky KG, Polis MA, Whitcup SM. **Immune recovery uveitis in patients with cytomegalovirus retinits taking highly active antiretroviral therapy.** *Am J Ophthalmol* 2000; **130**:49–56.
9. Connick E, Lederman MM, Kotzin BL, Spritzler J, Kuritzkes DR, St Clair M, *et al*. **Immune reconstitution in the first year of potent antiretroviral therapy and its relationship to virologic response.** *J Infect Dis* 2000; **181**:358–363.
10. Yeni PG, Hammer SM, Hirsch MS, Saag MS, Schechter M, Carpenter CC, *et al*. **Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society – USA Panel.** *JAMA* 2004; **292**:251–265.
11. Mutimer HP, Akatsuka Y, Manley T, Chuang EL, Boeckh M, Harrington R, *et al*. **Association between immune recovery uveitis and a diverse intraocular cytomegalovirus-specific cytotoxic T cell response.** *J Infect Dis* 2002; **186**:701–705.
12. Nguyen QD, Kempen JH, Bolton SG, Dunn JP, Jabs DA. **Immune recovery uveitis in patients with AIDS and cytomegalovirus retinitis after highly active antiretroviral therapy.** *Am J Ophthalmol* 2000; **129**:634–639.