HIV phenotype switching during antiretroviral therapy: emergence of saquinavir-resistant strains with less cytopathogenicity

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Objectives: The aim of the study was to investigate changes in virological characteristics of HIV strains isolated from 38 HIV-seropositive subjects during antiretroviral therapy.

Design and methods: Patients with a CD4+ cell count $\leq 300 \times 10^6$/l were treated with zidovudine (12 individuals) and saquinavir (10 individuals) alone or in combination (16 individuals). CD4+ cell count, viral load, HIV biological phenotype and drug resistance were evaluated during the study period.

Results: After 52 weeks, 28 subjects (74%) harboured drug-resistant strains. In patients with a syncytium-inducing (SI) strain, a decline of CD4+ cell count and an increase of viral load were observed aside from the emergence of drug resistance. Conversely, at the emergence of antiretroviral resistance, an immunological and virological deterioration was observed only in patients who had a non-syncytium-inducing (NSI) strain. During the study, a phenotype switching of HIV isolates was detected in eight (21%) patients and a temporal correspondence between the appearance of phenotype switching and the emergence of drug resistance was found in seven cases. Three patients harbouring saquinavir-resistant strains showed a switch from SI to NSI variants associated with a moderate increase in CD4+ cell count.

Conclusions: The emergence of resistant strains during antiretroviral therapy may be associated with the selection of viral strains with less cytopathogenicity, while it could become a poor prognostic sign in patients with NSI isolates.

AIDS 1997, **11**:1211–1217

Keywords: HIV biological phenotype, antiretroviral therapy, drug resistance, saquinavir

Introduction

The rate of clinical progression is variable among individuals infected with HIV. In most patients, the asymptomatic period may extend for many years, although CD4+ cell counts usually show a continuous gradual decline. In other patients, CD4+ cell counts decline rapidly, resulting in the onset of AIDS. To date, the underlying pathogenic mechanisms that govern the persistence of infection *in vivo*, and ultimately the transition from low-level to fulminant infection, are largely unknown [1,2].

So far, two distinct biological phenotypes of HIV have been described on the basis of their ability or inability to produce cytopathic effects in MT-2 cell line: the

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Sponsorship: Supported by grants from the Italian Ministry of Health (Istituto Superiore di Sanità) AIDS Research Projects, 1995 and 1996.

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Date of receipt: 20 August 1996; revised: 22 April 1997; accepted: 28 April 1997.

syncytium-inducing phenotype (SI) and the non-syncytium-inducing phenotype (NSI) [3]. SI variants have been detected at all stages of HIV-1 infection, but these phenotypic variants are found more commonly among patients with advanced disease and with accelerated CD4+ count decline [4,5]. Previous reports suggest that progression to AIDS is associated with increasing viral burden, deterioration of immunological status, emergence of drug-resistant viral strains and, finally, with a more cytopathic viral phenotype [6–8]. Although a strong association between SI variants, zidovudine (ZDV) resistance and CD4 cell counts decline has been reported [9,10], there is no evidence, as yet, to support the idea that antiviral drug resistance selects for the appearance of the SI and/or NSI phenotype.

In this study, the biological properties (antiviral resistance and viral phenotype) of sequential HIV-1 isolates obtained from patients treated with ZDV and saquinavir (SQV), alone or in combination, were evaluated. In addition, it was investigated whether changes in viral burden and CD4+ cell counts were temporally associated with the emergence of a distinct viral phenotype.

Methods

Study design

Ninety-two patients (62 males, 30 females) with symptomatic HIV infection and CD4+ lymphocyte count ≤ 300 cells $\times 10^6$/l, who had not received prior antiretroviral treatment, were enrolled in the study to evaluate the efficacy of SQV, a new protease inhibitor. The study was a 16-week, parallel, randomized double-blind, Phase I/II trial, with blinded monthly extensions of therapy in the absence of major disease progression and toxicity. HIV-1 isolates were obtained at baseline, after 16 weeks and after 52 weeks of therapy from only 38 subjects. These patients were treated thrice daily for 1 year with ZDV 200 mg (12 patients), SQV 600 mg (10 patients) and ZDV 200 mg plus SQV 600 mg (16 patients). All patients gave written informed consent.

Laboratory monitoring

Heparinized and EDTA-treated blood samples for plasma viraemia titration, HIV-RNA quantitative polymerase chain reaction (PCR) and CD4+ cell count were obtained from patients at baseline, weeks 2, 4, 8 and 16. Samples for CD4+ cell count were also collected monthly until week 52, and plasma for HIV-1 isolation was obtained at baseline, and after 16 and 52 weeks of therapy.

Virological evaluation

Plasma viraemia titration and Roche Molecular Systems (Branchburg, New Jersey, USA) RT PCR assay, which were used to quantify HIV-RNA copy numbers in plasma, were performed according to published techniques [11,12].

HIV was isolated from plasma as previously described [13]. One mililitre polyethylene glycol pretreated and untreated plasma samples were incubated in T25 flasks with 10^7 phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC), which had been obtained from HIV-seronegative donors. Cultures were placed in a humidified chamber at 37°C, in 5% CO_2, and were maintained for 40 days. Cultures were monitored twice weekly for p24 antigen production using a commercially available enzyme immunoassay (ELISA, Abbott Laboratories, North Chicago, Illinois, USA). A culture was considered positive if the concentrations of p24 antigen exceeded 1000 pg/ml in two consecutive determinations. Positive supernatants were harvested by centrifugation and stored in liquid nitrogen.

In order to determine whether the HIV isolates were SI or NSI, an aliquot of viral stock supernatant, containing $100 \times 50\%$ tissue culture infectious dose ($TCID_{50}$)/ml, was cultured with 10^6 MT-2 cells. Cultures were maintained for 4 weeks and were examined for syncytium formation twice a week [14].

HIV-1 isolates derived from plasma culture were tested for drug sensitivity by measuring growth in normal PBMC, obtained from seronegative donors, in the presence of different concentrations of drugs. Briefly, PHA-stimulated donor PBMC (4×10^6 cells) were infected with 2 ml medium containing viral stocks normalized to a multiplicity of infection of 2000 $TCID_{50}$/ml. After a 2-h adsorption period, aliquots of the cells were put into a 96-well plate containing five different concentrations of ZDV (0.001, 0.01, 0.1, 1 and 5 μM) or of SQV (0.01, 0.1, 1, 10 and 100 nM). All culture assays were performed in quadruplicate and monitored for p24 antigen production on day 7 after infection. A 50% inhibitory concentration (IC_{50}) of drug against virus was determined based upon comparative growth of isolates in untreated control cultures. HIV isolates were considered resistant to ZDV for $IC_{50} > 0.5$ μmol of drug, and resistant to SQV for $IC_{50} > 10$ nmol of drug. All HIV strains isolated from the same patient were tested for drug sensitivity simultaneously.

Statistical analysis

The joint analysis of 2×2 tables was performed using the Mantel–Haenszel χ^2 test. All *P* values were two-sided. Group means were compared by two-sample t tests and the analysis of variance; if the corresponding F test was statistically significant then individual means were compared using the Bonferroni additive inequality, which controls for the maximum experiment-wise error rate.

Results

Baseline patient characteristics

Baseline characteristics of patients are showed in Table 1. At the start of therapy 20 (53%) subjects carried NSI variants and 18 (47%) carried SI virus. No significant differences were observed in CD4+ cell counts and in viral load between the two groups of patients, even though subjects harbouring SI strains showed the lowest CD4+ cell counts and the highest HIV-RNA copy numbers and HIV plasma viraemia titres.

No degree of resistance to ZDV (mean IC_{50}, 0.006 μmol; range 0.004–0.02 μmol) or to SQV (mean IC_{50}, 0.83 nmol; range 0.37–1.0 nmol) was found in all baseline isolates.

Table 1. Baseline characteristics of patients according to biological phenotype of HIV isolates.

	NSI (n=20)	SI (n=18)
Mean CD4+ cell count	191 (± 79)	169 (± 110)
Mean HIV-RNA copies/ml	282275 (± 358712)	763585 (± 1607236)
Mean plasma viraemia titre ($TCID_{50}$/ml)	1.7 (± 2.9)	25.9 (± 74.5)
Antiviral treatment		
Zidovudine	6	6
Saquinavir	3	7
Zidovudine and saquinavir	11	5

NSI, Non-syncytium-inducing isolates; SI, syncytium-inducing isolates; $TCID_{50}$, 50% tissue culture infectious dose.

Correlation between viral phenotype, CD4+ cell counts and viral load

A gradual but significant increase of CD4+ cell count ($P = 0.03$) could be observed in the patients carrying NSI variants throughout the 52-week study period (Fig. 1). Conversely, in patients with SI variants, after a temporary increase during the first 4 weeks of treatment, CD4+ cell counts declined gradually returning to the baseline value at week 52. At the end of study, the two groups of patients showed a significant difference in the means of CD4+ cell counts ($P = 0.04$). A decrease in HIV-RNA copy number could be observed in all patients (Fig. 1). However, from the

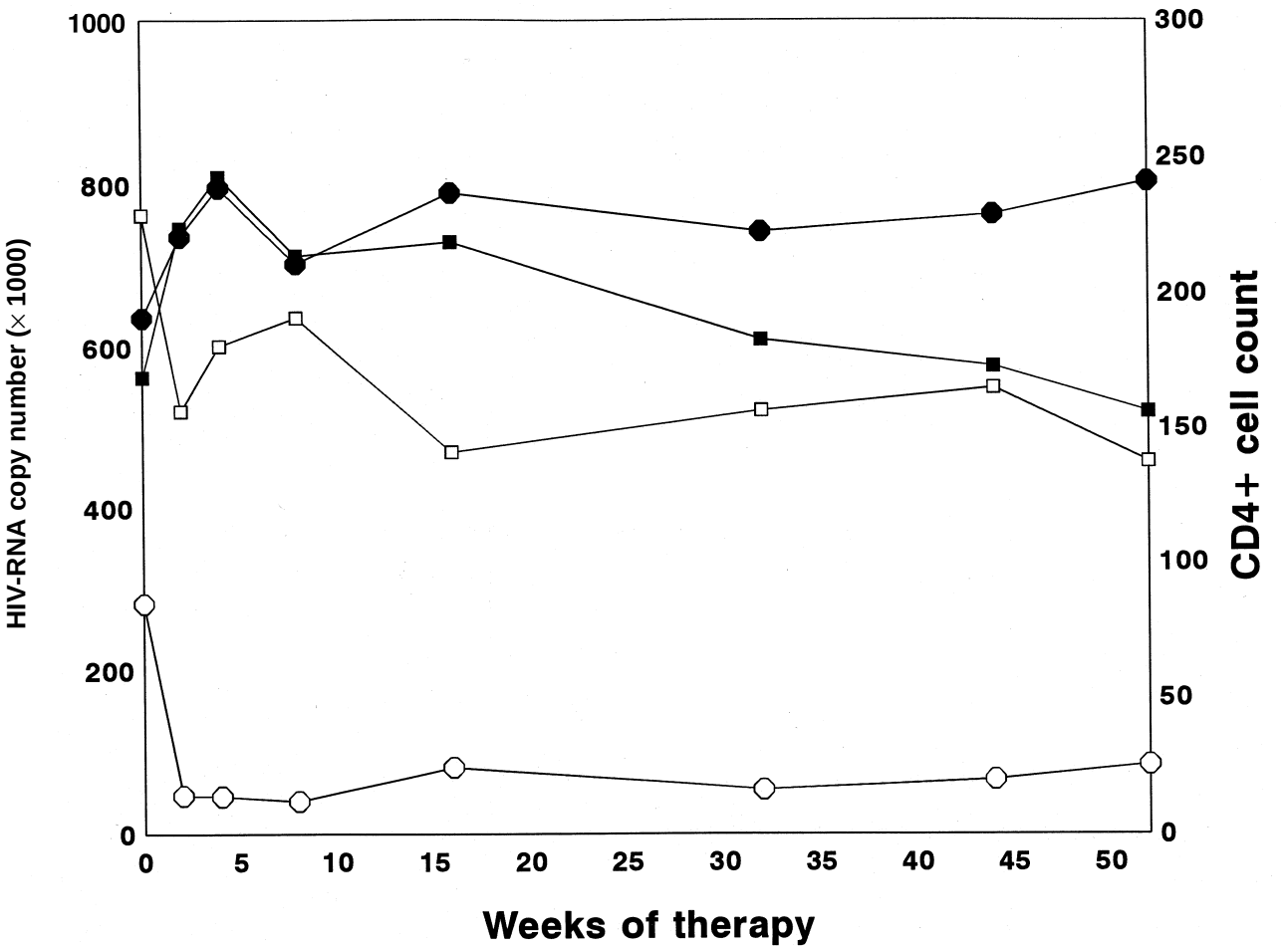

Fig. 1. CD4+ cell counts and HIV-RNA copy number in patients with non-syncytium-inducing (NSI) or syncytium-inducing (SI) viral isolates during 52 weeks of antiretroviral therapy. CD4+ cell count (●), and HIV-RNA copy number (○) in patients with NSI strains; CD4+ cell count (■) and HIV-RNA copy number (□) in patients with SI strains.

second week of treatment a significant difference in viral burden could be detected between the two groups (P=0.01). Furthermore, after 52 weeks of treatment only individuals with NSI strain had a significant reduction in HIV-RNA copies as compared with baseline values (P=0.006).

Relationship between biological phenotype and drug sensitivity

After 16 weeks of treatment, drug-resistant strains were isolated from nine patients (four were SQV-resistant and five ZDV-resistant). At the end of study, HIV-resistant strains were detected in 28 (74%) out of 38 patients (14 to SQV and 14 to ZDV); no isolate was resistant to both drugs. No significant difference was seen between subjects with SI or NSI strains with respect to the emergence of antiviral resistance, however, resistant strains were more frequently isolated from patients with SI variants (15 out of 18; 83%) compared with patients with NSI variants (13 out of 20; 65%).

Patients harbouring SI or NSI strains were subdivided into four groups according to sensitivity or resistance to antiretroviral drugs of HIV strains isolated after 52 weeks of treatment: SI-resistant (15 patients), SI-sensitive (three patients), NSI-resistant (13 patients) and NSI-sensitive (seven patients). No significant difference in mean CD4+ cell baseline values was observed between the four groups of patients (SI-resistant, $173 \pm 111 \times 10^6$/l; SI-sensitive, $150 \pm 122 \times 10^6$/l; NSI-resistant, $172 \pm 79 \times 10^6$/l; NSI-sensitive, 227 ± 71 cells $\times 10^6$/l). After 1 year of treatment, only patients carrying NSI-sensitive strain showed a significant increase in CD4+ cell counts (from 227 to 339 cells $\times 10^6$/l, $P = 0.01$). Moreover, after 1 year, these subjects had significantly higher CD4+ cell counts than the other three groups of patients ($P < 0.05$).

No significant differences were observed between the four groups relative to baseline values of viral load (ranges: SI-sensitive, 785 326 ± 709 944; SI-resistant, 759 237 ± 1 750 608; NSI-sensitive, 150 668 ± 116 050, NSI-resistant, 359 046 ± 431 000 copies/ml), however, patients carrying NSI-sensitive strain had the lowest HIV-RNA copy number at the beginning of the study. Figure 2 shows the median changes in plasma HIV copy number. After 4 weeks of therapy, a significant decrease of viral load (> 0.5 log) was detected in all groups but not in subjects with SI-resistant strains. This last group of patients included four subjects with very

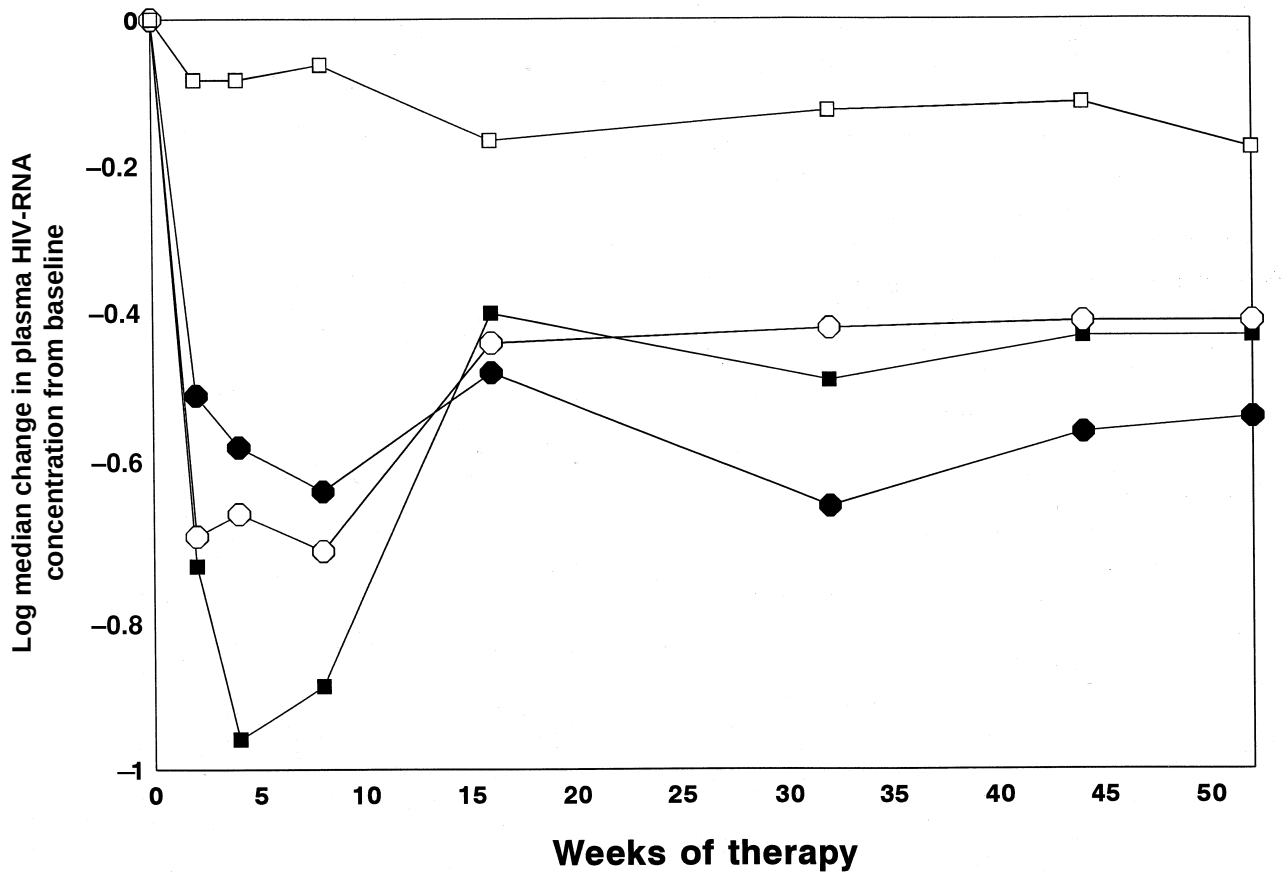

Fig. 2. Log median change in plasma HIV-RNA copy number during 52 weeks of antiretroviral therapy in patients with non-syncytium-inducing (NSI) strains sensitive to drugs (●), NSI strains resistant to drugs (○), syncytium-inducing (SI) strains sensitive to drugs (■), strains resistant to drugs (□).

high baseline HIV-RNA copy levels (2 452 612 ± 2 310 740 copies/ml) who developed early drug resistance (during the first 4 months of therapy) and showed only slight reduction (0.05 log) of HIV-RNA copy number. Conversely, the other 11 patients with SI late-resistant strain had low baseline HIV-RNA copy number (142 909 ± 158 610 copies/ml) and showed a significant decrease in HIV-RNA copy number (0.54 log) during the first weeks of therapy followed by a progressive increase of viral load up to baseline values (data not shown).

After 1 year of treatment, only subjects carrying NSI-sensitive strains had a decrease in HIV-RNA copy number of more than 0.5 log with respect to baseline value. Moreover, this group of patients had significantly lower ($P < 0.05$) HIV-RNA copy number than patients with SI-resistant strains. No differences in CD4+ cell count and HIV-RNA copy number were detected between the three groups of patients harbouring SI-sensitive strains, and NSI-resistant and SI-resistant strains.

Analysis of phenotype switching of HIV isolates

MT-2 tropism of HIV-isolates was monitored at baseline, and after 16 and 52 weeks. During the period of study, 30 out of 38 patients (15 with SI strains and 15 with NSI strains) did not change the viral biological phenotype (Table 2). A phenotype switching was observed in eight (21%) patients, five with a NSI strain and three with a SI strain at baseline. In particular, a NSI/SI conversion was demonstrated in one patient harbouring a sensitive strain to drugs (treated with combination therapy), in three patients with a ZDV-resistant strains (two subjects treated with ZDV and one subject treated with ZDV plus SQV) and in one patient harbouring SQV-resistant strain (treated with ZDV plus SQV). The NSI/SI phenotype switching occurred in two patients after 16 weeks and in three patients after 52 weeks of therapy. On the other hand, a SI/NSI conversion was observed in three patients with SQV-resistant strains and treated with protease inhibitor alone. The SI/NSI switch appeared in two cases after 16 weeks and in one case after 52 weeks of therapy. No patient harbouring a ZDV-resistant strain presented this phenotype conversion.

Table 2. Analysis of biological phenotype and sensitivity to zidovudine and saquinavir of HIV-1 strains isolated at baseline and after 52 weeks of antiretroviral treatment.

Drug sensitivity	HIV phenotype at baseline and at 52 weeks			
	Patients with phenotype switching		Patients without phenotype switching	
	NSI/SI	SI/NSI	NSI/NSI	SI/SI
Sensitive	1	0	6	3
SQV-resistant	1	3	4	6
ZDV-resistant	3	0	5	6

NSI, Non-syncytium-inducing isolates; SI, syncytium-inducing isolates; SQV, saquinavir; ZDV, zidovudine.

The mean CD4+ cell count at the time of phenotype switching was 383×10^6/l and 72×10^6/l for patients presenting a NSI/SI and SI/NSI conversion, respectively. Interestingly, after 3 months from conversion, the mean CD4+ cell count decreased 2.5-fold (153×10^6/l) in patients with NSI/SI switch, whereas it slightly increased in individuals with the SI/NSI change (83×10^6/l). In the follow up of these patients, no data on viral load were available.

In order to assure the stability of the biological phenotype, a further determination of the MT-2 tropism was performed on isolates obtained 12 weeks after phenotype switching. All viral strains tested showed the same biological phenotype that had been detected 3 months before. To validate the changes in the phenotype of HIV isolates, the seven HIV-resistant strains (four to SQV and three to ZDV), which were replicated in culture medium supplemented with or without sub-inhibiting dose of drugs, were tested again for MT-2 tropism and the same phenotype was confirmed.

HIV strain with a fluctuating phenotype was isolated from only one patient. In this subject, the NSI strain isolated at baseline changed into an SI variant after 16 weeks of treatment and returned to an NSI phenotype after 7 months of treatment, at the same time that resistance to SQV appeared.

Discussion

The clinical course of HIV disease varies widely and may be partially explained by the emergence of HIV isolates displaying SI phenotype [15]. The SI variant has been correlated with a rapid decline of CD4+ cells and with an increase in viral burden [15,16]. Further, it has been found that the progression to AIDS was more rapid in treated individuals harbouring SI isolates at the start of therapy and in individuals showing a conversion from NSI to SI isolates [17,18]. In particular, at the moment of phenotypic transition from NSI to SI variants, which occurs in about half of the individuals before (± 15 months) progression to AIDS, the rate of decline of CD4+ cells increases about threefold [19].

In this study, 38 mildly symptomatic patients were evaluated, who were receiving treatment with ZDV and SQV alone or in combination, in order to improve understanding of the relationships between biological phenotype, the drug resistance phenomenon, CD4+ cell counts and viral load. In agreement with previous studies [2], our results showed a strong association between SI phenotype and CD4+ cell decline and a higher viral burden, which strengthens the theory that SI phenotype is a poor prognostic sign in patients receiving antiviral treatment.

Previous studies [10] have demonstrated that high-level antiretroviral resistance predicts an accelerated disease progression. Genotypic and phenotypic evidence of drug resistance appears before a decrease in CD4 cells and an increase in viral load [20]. Moreover, some authors [21,22] have reported that patients with SI virus are less likely to respond to ZDV and are more likely to develop drug resistance, although antiviral therapy did not appear to select or prevent the emergence of SI variant.

Our data indicate that individuals with SI or NSI variant had no significant differences in the development of antiviral resistance. In contrast with other authors [9,10,21], we found that, in the presence of SI phenotype, the emergence of a resistant strain does not further modify CD4+ cell number and viral load. Thus, in the only three patients with SI-sensitive strains, as in the subjects with SI late-resistant strain, an initial significant decrease in HIV-RNA copy number was followed by a progressive increase in viral load up to baseline values. These results support the idea that there is no relationship between the development of drug resistance and the progression rate of disease in patients carrying SI variants. We demonstrated that the emergence of drug resistance in patients harbouring an NSI variant was associated with a marked decrease of CD4+ cells and a gradual increase of viraemia. After 1 year of treatment, a significant decrease of viral load with respect to baseline value was observed only in patients with NSI-sensitive strain. These findings seem to indicate that, in patients with less cytopathogenic viral strains, the development of antiviral resistance could be contributing to the deterioration of the immune system.

Our data demonstrated a temporal correspondence between the appearance of phenotype switching and the emergence of drug resistance of HIV isolates. In particular, in four out of five patients, a switch from NSI to SI phenotype seemed to coincide with the emergence of a strain resistant to ZDV (three patients) and to SQV (one patient), and to correlate with an increase of 2.5-fold in the rate of CD4+ cell decline. On the other hand, a conversion from SI to NSI variants, which was associated with a slight increase in CD4+ cell count, was observed in three patients who were treated with protease-inhibitor alone and who were harbouring a strain resistant to SQV. Earlier reports [23] have demonstrated that protease inhibitor-resistant mutants lose most of their infectivity and cytopathogenicity to T-cell lines *in vitro*. The cause of impaired infectivity seems mainly due to the less efficient processing of Gag proteins by the mutant protease enzyme, resulting in the selection of less cytopathic viral strains. Based on these findings, we conclude that long-term antiviral therapy with a protease inhibitor, even if it induces the emergence of resistant viral strains [24–26], may contribute to the selection of less virulent forms of the virus. Nevertheless, further studies are needed to analyse the effect on viral load of the biological phenotype conversion.

In conclusion, the patient's immune system, viral drug resistance, HIV biological phenotype and virus burden are likely to be interdependent factors in their effects on disease progression. Patients with SI variants appear to receive limited benefit from treatment; nevertheless, a change in HIV biological phenotype in the course of antiretroviral therapy may be a relevant event that can modify the progression rate to AIDS. Thus, the monitoring of the HIV-1 phenotype during antiretroviral therapy may help to identify subgroups of patients who are more likely to benefit from treatment.

Acknowledgement

We are grateful to A. Rughetti for helpful discussions and critical reading of the manuscript.

References

1. Connor RI, Mohri H, Cao Y, Ho DD: **Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals.** *J Virol* 1993 **67**:1772–1777.
2. Koot M, Vos AH, Keet RPM, *et al.*: **Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS.** *Ann Intern Med* 1993, **118**:681–688.
3. Koot M, Vos AHV, Keet RP, *et al.*: **HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay.** *AIDS* 1992, **6**:49–54.
4. Tersmette M, De Goede REY, Al BJM, *et al.*: **Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS related-complex.** *J Virol* 1988, **62**:2026–2032.
5. Fenyö EM, Albert J, Asjö B: **Replicative capacity, cytopathic effect and cell tropism of HIV.** *AIDS* 1989, **3** (suppl 1):S5–S12.
6. Asjö B, Morfeldt-Månsson L, Albert J, *et al.*: **Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection.** *Lancet* 1986, **ii**:660–662.
7. Fiscus SA, Heggem-Snow A, Troiani L, *et al.*: **Transient high titers of HIV-1 in plasma and progression of disease.** *J Acquir Immune Defic Syndr* 1995, **9**:51–57.
8. Karlsson A, Parsmyr K, Sandstrom E, Fenyö EM, Albert J: **MT-2 cell tropism of human immunodeficiency virus type 1 isolates as a marker for response to treatment and development of drug resistance.** *J Infect Dis* 1994, **170**:1367–1375.
9. Saint Clair MH, Hartigan PM, Andrews JC, Vavro CL: **Zidovudine resistance, syncytium-inducing phenotype, and HIV disease progression in a case-control study.** *J Acquir Immune Defic Syndr* 1993, **6**:891–897.
10. Kozal MJ, Shafer RW, Winters MA, *et al.*: **HIV-1 syncytium inducing phenotype, virus burden, codon 215 reverse transcriptase mutation and CD4 cell decline in zidovudine-treated patients.** *J Acquir Immune Defic Syndr* 1994, **7**:832–838.
11. Andreoni M, Sarmati L, Parisi SG, Ercoli L, Rocchi G: **Efficient and reproducible new semimicromethod for the detection and titration of HIV in human plasma.** *J Med Virol* 1992, **38**:207–213.
12. Mulder J, McKinney N, Christopherson C, *et al.*: **Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection.** *J Clin Microbiol* 1994, **32**: 292–300.

13. Sarmati L, Ercoli L, Parisi SG, *et al.*: **High rate of HIV isolation from plasma of asymptomatic patients through polyethylene glycol (PEG) treatment.** *J Acquir Immune Defic Syndr* 1994, **7**:10–14.
14. Japour AJ, Fiscus SA, Arduino JM, Mayers DL, Reichelderfer TS, Kuritzkes DR: **Standardized microtiter assay for determination of syncytium-inducing phenotypes of clinical human immunodeficiency virus type 1 isolates.** *J Clin Microbiol* 1994, **32**:2291–2294.
15. Jurriaans S, Van Gemen B, Weverling GJ, *et al.*: **The natural history of HIV-1 infection: virus load and virus phenotype independent determinants of clinical course**. *Virology* 1994, **204**:223–233
16. Shafer RW, Aguiniga E, Merigan TC: **Quantitative analysis of syncytium-inducing and non-syncytium-inducing virus in patients infected with human immunodeficiency virus type 1.** *J Clin Microbiol* 1995, **33**:212–214.
17. Daar ES, Chernyavskiy T, Zhao JQ, Krogstad P, Chen ISY, Zack JA: **Sequential determination of viral load and phenotype in human immunodeficiency virus type 1 infection.** *AIDS Res Hum Retrovirus* 1995, **11**:3–9
18. Schellekens PT, Tersmette M, Roos MT, *et al.*: **Biphasic rate of CD4+ cell count decline during progression to AIDS correlates with HIV-1 phenotype.** *AIDS* 1992, **6**:665–669.
19. Schellekens PTA, Koot M, Roos MTL, Tersmette M, Miedema F: **Immunologic and virologic markers determining progression to AIDS.** J *Acquir Immune Defic Syndr* 1995, **10** (suppl 2):S62–S66.
20. D'Aquila RT, Johnson VA, Welles SL, *et al.*: **Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy.** *Ann Intern Med* 1995, **122**:401–408.
21. Karlsson A, Parsmyr K, Sandstrom E, Fenyö EM, Albert J: **MT-2 cell tropism as prognostic marker for disease progression in human immunodeficiency virus type-1 infection.** *J Clin Microbiol* 1994, **32**:364–370.
22. Boucher CA, Lange JM, Miedema FF, *et al.*: **HIV-1 biological phenotype and the development of zidovudine resistance in relation to disease progression in asymptomatic individuals during treatment.** *AIDS* 1992, **6**:1259–1264.
23. el Farrash MA, Kuroda MJ, Kitazaki T, *et al.*: **Generation and characterization of a human immunodeficiency virus type 1 (HIV-1) mutant resistant to an HIV-1 protease inhibitor.** *J Virol* 1994, **68**:233–239.
24. Eberle J, Bechowsky B, Rose D, *et al.:* **Resistance of HIV type 1 to proteinase inhibitor Ro 31-8959.** *AIDS Res Hum Retrovirus* 1995, **11**:671–676.
25. Markowitz M, Mo H, Kempf DJ, *et al.*: **Selection and analysis of human immunodeficiency virus type 1 variants with increased resistance to ABT-538, a novel protease inhibitor.** *J Virol* 1995, **69**:701–706.
26. Jacobsen H, Hänggi M, Ott M, *et al.*: ***In vitro* resistant to a immunodeficiency virus type 1 proteinase inhibitor: mutations, kinetics, and frequencies.** *J Infect Dis* 1996, **173**:1379–1387.