AIDS Rev 1999; 1: 147-155

Heterogeneity in the Response to Antiretroviral Therapy

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Abstract

The dynamics of viral suppression and the quality of immune reconstitution after initiation of potent antiretroviral therapy may vary considerably from person to person. The heterogeneity of response reflects a summation of viral and host factors. Thus, analysis of the different responses may provide clues on the biology of viral replication under the constrains of drug pressure, or on critical aspects of the mechanisms of CD4 T-cell depletion. On the clinical side, decisions on treatment switch or discontinuation might need to take into account unexpected benefits from suboptimal therapy. There is also an urgent need for understanding the phenomenon of progressive immunosuppression after exhausting all available treatment combinations.

Key words

Antiretroviral therapy. HAART. Drug resistance. Viral fitness.

Introduction

Clinicians have been confronted with a diversity of responses to potent antiretroviral therapy (Fig. 1). These responses include not only the optimal suppression of HIV-1 viremia and continuous increase of CD4 T-cell count under optimal therapy, but also various manifestations of treatment failure[1].

Treatment failure may present with depletion of CD4+ cells following rebound of viremia. However, the diversity of responses also includes a paradoxical failure to reconstitute the immune system despite successful suppression of viremia, and instances of increase in CD4+ cell count in spite of significant viremia. The latter form of discordant response occurs more frequently than the former and may constitute a common response in a substantial number of patients in whom potent antiretroviral therapy is considered to be failing[2-5]. Recently, we have recognized a fifth pattern of response: That of delayed viral suppression in individuals receiving suboptimal therapy (Fig. 1E).

This heterogeneity of response has clinical as well as biological relevance. It demands a detailed analysis of the influence of drug resistance on the physiology of the virus, as well as a reassessment of immunological response and recovery in situations where a *de novo* equilibrium between ongoing viral replication and CD4 depletion/production has been established.

In this article we review existing knowledge on drug resistance and viral fitness as well as emerging data on the immune recovery under conditions of incomplete viral replication. Importantly, we address the critical issue of progression of immune damage in individuals that have exhausted all available antiretroviral agents -described herein as «advanced treatment failure»-. These issues are of increasing relevance in view of the prevalence of multidrug resistance.

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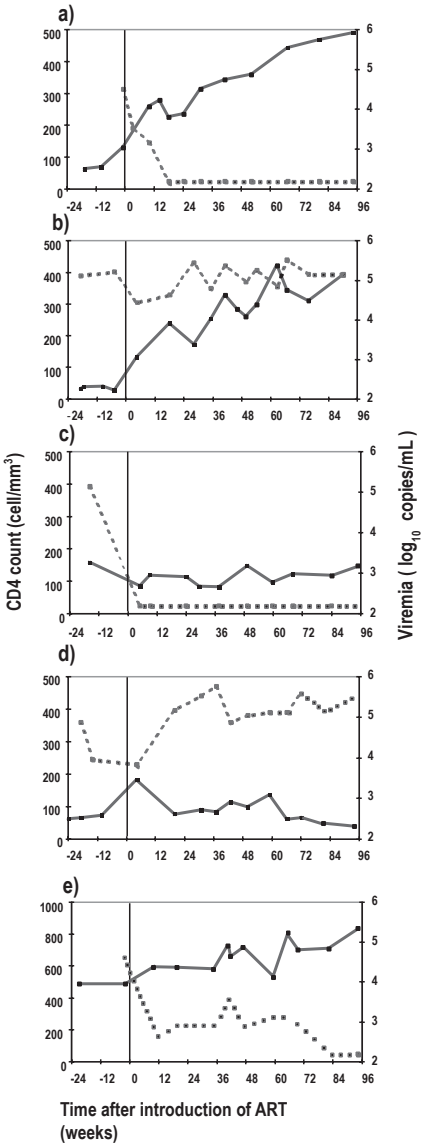

Fig.1. *Heterogeneity in the response to antiretroviral therapy. (A) Optimal virological and CD4 cell response, (B) discordant response with CD4 cell elevation despite uncontrolled viremia, (C) a discordant response with a blunted CD4 cell increase despite optimal viral suppression, (D) advanced treatment failure, (E) delayed viremia control in the presence of suboptimal therapy (NRTI bitherapy). Solid line: CD4 T cells, dotted line: viremia. (Modified from 1 with permission).*

Drug resistance and diminished viral fitness

Transient or partial reduction of viral load may be sufficient to allow cell proliferation or to decrease depletion rates, thus allowing a stabilization of CD4 T-cells at a higher level. This mechanism is widely accepted as central to the observation of clinical and immune stability following failure of potent antiretroviral therapy[2-5]. What this reasoning does not solve is the underlying mechanism explaining such partial reduction of viremia. Changes in infectivity, replication, or cell tropism -globally referred to as «viral fitness»- have to be invoked as potential mechanisms, as well as the possibility that moderate reductions in viremia should allow a certain degree of enhanced immune control.

Viral fitness. Methods for characterization of viral fitness have included comparisons of catalytic activities of mutant and sensitive viral enzymes, analysis of infectivity, replicative kinetic analyses, and detailed *in vitro* competition between two different virus variants mixed in a single culture or, *in vivo*, by observing the patterns of wild type regrowth in patients discontinuing antiretroviral therapy.

Modelling and kinetic analysis of consecutive proteolytic cleavages of the *gag-pol* polyprotein would suggest that HIV would cease being viable when the efficiency of a mutant protease is less than 61% of the wild type activity for each cleavage[6]. Thus, it has been proposed that convergent treatment with combined antiretroviral therapy would have limiting effect on processing, and result in growth kinetics sufficiently compromised to allow for sustained clinical benefits[7].

Most published experience (Table 1) indicates that viral fitness will be modified upon development of resistance. Mutations to NRTI and PI drugs generally involve changes of enzyme active site residues. This results in measurable changes in catalytic or processive efficiency, and for specific mutants, changes in infectivity due to accumulation of immature viral particles, and moderate to marked effects on viral replication (Table 1).

Given the remarkable plasticity of the HIV genome, selection of compensatory mutations takes place. This process involves structurally relevant substitutions (e.g., in the hinge or flap regions of the viral protease)[8], in enzyme substrate (e.g. *gag* cleavage sites)[7], or at as yet unrecognized sites elsewhere in the genome. *In vitro* data indicate that the pathways of compensation of fitness may not provide an immediate or full remediation of viral fitness deficits[9].

Most experiments have employed molecular clones constructed by site directed mutagenesis or by insertion or recombination of mutant viral cassettes (Table 1), and it must be emphasized that the environment of the viral background, T-cell line, and culture conditions used can be extrapolated to other situations only with great caution[10].

Our own experience with actual clinical isolates suggests that many multidrug-resistant strains have indeed a phenotype of diminished infectivity and replication, and that despite the observed reduction in fitness, there is medium term stability of multimutated genomes *in vitro* in the absence of drug selective pressure (Bleiber G, Telenti A, unpublished). This has been explained by a significant interlocking of primary and compensatory mutations and by the difficulties of back-tracking through less fit genotypes (e.g., reversion lamivudine-selected 184V to M184, through the unfit intermediate 184I)[11]. In the clinical setting, discontinuation of therapy may result, in up to two third of patients failing therapy, in rapid re-growth of wild type viral variants rather than actual reversion of mutated genomes to wild type.

All the above considerations should take into ac-

Table 1. *Representative studies on the influence of drug-resistance mutations on viral properties.*

Author, year publication (ref)	Virus (genotype)	Antiretroviral resistance	Cell type	Replication kinetics	Enzyme function	Comments
RT mutants						
Back '96 '97[11,30]	HXB2 (184V,I)	Lamivudine	SupT1, PBMCs	Replication WT > 184V (0.5 log) >184I (1 log). Differences accentuated in PBMCs.	RT activity: WT > 184V (35-67%) >184I (28-53%).	3TC-resistant viruses are sensitive to reduction of the intracelullar dNTP pool (i.e. primary cells, hydroxyurea).
Sharma '97[31]	NL4-3 (74V)	Didanosine	PBMCs	Replication WT>>74V (4-fold decrease). 11% loss of fitness of 74V in competition assays.	40-50% decrease in infectivity per unit of p24 or RT.	Unstable mutation, reversion possible.
Rayner '97[13]	HXB2, RF (100I)	DMP266 (NNRTI)	MT-2	Subtle difference in kinetics and competition		
Harrigan '98[10]	HXB2 with single or combination of mutation in codons 41,70,251	Zidovudine	MT-4	Competition assays: WT>70R(97%)>>215Y±41L (85%)>41L(80%).		
Caliendo '96[32]	HXB2 (67, 70, 215, 219)	Zidovudine	PBMCs	No differences in unstimulated cells. 2-6-fold higher p24 production for the mutant after mitogen stimulation.	Mutant RT exhibited 5-10-fold greater synthesis of high molecular weight DNA in a wide range of dNTP concentrations.	
Jellinger '97[33]	HXB2 (215 ± 41) or single or combination of mutations in codons 74, 77, 116, 151	Multinucleoside resistance	MT-2	Cytopathic effect. WT = 215 ± 41 = 74 > 151 = 75 + 77 ± 116		
Iversen '96[34] Maeda '98[35] Kosalaraksa '99[36]	HXB2 or NL4-3 with single or combination of mutations in codons 62, 75, 77, 116, 151, 215	Multinucleoside resistance	H9, HeLa CD4, MT-2, PBMCs, SubT1, H9, HeLa-CD4	Competition: (62, 75, 77, 116, 151) > (77, 116, 151) > (151) > WT > (75, 77, 116, 151) > (151, 215) > (215). Putative intermediate clones 151L and 151K were less fit than WT or 151M. Replication: WT @single or mutation constructs with the possible exception of (77), (77, 151) and (77, 116, 151).		
Schmit '96 [4]	Consecutive viral isolates from one patient. Isolate #1 (WT), #4 (68, 75, 77, 116, 151, 178, 202) and #6 (68, 70, 75, 77, 103, 116, 151, 178, 202)	Multinucleoside resistance + NNRTI (loviride).	PBMCs, C8166	Similar rates of replication WT and mutants. Competition analysis: Isolate n.4 > WT > no.6		Moderate increase in viral infectivity of resistant isolates. High viral load *in vivo*.
Goudsmit '97[37]	Plasma virions (41L + 215Y).	Zidovudine	*In vivo* viral dynamics off treatment.	215S > 215D (99%)>>215Y (75-90%)		Spontaneous reversion to more fit. 215D, S after transmission of AZT.

Table 1. *Representative studies on the influence of drug-resistance mutations on viral properties.*

Author, year publication (ref)	Virus (genotype)	Antiretroviral resistance	Cell type	Replication kinetics	Enzyme function	Comments
PR mutants						
Rayner '97[13]	HXB2, RF (84V).	DMP450	MT-2	Marked decrease in competition.	3-fold increase in unprocessed Pr55Gag polyprotein.	
Doyon '96[7] Croteau'97[38] Zhang'97[39] Carrillo'98[40]	NL4-3 (multimutated PR ± p7/p1/p6 cleavage site mutation).	BILA1906BS, BILA218BS, ABT-378, other PI	C 8166 T-cells, PBMCs, MT-2, MT-4, CEM	Markedly reduced replication for clones with 2 active site mutations. Other patterns may result from inability to grow without cleavage site compensation, to a 2 log diminished growht with compensator y p7/p1 or p1/p6 mutation. 46L+I54V+82A+p7/p1 431A+p1/p6 449F increase replication to 92% of WT.	From 42-fold decrease in PR catalytic efficiency (32I) to 2200-fold (32I,46I,71V,84A). 10-fold increase in mutant PR catalytic efficiency towards mutated cleavage site peptides.	No short term reversion of multimutated PR. No reversion of 84V mutants after 7-10 passages. 50% reversion 84A->V. Molecular clones from high-level ABT-378 resistant viruses were <u>not</u> infectious for various cell types in the presence of wild type p7/p1/p6 or only a p1/p6 L449F.
Zennou'98[41]	NL4-3 with cassettes from isolate resistant to saquinavir (46,48,90) or ritonavir multimutated PR.	Ritonavir Saquinavir	MT-4, HU578	Moderate to marked differences in kinetics normalized by p24 Ag.	Various degrees of polyprotein processing defects.	Clone-dependent decrease in infectivity from 2-to > 4-fold.
Martinez-Picado;99[42]	NLA4-3 (30±63P), (90±63P), (multimutated inc. simultaneous) 82/84 without p7/p1/p6 mutation.		MT-2, PBMCs	30N replication defect partially compensated by 63P; 90M minimal defect compensated by 63P; Multimutated cassette displayed no replication defect or competitive disadvantage.		Infectivity patterns parallel replication defects. Stability of multimutated clones.
RT/PR mutants						
Carron de la Carrière '99[43]	NL4-3 (multimutated PR ± site directed RT 41L, 215Y ± 184V).	Various	MT-4	AZT mutations partially rescued replication defect of PR mutant clones.	Mutant PR decreases RT activity due to poor Pol processing.	2-fold increase in AZT susceptibility of RT mutants in the presence of a mutant PR.
Miller, unpublished	Plasma virions after discontinuation of failing therapy.	Various	*In vivo* viral dynamics	67.7% shift to wild type phenotype.		Shift to susceptible phenotype was accompanied by increase in viremia vs. persistance of resistant viruses (0.98 vs. 0.34 log10 copies), and greater loss of CD4 count (-122 vs. -29 cells/mL).
Bleiber & Kaufmann, unpublished.	Clinical isolates, NL4-3 (multimutated RT ± multimutated PR).	Various Various	Ghost, CEM, PBMCs.	Various degrees of reduction in viral fitness among clinical isolates. Significant decrease in replication or PR mutant clones partially rescued by cognate mutant RT.		2- to > 4-fold decrease in infectivity among MDR clinical isolates. No reversion of mutations after 50-100 viral cycles in drug-free media.

count that some mutation pathways, such as the 151Q multinucleoside resistance, render viruses with apparently conserved fitness (Table 1), and that some individuals may harbour aggressive MDR-HIV strains with extreme values of peripheral viral load, and upon *in vitro* testing, fully fit isolates[12]. Resistance to non nucleoside reverse transcriptase inhibitors (NNRTI) appears to modify the viral physiology minimally[13,14]. This probably is a reflection of the drug binding away from the RT active site[15], and explains the occasional identification of NNRTI resistance mutations as natural polymorphisms[16].

Altered Cellular Tropism. Alternative hypotheses for the apparent reduced ability of MDR-HIV to deplete CD4 T-cells take into account the possibilities of (i) a shift in chemokine receptor usage, (ii) modification in the pathogenic potential of MDR-HIV on thymic precursors, and (iii) decrease in by-standing damage of HIV protease to cellular substrates.

We have recently analysed the first issue, that the emerging MDR virus would have switched from the more aggressive dual-chemokine receptor CCR5/CCXR4 to a non-syncytia-inducing CCR5 virus. Analysis of isolates from patients presenting various responses to potent antiretroviral therapy did not present differences in the distribution of receptor usage phenotypes, nor changes in the pre- and post-therapy charge of their *env* V3-loops[12].

In their analysis of the ability of multiresistant viruses to inhibit T-cell regeneration, Stoddart *et al.*[17] reported that while both wild-type and MDR viruses are capable of depleting mature CD4 T-cells *in vitro*, they displayed a different pathogenic potential on thymic precursors. In a SCID-hu mouse (a mouse containing human thymic tissue), only the wild-type virus caused productive infection and depletion of CD4 T-cells in the organ. Given the increasingly recognized role of the thymus in the pathogenesis of HIV disease[18], even minor changes in susceptibility of the thymus to particular virus mutants could result in greater CD4 cell production.

HIV protease may also play a role in the HIV-induced cytopathic effect both in tissue culture and *in vivo*. A viral protease with a T26S active site mutation was shown to have a reduced cleavage activity for cytoskeletal proteins *in vitro*[19] thus limiting the protease-mediated by-stander cytopathic effect. Whether the mutations implicated in clinical drug resistance lead to similar change in protease-induced cell damage has not been investigated.

Are there additional effects of antiretroviral therapy?

PIs could induce a sustained CD4 T-cell response through mechanisms independent of their direct antiviral activity. Despite the high degree of specificity of PIs for the viral protease, there has been some interest in identifying potential activities of PI against eukariotic targets. Until present, there have been reports on inhibition of cellular proteasome function, and on the inhibition of secreated aspartyl proteases of *Candida*, by PIs. There is controversial data on the potential for inhibition by PIs of cellular proteases implicated in apoptosis.

A recent study by André *et al*[20] suggests that protease inhibitors may, by inhibiting proteasome function and antigen processing, modify HIV-specific cytotoxic T-lymphocyte (CTL) activity. In the study, ritonavir, and to a lesser extent saquinavir, reduced MHC-1-restricted antigen presentation in mice infected with the lymphocytic choriomeningitis virus, and thus diminished CTL-mediated antiviral response and tissue damage.

PIs are *in vitro* inhibitors of secretory aspartic proteases SAP 1-3 of *Candida albicans*[21]. SAPs are virulence factors participating in mucosal adherence. In particular, ritonavir and saquinavir were found to inhibit adherence of *C. albicans* to Vero cells in a standard adherence assay. Whether these *in vitro* observations have any bearing on the diminution of episodes of candidiasis in patients receiving potent antiretroviral therapy remains speculative.

No direct effect of aspartyl protease inhibitors on apoptosis pathways (cysteinyl-aspartic proteases) has been shown. While apoptosis in lymphocytes from HIV-infected individuals is reduced following suppression of viral replication, no effect of PIs could be demonstrated for lymphocytes from non-infected subjects[22], and PIs did not directly inhibit apoptosis by acting on proteases implicated in programmed death (Borner C, Telenti A, unpublished). Our work used saquinavir at concentrations of up to 1 μM, and included analysis of DNA fragmentation, nuclear condensation/fragmentation, cytochrome C release, caspase-3 activation, cellular morphology, trypan blue exclusion and determination of live/dead cells by the LIVE/DEAD fluorescence kit of Molecular Probes. However, a recent publication by Sloand *et al*[23] describes a markedly decrease in CD4 cell apoptosis and interleukin-1ß converting enzyme (ICE, caspase 1) upon exposure of stimulated and unstimulated CD4 T-cells from HIV-infected and from non-infected donors to ritonavir.

Immune parameters under conditions of treatment failure

Immune recovery under conditions of persistent viremia could be explained by a relative improvment of CD4 cell production and a diminished rates of peripheral destruction. Deeks *et al*[24] reported a prolonged survival of CD4 T-cells generated under conditions of persistent viremia under treatment, compared with CD4 T-cells in patients with similar degrees of viremia who are not taking a PI-containing regimen.

However, does an increase in CD4 T-cells equal a potential for immune reconstitution? Table 2 includes a comparison of a patient with controlled viremia and wild-type virus, with a patient with significant viremia and multidrug-resistant virus. In the

Table 2. *Comparison of evolutionary parameters in two patients differing in type of viral drug resistance profile.*

	Patient 1 Wild type HIV	Patient 2 MDR-HIV
Baseline CD4 (cells/mm^3)	37	28
Baseline CD8 (cells/ mm^3)	730	204
Baseline viremia (copies/mL)	340244	169454
Current CD4 (cells/ mm^3)	438	424
Current CD8 (cells/ mm^3)	1238	992
Current viremia (copies/mL)	< 400	70600
Naïve CD4 T cells (% CD45RA+)	30	59,3
Memory CD4 T cells (% CD45RO+)	36	25,5
CD8 T-cells expressing CD28 (%)	50,1	47,5
CD4 T-cells in cycle (% Ki67+)	2,69	1,35
CD8 T-cells in cycle (% Ki67+)	0,67	0,77
IFNγ-producing CD4 cells (%)	18	21
IFNγ-producing CD4 memory cells (%)	24	34
IFNγ-producing CD8 cells (%)	58	53
IFNγ-producing CD8 memory cells (%)	42	48
Lymphoproliferative response to specific antigens (number of positive responses)	2/6	3/7

context of parallel increases in CD4 T-cell counts, comparable percentages of naïve CD4+ cells and cells in cycle (Ki67+) were found. Overall, an increase in naïve CD4 T-cells, as well as partial functional recovery of lymphoproliferative response to anti-CD3, PHA, and specific antigens can be observed both in patients with optimal suppression of viremia and among those with lesser degree of viremia control[5,12].

Advanced treatment failure

Despite the stability observed in clinical cohorts since the availability of potent antiretroviral therapies[25], there are patients that experience progressive immunosuppression after exhausting all therapeutic options.

Among patients receiving antiretroviral therapy, those with poor control of viremia and minor benefit on CD4 T-cells bear the burden of opportunistic disease today. In the Swiss HIV Cohort Study, the cumulative incidence of opportunistic diseases at 30 months after initiation of therapy was 16.6% among participants with 200 cells/mm^3 or fewer CD4 and detectable viremia at 6 months, whereas rates of 5% or less were observed among patients with CD4 T-cells above 200 cells/mm^3 or with viremia below the level of detection at 6 months[26].

There has been limited work devoted to the particular issue of advanced treatment failure. Our own data indicates that patients with aggressive, advanced virological and immunological failure generally display a high peripheral viral load despite of adequate adherence to treatment and a profound damage to the immune system and manifested by absence of naïve CD4+ cells, and low numbers of cells in cycle as measured by expression of Ki67+[12]. Viral isolates from these patients are comparatively more infectious and replicative *in vitro* than isolates from patients with virological failure and stable clinical course.

An additional concern relates to the future of patients currently stable despite poorly suppressed viral replication. The precarious nature of a clinical stability that depends on viral fitness and on theoretical bottlenecks in viral evolution,[27] could give way to renewed disease progression.

Unusual patterns of successful viral suppression

A limited number of patients (< 5%) with successful control of viremia present unusual patterns of response to antiretroviral therapy. In a first instance, optimal viral suppression does not result in a substantial recovery of CD4 T-cell count (Fig.1C), and patients may remain at significant risk for opportunistic events. In the Swiss HIV Cohort Study, 42 of 2410 patients who started potent antiretroviral therapy at very advanced state of immunosuppression (< 100 CD4 cells/mm^3), achieved and maintained undetectable viremia but failed to increase the CD4 T-cell count (< 50 cell/mm^3 increase). At two years of follow-up, progression rate to a new opportunistic disease was very high (29% rate).

In a second model of response, patients receiving suboptimal therapy (NRTI bitherapy, or sequen-

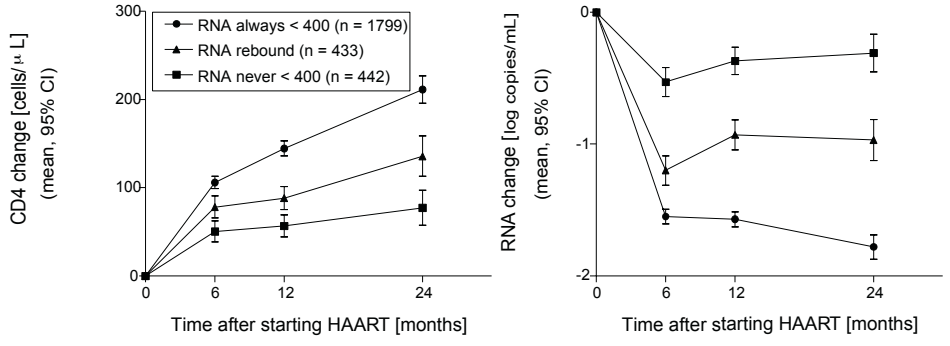

Fig. 2. *Changes in CD4 T cell count and plasma viremia according to magnitude of control of viremia in patients receiving potent antiretroviral therapy in the Swiss cohort.*

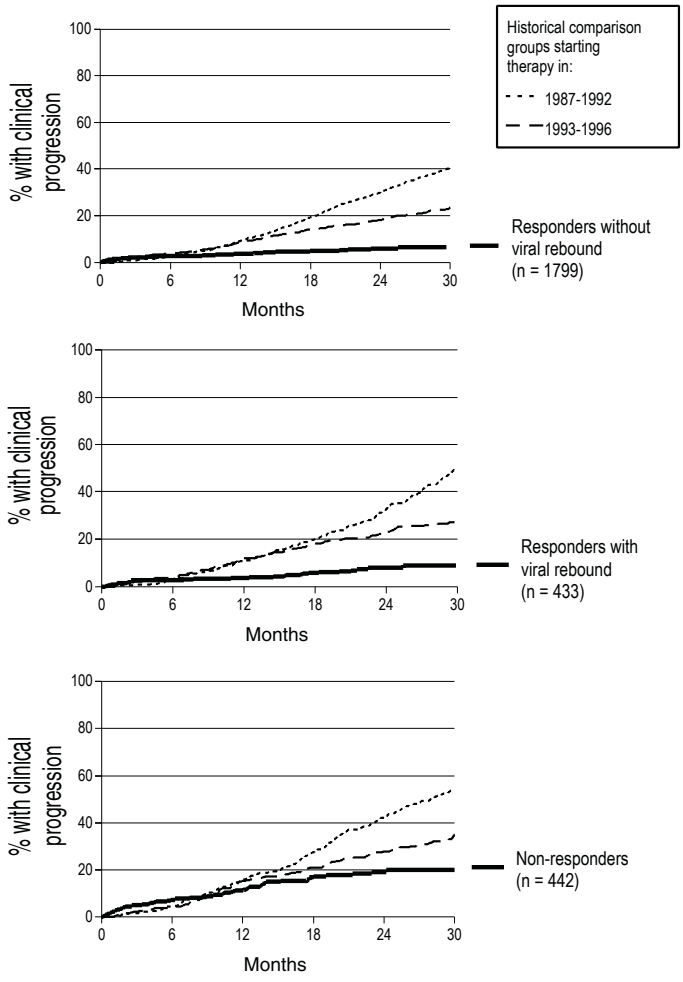

Fig. 3. *Rates of progression to death or opportunistic disease according to prior control of viremia in the Swiss cohort compared with rates in patients with similar control of viremia treated in years prior to availability of potent antiretroviral regimens. (Adapted from[25] with permission).*

tial addition of PI to a regimen) achieved optimal viral suppression only after a prolonged period, i.e. after more than 1 year (Fig. 1E).

Little is known about the immunological bases for these particular evolutions. In the first situation, the possibility of ongoing immune damage by viral products[28] or other factors needs to be considered. In the second pattern, there could be a role for CTL response in the late viral control. Further research should provide valuable data on these basic issues.

Clinical relevance of heterogeneous responses

The sustained CD4 T-cell benefit in patients with rebound or persistent viremia during PI therapy (Fig. 2) appears to predict a sustained clinical benefit. Two recent analyses of the Swiss HIV Cohort Study allowed quantification of the positive effect of potent antiretroviral therapy even in the absence of optimal control of viremia. A first study[25], demonstrated that in comparison with matched historical controls, clinical progression was rare over two years of follow-up, even in patients receiving potent antiretroviral therapy with evidence of ongoing viral replication (Fig. 3). A second analysis[26] quantified the immune benefit of potent antiretroviral therapy: An increase in the CD4 count of 50 cells/mm^3 after 6 months of treatment leads to a risk reduction of 70% for a new AIDS-defining event. Furthermore, reaching 200 cells/mm^3 at 6 months continues to distinguish between more profound and moderate immunodeficiency, as only a small percentage of all observed events developed above that threshold, even in the presence of incomplete viral suppression.

On a pragmatic standpoint, clinicians should consider several issues when evaluating a patient with increasing viremia but a stable CD4 T-cell count:

- Continuing therapy may represent the only alternative for patients with extensive exposure to antiretroviral and limited additional options.
- Whether failure under a non-nucleoside RT inhibitor-containing regimen (instead of PI) results in the same degree of stability is not established.

- Stopping therapy or poor adherence may result in progression of immunosuppression[2,29].

Continuing antiretroviral therapy in the face of virological failure may result in clinical benefit. However, continued pressure will almost certainly result in evolution of drug resistance and, most likely, increased viral fitness. Advanced treatment failure may thus become a major management problem. Decisions on therapy should be guided by the degree of immunosuppression, availability of new drug options, and judicious use of genotype/phenotype resistance data.

Conclusions

Heterogeneity in the response to antiretroviral therapy represents a clinical and research challenge. Relevant biological information may be drawn from the investigation of different patterns of response, in particular on viral fitness and evolution, and on immune reconstitution. Whether the available *in vitro* and theoretical data justifies the notion that less viral fitness contributes to a measurable clinical benefit remains highly controversial.

Acknowledgements

We thank Martínez R, Peters S, and Meylan P for assistance and helpful commentaries. Work in our laboratory is funded by the Swiss Federal Office of Public Health (Grant 97-7339) and by the Santos Suárez Foundation.

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