Changes in Cellular Virus Load and Zidovudine Resistance of Syncytium-Inducing and Non-Syncytium-Inducing Human Immunodeficiency Virus Populations under Zidovudine Pressure: A Clonal Analysis

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Zidovudine treatment preferentially benefits persons with only non-syncytium-inducing (NSI) human immunodeficiency virus type 1 (HIV-1) variants. To understand this differential efficacy, changes in cellular virus load, clonal composition of HIV-1 populations, and development of resistance-conferring reverse transcriptase mutations were studied in 17 persons initiating zidovudine therapy. Zidovudine treatment resulted in larger and more sustained decreases in cellular virus load in persons with NSI variants only compared with persons also carrying syncytium-inducing (SI) variants. Although the former group had a delayed emergence of resistance mutations, differences in initial responses between the 2 groups were independent of the emergence of resistance mutations. Changes in virus load in subjects also carrying SI variants were due mainly to loss of coexisting NSI virus. Resistance mutations emerged at similar rates in both coexisting variants. Data suggest that mechanisms other than drug resistance are necessary to completely explain the phenotype-dependent benefit of zidovudine.

The early asymptomatic phase of human immunodeficiency virus type 1 (HIV-1) infection is characterized by a low prevalence of productively infected cells carrying non-syncytium-inducing (NSI), preferentially macrophage-tropic variants. With progression of disease, an increase in cellular virus load due to an expansion of preferentially T cell-tropic variants is seen in ~50% of infected persons in association with the emergence of syncytium-inducing (SI) variants [1, 2]. The presence of SI variants is associated with more rapid progression to AIDS than is the presence of NSI variants only [3]. Of interest, zidovudine (3'azido-3'deoxythymidine) treatment significantly reduces the speed of disease progression in persons with NSI variants only but not in those with SI variants [4]. The mechanism for this viral phenotype-dependent efficacy of zidovudine treatment is not known.

Another determinant for the clinical outcome of zidovudine treatment is the development of zidovudine resistance [5], which is conferred by specific mutations in the reverse transcriptase (RT) gene that result in the following amino acid substitutions: Met41→Leu, Asp67→Asn, Lys70→Arg, Thr215→Tyr/Phe, and Lys219→Gln [6, 7]. Decreased in vitro zidovudine sensitivity of virus isolates is associated with high levels of viremia [8] and poor clinical outcome [5]. We previously observed a trend toward an increased prevalence of the combined presence of mutations at codons 215 and 41 in SI isolates compared with NSI isolates from zidovudine-treated subjects [9]. In the current study, we investigated whether differences in development of resistance may contribute to the observed viral phenotype-dependent efficacy of zidovudine treatment.

Patients and Methods

Ten asymptomatic HIV-1-infected participants from the Amsterdam Cohort Studies of HIV infection and AIDS in homosexual men who were included in a previously described zidovudine study [9] were studied for 6 months before and up to 84 months after the start of treatment (table 1). Seven other participants from the Amsterdam Cohort Studies started zidovudine treatment between July and December 1993 and were followed for 12 months (table 1). Peripheral blood mononuclear cells (PBMC) were obtained from all 17 persons (group A) at sequential time points before and after initiation of treatment and analyzed. Determination of the

Received 14 December 1995; revised 17 May 1996.


This study is part of the Amsterdam Cohort Studies on AIDS, a collaboration between the Municipal Health Service, Academic Medical Centre, and Central Laboratory of the Netherlands Red Cross Blood Transfusion Service. Written informed consent was obtained from all participants. In the conduct of clinical research, human experimentation guidelines of the authors’ institutions were followed.

Grant support: Netherlands Ministry of Public Health; Netherlands Foundation for Preventive Medicine.

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Table 1. Characteristics of 17 zidovudine-treated subjects.

<table>
<thead>
<tr>
<th>Baseline phenotype,* subject (mg/day)</th>
<th>Zidovudine cell count</th>
<th>Baseline Cellular virus load</th>
<th>AIDS</th>
<th>AIDS-defining illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>Zidovudine (mg/day)</td>
<td>Baseline CD4 (10^3/mm³)</td>
<td>1 month</td>
<td>3 months</td>
</tr>
<tr>
<td>SI</td>
<td>8</td>
<td>1200</td>
<td>430</td>
<td>193</td>
</tr>
<tr>
<td>SI</td>
<td>30</td>
<td>1200</td>
<td>170</td>
<td>250</td>
</tr>
<tr>
<td>SI</td>
<td>105</td>
<td>1000</td>
<td>190</td>
<td>390</td>
</tr>
<tr>
<td>SI</td>
<td>199</td>
<td>2000</td>
<td>190</td>
<td>61</td>
</tr>
<tr>
<td>SI</td>
<td>316</td>
<td>1000</td>
<td>240</td>
<td>284</td>
</tr>
<tr>
<td>SI</td>
<td>545</td>
<td>1200</td>
<td>450</td>
<td>292</td>
</tr>
<tr>
<td>SI</td>
<td>6092</td>
<td>1200</td>
<td>320</td>
<td>203</td>
</tr>
<tr>
<td>SI</td>
<td>6116</td>
<td>1200</td>
<td>250</td>
<td>426</td>
</tr>
<tr>
<td>NSI</td>
<td>53</td>
<td>1000</td>
<td>420</td>
<td>26</td>
</tr>
<tr>
<td>NSI</td>
<td>107</td>
<td>2000</td>
<td>400</td>
<td>93</td>
</tr>
<tr>
<td>NSI</td>
<td>181</td>
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<td>340</td>
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<td>NSI</td>
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<tr>
<td>NSI</td>
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<td>1000</td>
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</tr>
<tr>
<td>NSI</td>
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<td>2000</td>
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<tr>
<td>NSI</td>
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<td>1200</td>
<td>90</td>
<td>1509</td>
</tr>
<tr>
<td>NSI</td>
<td>6118</td>
<td>1200</td>
<td>400</td>
<td>230</td>
</tr>
<tr>
<td>NSI</td>
<td>346</td>
<td>1000</td>
<td>290</td>
<td>239</td>
</tr>
</tbody>
</table>

**NOTE.** PCP = *Pneumocystis carinii* pneumonia; TXC = cerebral toxoplasmosis; CMVR = cytomegalovirus retinitis; CE = *Candida* esophagitis; NT = not tested.

* Determined on MT-2 cells. Subjects ACH105, ACH199, and ACH316 harbored SI viruses for at least 23, 4, and 1 month, respectively. SI viruses were first detected in ACH346 7 months after initiation of therapy.

† HIV-1 TCIDs/10^6 CD4 cells.

‡ % change from baseline.

§ Months after initiation of therapy.

∥ No AIDS-defining illness during 12 months of zidovudine treatment.

§§ During first 4 weeks of therapy, different dose regimens were used in these subjects; thereafter, they received 1000 mg of zidovudine daily.

** No AIDS-defining illness during 84 months of zidovudine treatment.

The proportion of infected cells and isolation of biologic virus clones was done as described [1, 2]. About 10 biologic clones for each viral phenotype were randomly selected at each time point for use in further analyses. The presence of RT mutations at codons 41 (Met→Leu) and 215 (Thr→Tyr) was analyzed with a selective polymerase chain reaction procedure as described previously [10].

Group B consisted of 6 untreated asymptomatic participants of the Amsterdam Cohort Studies who were known to have been infected with SI HIV-1 variants for <1 to >41 months (median, 20). PBMC from this group were used to compare preexisting zidovudine sensitivity of coexisting SI and NSI variants. Biologic virus clones from patient PBMC were isolated as described [1, 2] but in the presence of increasing concentrations of zidovudine (range, 0–2.5 µg/mL). Before initiation of the cocultures with patient PBMC, phytohemagglutinin-stimulated PBMC from healthy blood donor volunteers were incubated with increasing zidovudine concentrations at 37°C. After 1 h, patient PBMC were added, and cultures were maintained as described [1, 2]. Each week, a new dose of zidovudine was added together with fresh medium and phytohemagglutinin-stimulated healthy donor PBMC. After 3–4 weeks, the number of cultures showing evidence of p24 production and the number with MT2-tropic (i.e., SI) viruses were scored for each concentration of zidovudine.

The Mann-Whitney rank sum test was used to compare the virus loads of the groups. The Wilcoxon signed ranks test was used to compare the responses of SI and NSI variants to zidovudine in vitro.

**Results**

**Effect of zidovudine treatment on cellular virus load.**

Changes in cellular virus load in the 17 group A subjects during the first 15 months of zidovudine treatment are shown in table 1. Virus load and CD4 cell counts at baseline did not differ significantly among subjects with NSI variants only and those also carrying SI variants (table 1, *P* = .172). The magnitude and duration of the decline in virus load were superior in persons with NSI variants only. The median changes at 1, 3, and 6 months for persons with NSI variants only were −82%, −60%, and −48%, respectively. The decreases in virus load were accompanied by increases in CD4 cell counts (data not shown). Within the group with NSI variants only, no clear differences in the responses to treatment were observed between individuals who progressed to AIDS and those who did not.

The Mann-Whitney rank sum test was used to compare the virus loads of the groups. The Wilcoxon signed ranks test was used to compare the responses of SI and NSI variants to zidovudine in vitro.
Given these results, we questioned whether coexisting NSI and SI variants also showed a differential response in virus load. For the 8 subjects with both SI and NSI variants, we analyzed the composition of clonal virus populations during the first 6 months of zidovudine treatment. The contribution of SI variants to the total virus load before the start of zidovudine treatment ranged from 5% to 89% (median, 67%). After the initiation of zidovudine, the NSI load in 6 of the 8 subjects decreased by -6% to -100% (median, -65%) of the baseline level, whereas the SI load decreased only slightly or increased up to 141% (median, 10%; range, -44% to 141%) after 6 weeks of treatment.

Kinetics of development of zidovudine resistance–conferring mutations. The emergence of RT mutations at codons 41 and 215 was analyzed in virus clones obtained from 7 members of group A (figure 1). In general, mutations appeared first at codon 215 and subsequently at codon 41 [9]. Both mutations were detected earlier in viruses from subjects with both NSI and SI variants than in those with NSI variants only, confirming our previous finding [9]. Of the virus clones (both SI and NSI) obtained from SI–carrying subjects, 83%–100% were mutant at codon 215 within 6 months of treatment, compared with at least 12 months in subjects with NSI variants only. The rate of acquisition of this mutation was very similar within each phenotype group. Similar differences between both groups were observed for the mutation at codon 41, although there was much more variation within each group.

No clear differences were observed between coexisting SI and NSI virus clones in the rate of appearance of mutations at codons 41 and 215 in 3 subjects (ACH105, ACH316, ACH346). In general, a lysine-to-arginine change at codon 70 is the first to emerge during zidovudine treatment, and differences in the rate of acquisition of this mutation may have been responsible for the observed differences in the early response to zidovudine. However, in these 3 persons, the codon 70 mutation could not be detected in either SI or NSI clones after 3 months of zidovudine treatment (data not shown).

In vitro zidovudine sensitivity in coexisting SI and NSI clones. Since we did not observe major differences between coexisting SI and NSI clones in the rate of acquisition of three important zidovudine resistance–conferring mutations, we questioned whether viral phenotype–related preexisting differences in zidovudine sensitivity could account for the differences in the variants’ responses to treatment. Using PBMC from 6 group B subjects who had both NSI and SI variants and who had not received zidovudine, we tested the in vitro zidovudine sensitivity of coexisting SI and NSI variants. In total, 15 different clones were isolated according to the protocol outlined in the Patients and Methods section. Without zidovudine, the percentage of SI clones varied from 20% to 90% (median, 58%) in the respective PBMC samples. The slopes of the decline of the number of virus-producing wells with increasing zidovudine concentrations were not significantly different for SI and NSI clones (NSI: median, -5.9; range, -91.1 to -0.8; SI: median, -4.6; range, -64.3 to 0.3, P = 0.11), thus excluding viral phenotype–related preexisting differences in zidovudine sensitivity.

Discussion

The development of zidovudine resistance is an important determinant for the clinical outcome of zidovudine treatment [5]. In addition, HIV-1–infected persons with only NSI variants receive more clinical benefit from zidovudine treatment than do subjects also carrying SI variants [4]. In agreement with a larger clinical benefit, in this study the declines in virus load after starting treatment were larger and lasted longer in persons with NSI variants only. Although we did not measure plasma viremia in all subjects, preliminary data show a correlation between plasma HIV-1 RNA and cellular virus load, indicating that similar differences in response of plasma HIV-1 RNA load may be expected (data not shown). The declines in virus load were accompanied by increases in the number of CD4 cells, which were more sustained in persons with NSI variants only.

Since viral phenotype–related differences in zidovudine efficacy may indirectly be based on differences in RT resistance, we investigated whether SI and NSI variants differ in the rate of development of zidovudine resistance mutations. Indeed, zidovudine resistance–conferring mutations emerged more slowly in viruses from individuals with NSI variants only. We obtained similar results when comparing the responses to lamivudine treatment in subjects with different viral phenotypes [11]. SI isolates have faster replication rates than virus isolates obtained from persons with NSI variants only. This may contribute to faster acquisition of mutations under zidovudine pressure. The equally rapid acquisition of resistance mutations by coexisting NSI and SI variants, as observed in our study, is in agreement with our observation that coexisting virus clones may have similar replication kinetics. Although SI variants did not seem to be affected much by zidovudine treatment, the equal kinetics of resistance development suggest that even a small advantage is apparently sufficient to establish the outgrowth of a mutant virus population [12].

While differences in the rate of resistance development were observed, several lines of evidence indicate the existence of differences in zidovudine efficacy that are independent of genomic resistance. First, the differences in initial responses between subjects with NSI variants only and those also carrying SI variants were observed in the absence of resistance mutations in either group. Similarly, the rebound of virus load after the initial decline was also preceded by the emergence of resistance mutations. This rebound may be explained by predator–prey dynamics, in which the increase in CD4 cells due to suppression of viral replication provides the virus with new target cells [13]. Infection of these cells by unsuppressed virus may result in increased replication and a rebound in virus load. Accordingly, the limited pressure of zidovudine on SI variants...
in combination with a more rapid replication of these variants may result in an accelerated rebound in virus load despite the moderate increase in the number of CD4 cells.

The most striking evidence for a viral phenotype-dependent, RT resistance-independent efficacy of zidovudine was provided by our clonal analysis of coexisting SI and NSI variants. This showed that the decline in virus load in subjects with both variants was mainly due to the loss of NSI virus. Declines in SI virus burden were either small or did not occur at all. In contrast, no differences between coexisting SI and NSI variants were observed in the rate of resistance development or in preexisting in vitro zidovudine susceptibilities. The latter observation has been confirmed using standard virus inocula (data not shown). The similar rates of resistance development indicate that the differential efficacy of zidovudine could also not be explained by a difference in replication rates between both variants. The fact that the viral phenotype-dependent differential activity of zidovudine appears independent of RT resistance is supported by the recent finding that zidovudine resistance and SI phenotype are independent predictors of disease progression during zidovudine treatment [5].

The mechanism of the differential activity of zidovudine remains unclear. Cells may display differences in intracellular pharmacology of zidovudine and, depending on the activation state of the cell, there may be differences in phosphorylation patterns and nucleotide pools [14]. This may lead to differences between cells in intracellular zidovudine concentrations. We recently demonstrated that SI variants have a broader tropism within the heterogeneous CD4 cell population than do NSI variants [15]. This might enable SI variants to selectively replicate in cells displaying cellular zidovudine resistance.

Currently, in vitro research is in progress in our laboratory to unravel the genomic resistance-independent phenotype-dependent differences in efficacy of zidovudine treatment in HIV-
I-infected persons. Our observations, in combination with clinical data showing that the biologic phenotype of HIV-1 is a predictor of disease progression under zidovudine pressure, independent of resistance-conferring mutations [5], suggest that these RT resistance-independent mechanisms may be relevant for the clinical outcome of zidovudine treatment.

Acknowledgments

We are indebted to all subjects for their continuous participation, to Marijke Roos and colleagues for excellent technical assistance, to Maarten Koot, Matthijs Tersmette, and Rob Schuurman for helpful discussions, and to Michel Klein, Ana Maria de Roda Husman, Hetty Blaak, and Frank Miedema for critically reading the manuscript.

References