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# HIV-1 Syncytium-Inducing Phenotype, Virus Burden, Codon 215 Reverse Transcriptase Mutation and CD4 Cell Decline in Zidovudine-Treated Patients

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**Summary:** The variable rate of disease progression in HIV-1-infected patients treated with zidovudine may be related to certain viral characteristics, such as, antiviral drug resistance, virus burden, and viral syncytium-inducing (SI) capacity. Thirty-two HIV-1-infected patients treated with zidovudine (mean of 34 months) were studied to determine the relationship of SI phenotype and the codon 215 *pol* gene mutation (a marker of zidovudine resistance) to virus burden and CD4 cell decline. Patients with SI strains and the codon 215 mutation in their proviral DNA had a 54% decline in CD4 cells and a virus burden of 21,480 proviral DNA copies/$10^6$ CD4 cells. In contrast, patients with non-SI (NSI) strains and wild-type at codon 215 had a 10% increase in CD4 cells and had a viral burden 1/46 that of patients with SI and the 215 mutation. Among patients with NSI strains, changes in CD4 cells depended on the presence of the codon 215 mutation ($-160$ CD4 cells/μl), compared with those wild-type at codon 215 ($+28$ CD4 cells/μl) ($p < 0.01$). There was a concordant rise in virus burden between proviral DNA and plasma HIV RNA depending on HIV phenotype and genotype. Using multiple linear regression, SI phenotype and the codon 215 mutation were found to independently predict CD4 cell decline and increased virus burden in zidovudine-treated patients. **Key Words:** Zidovudine resistance—Virus burden—Syncytium-inducing phenotype—HIV-1—$CD4^+$ T-cell decline.



It has been shown that multiple mutations in the HIV reverse transcriptase (RT) gene confer zidovudine resistance (1–4). A mutation resulting in an amino acid change from Thr to Tyr or Phe at codon 215 has been shown to cause a 16-fold decrease in HIV's susceptibility to zidovudine (2,3). In a previous report (5), the development of this mutation in proviral DNA in peripheral blood mononuclear cells (PBMCs) and viral RNA in patients on zidovudine monotherapy was found to be strongly associated with CD4 cell decline. Certain biological properties of HIV strains from HIV-infected patients can predict disease progression (6), specifically the ability of HIV strains to induce syncytia in the MT2 cell line. HIV isolates that induce syncytia (SI) as compared with non-syncytium-inducing (NSI) strains have a greater tropism and are more cytopathic in vitro for $CD4^+$ T lymphocytes (7,8). Terssmette and others (6,9–12) have shown a strong association between the emergence of SI isolates in HIV-infected patients and subsequent accelerated CD4 cell decline and disease progression.

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In HIV-infected patients, HIV biological phenotypes (6,9–12), zidovudine resistance (12–14), and increased virus burden (15) have each been associated with disease progression. However, no study as yet has addressed whether these viral characteristics are independent of each other or are dependent in their association with CD4 cell decline. In this study, HIV isolates obtained from patients in which the correlation between the codon 215 mutation and CD4 cell decline was reported (5) were examined for their HIV biological phenotype. Using multiple linear regression, the biological phenotype of the virus and the RT mutation at codon 215 were then compared to determine if these viral characteristics were independent of each other or dependent in their association with $CD4^+$ T-cell decline and virus burden. We also present how virus burden in both serum and PBMCs relates to SI phenotype and codon 215 RT genotype.

## PATIENTS AND METHODS

### Patients

Study subjects included 32 HIV-seropositive patients who participated in the AIDS Clinical Trial Group protocols 016 and 019 at Stanford University. Patients were selected from a previously described group (5) based on the availability of stored PBMCs. At study entry patients had not been previously treated and had >200 CD4 cells/μl. They were subsequently treated with zidovudine for 2–4 years with $CD4^+$ T-cell measurements performed about every 3 months. At the end of the study period, serum, plasma, and PBMCs were obtained for SI/NSI determination, proviral DNA and viral RNA quantification, and codon 215 RT genotypic analysis.

### HIV Biological Phenotype Analysis

To ensure the infectivity of HIV from the cryopreserved PBMCs, we created viral stocks by thawing the cryopreserved PBMCs and cocultivated them with $10^7$ phytohemagglutinin-stimulated PBMCs from healthy seronegative blood donors. Viral stocks were harvested when p24 antigen production exceeded 10 ng/ml. Two hundred microliters of viral stock supernatant (tissue culture median infective dose ~2000) were cultured with 8 ml of MT-2 cells (at a concentration of $0.5 \times 10^6$ cells/ml) in duplicate. Twice weekly, 80% of the culture (cells and supernatant) was replaced by culture medium (RPMI 1640 medium with L-glutamine supplemented with 10% heat-inactivated fetal bovine serum (Irvine Scientific, Santa Ana, CA, U.S.A.), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cultures were maintained for ≥3 weeks and were examined for syncytia twice a week as described by Koot et al. (8).

### Analysis of Codon 215 RT Gene

To determine the presence of a wild-type (amino acid Thr) or mutant (amino acid Tyr or Phe) sequence at codon 215 in proviral DNA from cryopreserved (−180°C) PBMCs or viral RNA from cryopreserved serum (−70°C) (after reverse transcription of RNA to c-DNA), duplicate samples were amplified in a nested polymerase chain reaction (PCR) assay, and then the product was scored as wild-type or mutant, as previously described (2,5, 16).

### Quantification of Plasma HIV RNA and Proviral DNA in PBMCs

To determine the number of proviral DNA copies present in $1 \times 10^6$ CD4 cells, duplicate 1-μg samples of PBMC DNA extracted from patients were amplified in a quantitative PCR assay (17); reaction conditions and methods have been previously described (8). The proviral DNA copy number per $10^6$ PBMCs was transformed to copies per $10^6$ CD4 cells based on the patient's CD4 cell count from the same time point.

Cryopreserved (−70°C) plasma samples were thawed, and then 0.1–0.5 ml of plasma was ultracentrifuged. The resulting pellet was dissolved in 400 μl 5 *M* guanidine thiocyanate. HIV RNA was purified and quantitated essentially as previously described (17,18). The RNA from duplicate samples was purified by phenol-chloroform extraction and alcohol precipitation. Each sample was reverse-transcribed along with a dilution series of purified RNA standard of known copy number. PCR amplification using the HIV *gag* gene primers SK38/39 was then performed for 33 cycles on the reverse-transcribed RNA samples, and the amount of product in each amplification was measured in a nonisotopic enzyme-hybridization assay (18). Results were then expressed as log RNA copies per milliliter of plasma.

### Statistics

The Student's *t* test was used for the analysis of changes during the study in $CD4^+$ T cells, proviral burden, and months of therapy from one group of patients to another. Fisher's exact test was used for the difference in the proportion of mutations in the SI and NSI groups. Multiple linear regression was used to analyze the point effect of several independent variables on CD4 cell decline and changes in viral burden.

## RESULTS

### Patients' Characteristics

Serum, plasma, and PBMCs were obtained from 32 patients who had been treated with zidovudine for a mean of 34 ± 8 months. The mean starting $CD4^+$ T-cell count for the patients was 388 ± 104 CD4 cells/μl. Over the 34-month treatment period there was a mean CD4 cell fall for the 32 patients of −100 ± 156 CD4 cells/μl. All the patients remained in the study, and none developed AIDS during this time period. Virus isolates from 12 of 32 (37%) patients were found to have SI strains. Nested PCR amplification of proviral DNA from PBMCs showed that 16 of 32 (50%) patients had a mutation of codon 215. Nested PCR after reverse transcription of serum HIV RNA confirmed that 21 of 32 patients had a codon 215 mutation; these patients included the 16 who had the mutation in their proviral DNA from PBMCs plus five others who had the mutation only in HIV virion RNA in serum.

### Codon 215 RT Genotype, Virus Burden, and CD4 Cell Changes

The initial CD4 cell counts at the start of zidovudine therapy were similar for patients mutant or wild-type at codon 215 in their PBMCs [404 ± 102 versus 374 ± 105 CD4 cells/μl ($p$ = NS)], as were their months on therapy (34 versus 33 months). Patients who developed a codon 215 mutation in their PBMCs had a significantly greater decline in their CD4 cell counts than the 16 patients remaining wild-type at codon 215, −194 ± 133 versus −8 ± 119 CD4 cells/μl, ($p < 0.01$) (Table 1). Patients with the 215 mutation also had a mean greater viral burden compared with those who remained wild-type—5,636 versus 698 HIV proviral DNA copies/$10^6$ CD4 cells ($p$ = 0.003). Plasma HIV RNA burden was also greater in patients with the mutation compared with those patients without it [131,830 versus 48,980 HIV plasma RNA copies/ml plasma ($p < 0.02$)].

Sixty-six percent of patients (21 of 32) were found to have the 215 mutation in their serum HIV RNA. The starting CD4 cell counts and the months of zidovudine therapy were similar for both groups, mutant (391 ± 98 CD4 cells/μl, 34 months of therapy) and wild-type (381 ± 122 CD4 cells/μl, 33 months of therapy) ($p$ = NS). Patients with the mutation in their serum had a significantly greater decline in their CD4 cell counts than did those patients with a wild-type sequence in their serum HIV RNA, −146 ± 161 versus −2.6 ± 91 CD4 cells/μl ($p$ = 0.003). Patients with the 215 mutation in their serum compared with those remaining wild-type had a trend toward a greater viral burden in their CD4 cells [3,162 versus 710 HIV proviral DNA copies/$10^6$ CD4 cells ($p$ = 0.06)] and in their plasma [104,700 versus 46,800 HIV RNA copies/ml plasma ($p$ = 0.08)].

### HIV Biological Phenotype Related to Virus Burden and CD4 Cell Decline

The starting CD4 cell counts (401 ± 121 versus 380 ± 95 CD4 cells/μl ($p$ = NS)) and the months of zidovudine therapy (32 versus 34 ($p$ = NS)) for patients with and without the SI phenotype were similar. Patients with the SI phenotype had a higher virus burden [5,495 versus 1,070 HIV proviral DNA copies/$10^6$ CD4 cells ($p$ = 0.03)] and a trend toward a greater fall in their CD4 cells [−156 ± 129 versus −66 ± 161 CD4 cells/μl ($p$ = 0.09)] than the 20 patients with the NSI phenotype (Table 1).

TABLE 1. *Differences in CD4 cells after 34 months of zidovudine therapy for 32 patients depending on HIV-1 biological phenotype and codon 215 proviral DNA genotype*

| | Codon 215 genotype | | | HIV biological phenotype | | |
|---|---|---|---|---|---|---|
| | Wild-type | Mutant | p[a] | NSI[b] | SI[c] | p[a] |
| No. of patients | 16 | 16 | | 20 | 12 | |
| Months of therapy | 33 | 34 | NS | 34 | 32 | NS |
| Starting CD4 cells (cells/μl ± SD) | 374 ± 105 | 404 ± 102 | NS | 380 ± 95 | 401 ± 121 | NS |
| End of study CD4 cells (cells/μl ± SD) | 378 ± 142 | 205 ± 104 | <0.001 | 322 ± 173 | 241 ± 90 | 0.09 |
| Absolute CD4 cell change (cells/μl ± SD) | −8 ± 119 | −194 ± 133 | 0.0002 | −66 ± 161 | −156 ± 126 | 0.09 |
| HIV copies/$10^6$ CD4 cells | 698 | 5,636 | 0.003 | 1,070 | 5,495 | 0.03 |

[a] Student's $t$ test.
[b] Non-syncytium-inducing HIV phenotype.
[c] Syncytium-inducing HIV phenotype.

### HIV Biological Phenotype and Codon 215 RT Gene Mutation Related to Virus Burden and CD4 Cell Decline

To determine the relative contributions of the SI/NSI phenotype and the codon 215 genotype on CD4 cell decline and virus burden, patients were grouped by the following PBMC viral characteristics: I—wild-type at codon 215 and NSI phenotype (WT/NSI) (10 patients); II—wild-type at codon 215 and SI phenotype (WT/SI) (six patients); III—a codon 215 mutation and NSI phenotype (MUT/NSI) (10 patients); and IV—a codon 215 mutation and SI phenotype (MUT/SI) (six patients) (Table 2). Patients in group IV (MUT/SI) had the greatest decline in CD4 cells (−54%) and the greatest virus burden (21,480 HIV proviral DNA copies/$10^6$ CD4 cells and 210,000 HIV RNA copies/ml plasma) compared with the other three groups (Table 2). The three remaining groups had a decreasing proviral burden and had less of a CD4 cell decline as follows: MUT/NSI > WT/SI > WT/NSI (see Fig. 1). Patients with the wild-type genotype at codon 215 and NSI phenotype had an increase in their CD4 cell counts (+10%) and the lowest proviral burden (467 HIV proviral DNA copies/$10^6$ CD4 cells and 43,650 HIV RNA copies/ml plasma) after 34 months of therapy.

Patients with the NSI phenotype (groups I and III) had significantly different changes in their CD4 cells depending on the presence of the 215 mutation, [group III (MUT/NSI) CD4 cell change of −160 ± 140 CD4 cells/μl versus group I (WT/NSI) CD4 cell change of +28 ± 122 CD4 cells/μl ($p < 0.01$)], (see Fig. 1A). Both these groups had similar starting CD4 cell counts 363 ± 87 versus 397 ± 104 CD4 cell/μl ($p$ = NS) and similar lengths of therapy 34 versus 37 months ($p$ = NS).

In a multiple linear regression with both SI phenotype and PBMC codon 215 mutation as the explanatory variables, each added significantly to the other in predicting the outcome variable and proviral burden (PBMC codon 215 mutation $p = 0.001$ and SI phenotype $p = 0.01$). The data also showed that each added significantly to the other in predicting $CD4^+$ T-cell decline (PBMC codon 215 mutation $p = 0.0001$ and SI phenotype $p = 0.04$). Patients with SI or NSI isolates had the same frequency of a codon 215 mutation in their PBMCs (six of 12 versus 10 of 20, respectively, $p$ = NS) (Fig. 2). However, patients with the SI phenotypes were more likely to have a codon 215 mutation in their serum HIV RNA than the patients with the NSI isolates (11 of 12 versus 10 of 20, respectively, ($p = 0.04$)) (Fig. 2). Of the 11 patients with the SI phenotype and the codon 215 mutation in their serum virus, six also had the mutation in their PBMCs, whereas, five patients were wild-type at codon 215 in their PBMCs.

TABLE 2. *HIV-1 proviral DNA codon 215 genotype and SI/NSI phenotype vs. CD4 cell changes and virus burden among 32 zidovudine-treated patients*

| | Wild-type codon 215 NSI phenotype | Wild-type codon 215 SI phenotype | Mutant codon 215 NSI phenotype | Mutant codon 215 SI phenotype |
|---|---|---|---|---|
| No. of patients | 10 | 6 | 10 | 6 |
| Absolute CD4 change (cells/μl) | +28 | −66 | −160 | −252 |
| % CD4 cell change | +10% | −16% | −41% | −54% |
| HIV copies/$10^6$ CD4 cells | 467 | 1,380 | 2,510 | 21,480 |
| HIV RNA copies per ml plasma | 43,650 | 60,230 | 100,460 | 210,000 |

NSI, non-syncytium-inducing HIV phenotype; SI, syncytium-inducing HIV phenotype.

## DISCUSSION

In this study we evaluated 32 patients with no symptoms (ACTG 019) or mild symptoms (ACTG 016) of HIV infection on zidovudine monotherapy (mean starting $CD4^+$ T-cell count ~400 CD4 cells/μl) for the presence of the SI/NSI phenotype and a codon 215 RT mutation in proviral DNA and serum virion RNA and related them to $CD4^+$ T-cell changes and virus burden. Using multiple linear regression, SI phenotype and proviral DNA 215 mutation were found to be independently associated with $CD4^+$ T-cell decline and increased virus burden. The presence of both SI phenotype and codon 215 mutant genotype in patients on zidovudine was the strongest predictor of $CD4^+$ T-cell decline (−54%) and increased virus burden (21,500 HIV copies/$10^6$ $CD4^+$ T cells). Patients receiving zidovudine who remained wild-type at codon 215 and had only NSI strains had an increase in their $CD4^+$ T cells (+10%) and 1/46 the proviral burden compared with patients with the 215 mutation and SI strains. There was a concordant pattern of increasing viral burden for both proviral DNA and plasma HIV RNA across the subgroups of patients (WT/

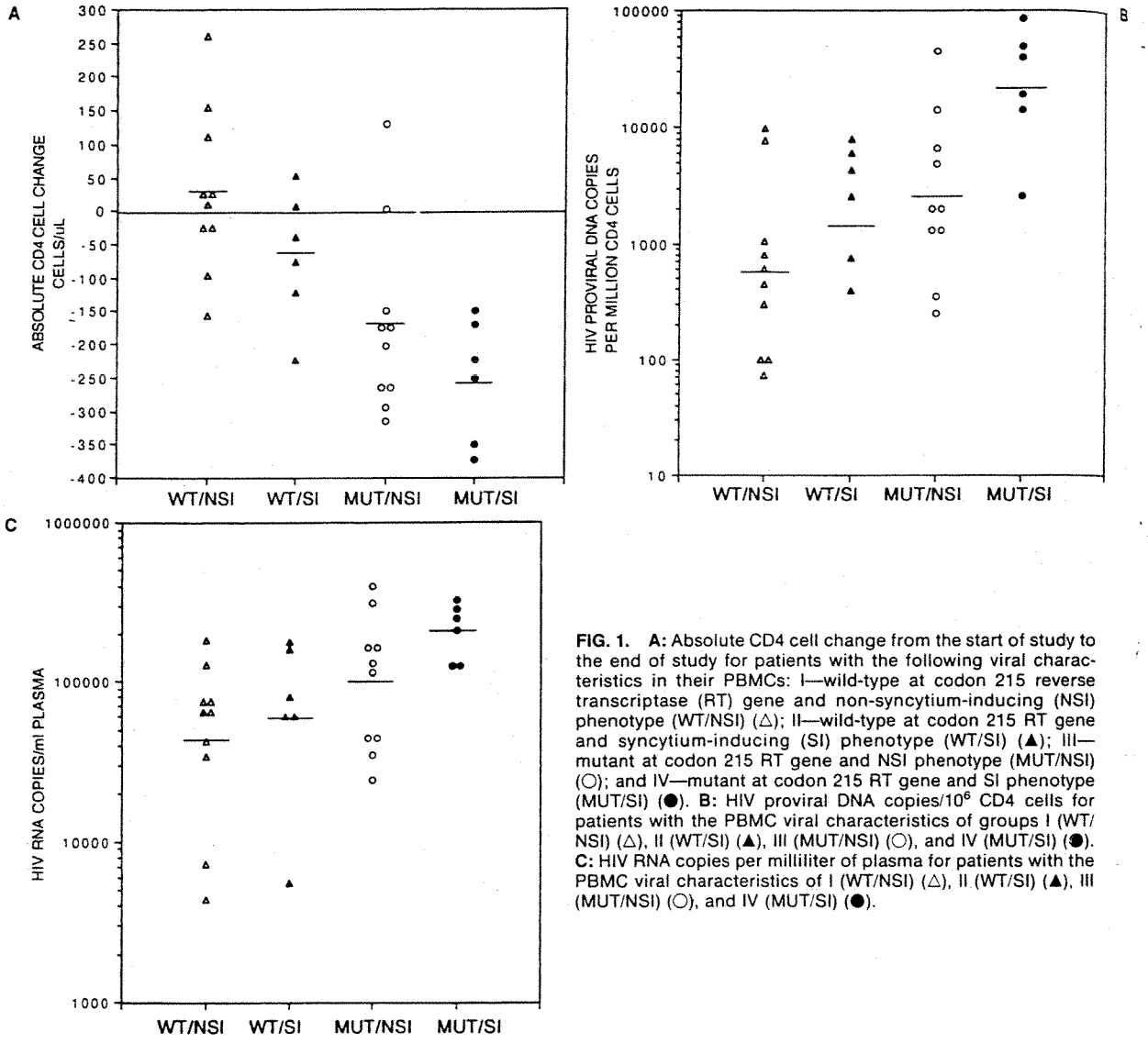


**FIG. 1.** **A:** Absolute CD4 cell change from the start of study to the end of study for patients with the following viral characteristics in their PBMCs: I—wild-type at codon 215 reverse transcriptase (RT) gene and non-syncytium-inducing (NSI) phenotype (WT/NSI) (△); II—wild-type at codon 215 RT gene and syncytium-inducing (SI) phenotype (WT/SI) (▲); III—mutant at codon 215 RT gene and NSI phenotype (MUT/NSI) (○); and IV—mutant at codon 215 RT gene and SI phenotype (MUT/SI) (●). **B:** HIV proviral DNA copies/$10^6$ CD4 cells for patients with the PBMC viral characteristics of groups I (WT/NSI) (△), II (WT/SI) (▲), III (MUT/NSI) (○), and IV (MUT/SI) (●). **C:** HIV RNA copies per milliliter of plasma for patients with the PBMC viral characteristics of I (WT/NSI) (△), II (WT/SI) (▲), III (MUT/NSI) (○), and IV (MUT/SI) (●).

NSI < WT/SI < MUT/NSI < MUT/SI) (Fig. 1B and C), although not of the same order of magnitude (46-fold increase for proviral burden and five-fold increase for plasma HIV RNA). Patients with the largest declines in their $CD4^+$ T cells had, on average, the greatest virus burden (Fig. 1).

St. Clair et al. (12) previously reported that SI phenotype and ZDV resistance are predictive of disease progression in patients on ZDV monotherapy and that the SI phenotype in their study population was the stronger predictor of disease progression. Our results similarly show a strong association between a marker of ZDV resistance (a codon 215 *pol* gene mutation) and SI phenotype with CD4 cell decline. However, given our small sample size we could not analyze the data to determine which viral characteristic is the stronger predictor. Our finding that SI phenotype and codon 215 *pol* gene mutation are strongly associated with an increased virus burden in both proviral DNA (PBMCs) and serum HIV RNA adds additional evidence that these viral characteristics are poor prognostic signs in patients on ZDV monotherapy.

Boucher and co-workers (11) reported that 18 p24

| | | NSI | SI | |
|---|---|---|---|---|
| PBMC codon 215 | WT | 10 | 6 | p=NS |
| | MUT | 10 | 6 | |

| | | NSI | SI | |
|---|---|---|---|---|
| SERUM codon 215 | WT | 10 | 1 | p=0.04 |
| | MUT | 10 | 11 | |

**FIG. 2.** Frequency of the codon 215 mutation for 32 patients on zidovudine monotherapy in proviral DNA from peripheral blood mononuclear cells (PBMCs) and in serum HIV RNA.

antigen–positive individuals with SI or NSI had no significant differences in the development of zidovudine-resistant mutations. Also, in our 32 patients no difference was found in the presence of the codon 215 mutation in PBMC DNA for those patients with (six of 12) and without (10 of 20) SI isolates. However, we did find a difference in the presence of the codon 215 mutation in serum RNA. Of the patients with SI isolates 11 of 12 had the 215 mutation in their serum RNA, while only 10 of 20 of the NSI patients had the mutation after 34 months of therapy ($p = 0.04$). By analyzing both proviral DNA and serum HIV RNA, we were able to find five patients who had SI isolates that had a difference in the codon 215 genotype depending upon the site tested, i.e., their proviral DNA was wild-type at codon 215, but their serum HIV RNA contained a mutation. Previously, Smith et al. (19) reported two patients, and we reported 18 patients (5), in whom mutations that cause ZDV resistance appeared first in serum virus. We also found that PBMCs can remain wild-type at these codons for months after the serum virus has mutated. This discrepancy in viral genotypes between the serum and the PBMCs suggests that virus in the serum may originate from sites of viral replication other than the circulating PBMCs, such as the lymphatic system (20,21). Cell-free virus in the serum is the result of current virus replication. The five patients in whom the mutation was detected only in serum may represent a group showing early emergence of a new viral genotype resulting from the SI phenotype's greater replication rate and higher virus burden. Alternatively, zidovudine-resistant virus could be contributing to the deterioration of the immune system and be facilitating the emergence of the SI strains by damaging host immune surveillance for the more virulent forms of the virus.

With long-term zidovudine therapy and disease progression, the prevalence of both SI (10) and the codon 215 mutation (11,22) increases, and thus their independent effect on disease progression is likely to become more difficult to discern, since each could contribute to the likelihood of the other arising by adversely affecting the immune system and increasing virus burden. However, only ~50% of all HIV-infected patients who progress to AIDS develop SI strains (10). Another finding that suggests the independent association of the codon 215 mutation with CD4 cell decline is that among the 20 patients with only NSI strains, the 10 patients with the codon 215 mutation had a marked decline in CD4 cells (~41%) and a high viral burden (2,510 HIV copies/$10^6$ CD4 cells), which differed significantly from the 10 patients wild-type at codon 215, who experienced a 10% increase in CD4 cells and had a proviral burden of 467 HIV copies/$10^6$ CD4 cells. This difference could not be explained by differing initial CD4 cell counts or duration of therapy. This finding suggests that zidovudine exerts a suppressive effect on HIV and the decline in CD4 cells in patients developing the 215 mutation could be due to zidovudine resistance.

The complex interactions among the patient's immune system, viral drug resistance to zidovudine, HIV biological phenotype, and virus burden are likely interdependent in their effects on disease progression. Each factor may have an independent effect on the failure of the immune system and when combined with each other an additional if not synergistic effect. Despite this complicated picture in zidovudine-treated patients, determination of HIV biological phenotype, virus burden, and codon 215 genotype all provide valuable information on the likelihood of immunologic deterioration. Identification of these viral characteristics in patients on zidovudine could be used to help predict which patients will experience disease progression and thus allow a therapeutic change to be made.

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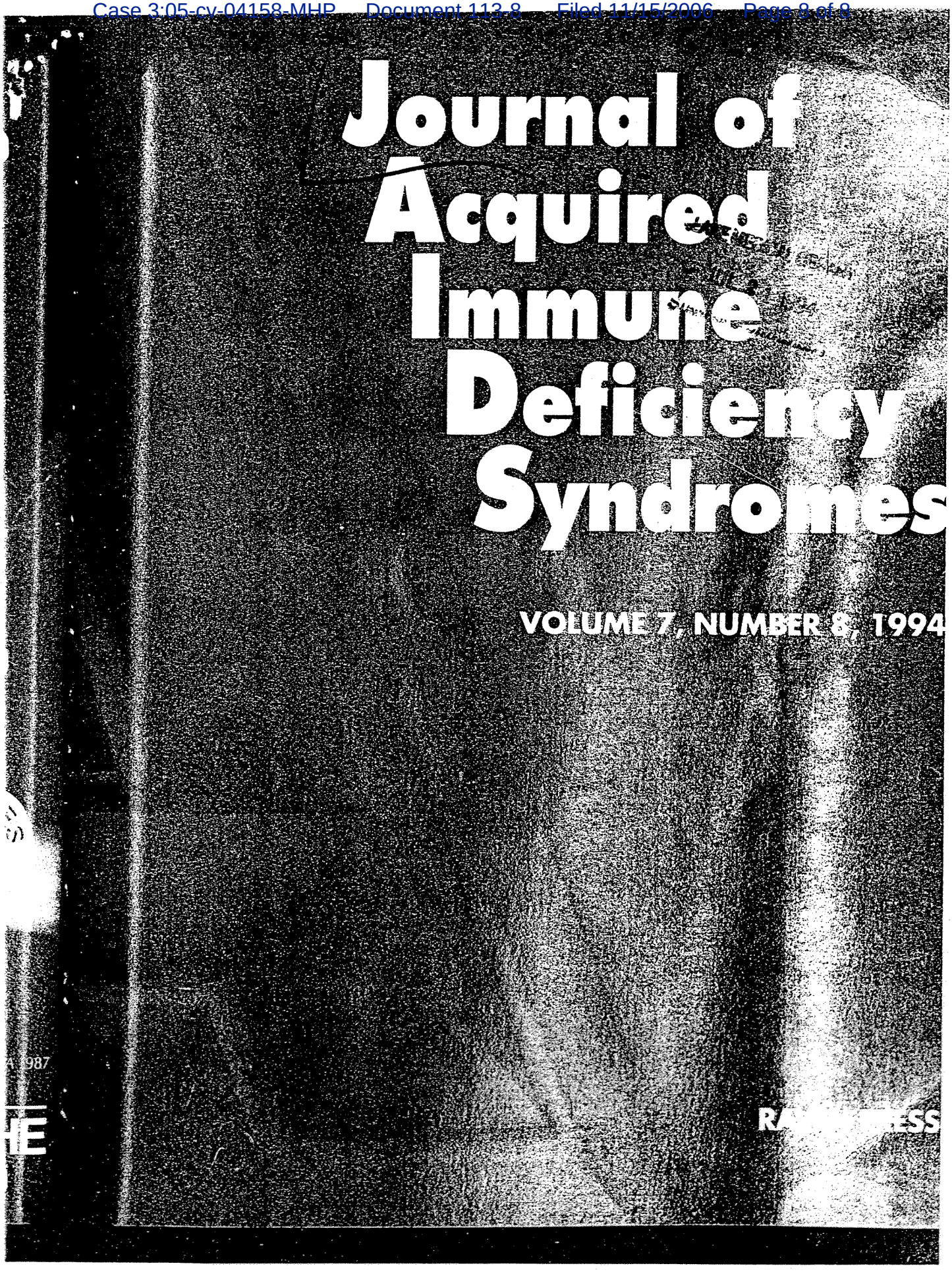
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