

Case study

Absence of detectable viremia in a perinatally HIV-1-infected teenager after discontinuation of antiretroviral therapy

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A 15-year-old girl with perinatal HIV-1 infection has remained asymptomatic with undetectable plasma HIV-1 viremia for more than 5 years after discontinuing all antiretroviral therapy. Viral sequence analysis of proviral HIV-1 DNA revealed no evident fitness-attenuating deletions or mutations. This subject exhibited an unusually robust HIV-specific T-cell response, with an intact CD4⁺ T cell-proliferative response to HIV-1 antigens. In addition, the subject was found to be heterozygous for the 32-bp deletion in the *CCR5* gene, which encodes the primary coreceptor for HIV-1 entry into cells. This mutation mediates profound resistance to HIV infection in homozygotes and has been associated with delayed disease progression in heterozygotes after both horizontal and vertical HIV-1 infection. Although adults with long-term nonprogressive HIV disease have been studied at length, there is no prior description in the literature of a perinatally HIV-infected child whose plasma HIV-1 viremia is controlled to undetectable levels in the absence of antiretroviral therapy. (*J Allergy Clin Immunol* 2006;118:324-30.)

Key words: Perinatal HIV, long-term nonprogressor, *CCR5*, cytotoxic T lymphocytes, CD4 T_H cells

CASE PRESENTATION

A 15-year-old African American girl presented to the Infectious Disease Clinic at Children's Hospital in Boston, Massachusetts, with asymptomatic vertically acquired HIV-1 infection. The patient was born to a mother with known HIV-1 infection who had received no antiretroviral

Abbreviations used

GBV-C: GB virus C

HAART: Highly active antiretroviral therapy

prophylaxis in the perinatal period. The child's HIV-1 ELISA and Western Blot test results remained positive at 18 months of age, and she was therefore judged to be HIV-1 infected. At that time (in 1991), she began antiretroviral therapy with zidovudine and didanosine in the absence of any symptoms or physical findings suggestive of HIV-1 infection. The patient's plasma viral load, first measured at 35 months of age, was consistently less than 400 HIV RNA copies/mL on this minimal therapeutic regimen. At age 7 years, the patient enrolled in a study protocol, and her medications were changed to stavudine and zalcitabine, which was later replaced by didanosine because of supply issues. Over the next 3 years, she remained clinically well, and a stable CD4⁺ T-cell count was maintained on this regimen. Her viral load was persistently less than 400 copies/mL, although viral RNA was occasionally quantifiable with ultrasensitive assays. At age 10 years, the patient self-discontinued all antiretroviral therapy, initially without informing her caregivers or medical team. After a brief increase to 1000 copies/mL, her HIV-1 viral load spontaneously decreased to undetectable levels.

The patient's past medical history was otherwise unremarkable, with no opportunistic infections or evidence of immunocompromise. Her growth and development were normal, and she did not exhibit abnormal lymphadenopathy or other physical findings associated with HIV-1 infection. The family history was notable only for HIV-1 infection in the patient's mother, who died in a car accident when the patient was 2 years old. The mother had a history of intravenous drug abuse. The clinical details of the mother's HIV disease course are unknown.

Results of longitudinal HIV-1 viral load and CD4⁺ T cell measurements are shown in Fig 1. Plasma HIV-1 RNA was detectable at low levels on only a few occasions (maximum 1000 copies/mL). The subject's CD4⁺ T-cell percentage has remained stable at approximately 30%, and her absolute CD4⁺ T-cell count has remained within

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age-based norms. More than 5 years after discontinuing therapy, her viral load currently remains less than 400 copies/mL.

DIFFERENTIAL DIAGNOSIS

Although rare, spontaneous control of HIV viremia has been well described in the adult population.¹ However, lack of detectable plasma viremia in the absence of therapy has not previously been reported in a perinatally HIV-1-infected child. Much of our current understanding of the factors that govern the rate of progression to AIDS has come through the study of adult long-term nonprogressors who maintain very low viral loads in the absence of therapy. Long-term nonprogressive HIV-1 infection has been associated with mutations in the HIV-1 viral genome that attenuate viral fitness,²⁻⁶ with unusually robust HIV-1-specific immune responses⁷⁻¹¹ and with host genetic factors.¹²⁻¹⁸ We therefore sought to determine whether any of these rare viral, immunologic, or genetic factors might help to explain the unusually benign clinical course observed in our patient.

In rare cases individuals with long-term nonprogressive HIV-1 disease have been shown to harbor unusual mutations within the HIV genome. Perhaps the most convincing demonstration of a viral genetic mutation leading to HIV attenuation comes from the Sydney Blood Bank Cohort, which is comprised of a blood donor and 8 recipients infected between 1981 and 1984 with a strain of HIV-1 containing a large deletion in the region of overlap of the *nef* gene and the long terminal repeat.¹⁹ All subjects in this cohort experienced an unusually long asymptomatic phase with very low levels of plasma viremia, in some cases less than the limit of detection. However, evidence of clinical disease progression or CD4⁺ T-cell decrease developed in most cohort members during the second decade of infection.¹⁹ Other studies have uncovered a variety of unique, small, in-frame deletions and insertions within HIV-1 *gag*, *vif*, and *nef* gene sequences in long-term nonprogressors,²⁻⁶ although the effect of these mutations on viral replicative fitness was not assessed in all cases. In those studies that did include *in vitro* assessments of viral fitness, many of the observed viral sequence polymorphisms did not significantly affect viral infectivity or replication kinetics.^{3,20} In the context of a highly heterogeneous virus such as HIV-1, the extent to which observed viral sequence variants cause a substantial decrease in viral fitness and pathogenicity is often difficult to assess, and clear cases of viral attenuation are rare.

Another virologic factor known to influence the rate of HIV-1 disease progression is concurrent viremia with GB virus C (GBV-C), a flavivirus closely related to the hepatitis C virus. GBV-C is transmitted through parenteral, sexual, and vertical routes but is not known to cause any clinical syndrome in human subjects. Up to 85% of HIV-1-infected homosexual males have evidence of current or past infection.²¹ HIV-1-infected individuals with detectable GBV-C RNA in peripheral blood

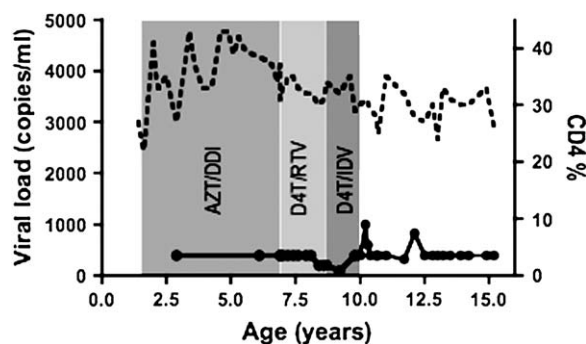


FIG 1. Longitudinal HIV RNA measurements and CD4 percentages. HIV RNA levels (solid line, left axis) and CD4⁺ T-cell percentages (dotted line, right axis) are shown. Subthreshold viral load measurements of less than 400 are graphed as 399 copies/mL, and measurements of less than 50 are graphed as 49 copies/mL. Antiretroviral medication regimens are indicated by shading. The patient has remained off all antiretroviral therapy since 10 years of age.

experience prolonged survival, as well as lower HIV-1 RNA levels, higher CD4⁺ T-cell counts, and slower rates of CD4⁺ T-cell number decrease compared with uninfected individuals or those with resolved GBV-C viremia. GBV-C replicates in CD4⁺ T cells and reduces HIV-1 replication in coinfecting lymphocyte cultures,²² but the mechanism of its effect on HIV-1 replication and clinical progression are unknown.

Several lines of evidence suggest that the adaptive immune response to HIV-1 can contribute to spontaneous control of viremia in some individuals. Detailed studies of the HIV-specific immune responses generated by adults with long-term nonprogressive infection have revealed elements of the immune response that appear to correlate effective viral control.⁷⁻¹¹ The first such immunologic correlate of spontaneous viral control to be identified was the CD4-proliferative response to the HIV Gag protein, which was noted to be particularly robust among long-term nonprogressors with undetectable plasma viremia.^{7,8} Such responses are exceedingly rare in viremic individuals. However, subsequent studies have demonstrated the emergence of HIV-specific CD4-proliferative responses after prolonged viral suppression on highly active antiretroviral therapy (HAART),²³ and it is therefore unclear whether these responses represent a cause or merely a consequence of the successful resolution of viremia. More recent studies have shown that the proliferative capacity of CD8 T cells is also uniquely maintained in subjects in whom viremia is spontaneously controlled.¹⁰ In addition, HIV-specific IL-2 production by both CD4 and CD8 T cells has been reported among long-term nonprogressors^{11,24} and might be a marker for functionally intact virus-specific T cells that are capable of antigen-induced proliferation and self-renewal.

Immunogenetic studies have shown that certain class I HLA molecules, particularly HLA-B27 and B57, are strongly associated with lower viral loads and delayed disease progression.^{17,25} Although the mechanism underlying the protective effect of these alleles is not

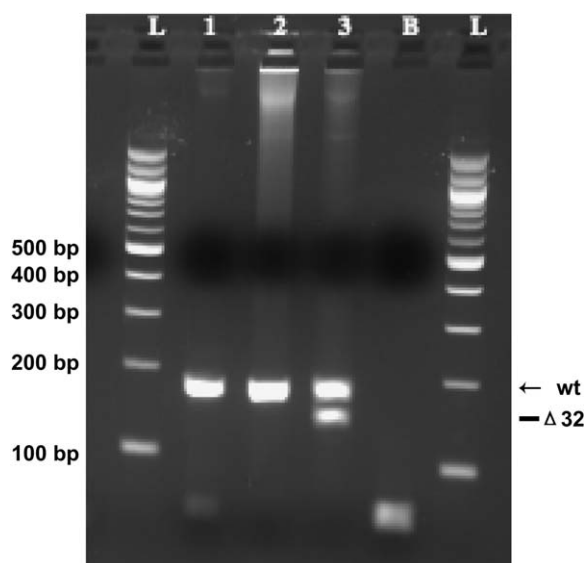


FIG 2. Heterozygosity for the CCR5 Δ 32 mutation. CCR5 was amplified from genomic DNA of our patient (*lane 3*) and 2 wild-type/wildtype controls (*lanes 1 and 2*). Fragment sizes of both 150 and 182 bp were generated in our subject, indicating heterozygosity for the 32-bp deletion. This deletion was confirmed by sequencing of the CCR5 gene.

understood, it is suspected that these HLA molecules exert their beneficial effect through their role in presenting viral epitopes to CD8⁺ T cells. The majority of HLA-B27⁺ long-term nonprogressors who exhibit exceptional control of viremia mount a highly immunodominant response to a particular epitope in the Gag protein.²⁶ Although the viral sequence of this epitope is highly conserved, a number of HLA-B27⁺ subjects have been shown to develop a viral mutation at the amino-terminal anchor position of this epitope after many years of infection.²⁷ This mutation prevents binding of the epitope to the HLA-B27 molecule, allowing the virus to escape recognition by B27-restricted CD8⁺ T cells. The emergence of this escape mutation is often followed by an increase in viral load, clinical disease progression, or both.^{26,28} In contrast, among subjects who express HLA-B57, an allele that is even more strongly associated with benign outcome,²⁵ no single epitope has been identified that is associated with viral control. However, B57-restricted CD8⁺ T cells have been shown to exhibit superior proliferative capacity, and this preserved functional capacity might translate into a more efficacious cytotoxic T-cell response to HIV.¹⁰

In addition to HLA, several host genetic factors have been identified that are associated with prolonged survival, delayed progression to AIDS, or both. The most striking of these is a 32-bp deletion in the chemokine receptor CCR5, which acts as a coreceptor for HIV-1 entry into CD4⁺ T cells.²⁹⁻³² Homozygosity for the CCR5 Δ 32 deletion renders T cells profoundly resistant to infection with HIV-1 and accounts for a substantial portion of highly exposed yet uninfected individuals.^{12,33-35} The population prevalence of this deletion differs widely among different

ethnic groups, ranging from less than 1% in Africans to greater than 10% among some northern European groups. Heterozygous individuals have been shown to exhibit delayed HIV-1 disease progression after both horizontal and perinatal infection, although this effect has not been observed in all cohorts studied.^{12,13,36-40} Additional polymorphisms have been described in the CCR5 promoter region that also affect HIV-1 progression rates.⁴¹ Other genetic polymorphisms that have been associated with slow progression to AIDS include the 64I polymorphism in the cellular coreceptor CCR2,^{15,16} which is in tight linkage disequilibrium with CCR5, as well as polymorphisms in stromal-derived factor 1,¹⁴ the ligand for CXCR4, which serves as the cellular coreceptor for syncytium-inducing strains of HIV-1.

LABORATORY TESTING

Viral, immunologic, and genetic factors were all considered potential contributors to the unusual clinical phenotype of our patient. Full-genome viral sequencing was performed with previously described PCR primers and conditions to rule out the possibility that the patient was infected with an attenuated strain of HIV-1.⁴² The virus was readily amplified from proviral DNA extracted from PBMCs at age 12.1 years, and no deletions or previously described fitness-attenuating polymorphisms were detected. The patient's virus was noted to contain a 3-amino-acid duplication within the PTAP motif of the p6 Gag protein, which overlaps with the alternate reading frame for *pol*. This tetrapeptide motif interacts with the cellular protein tumor susceptibility gene 101 to mediate viral budding.⁴³ However, duplications in this region are common, occurring in up to 9% to 27% of clinical HIV-1 isolates, and are not associated with differences in transmission, viral load, or clinical progression.⁴⁴ A plasma sample from our patient was negative for GBV-C RNA, as determined by using a nested RT-PCR assay.

As discussed above, genetic and immunologic contributions to host defense are to a large extent interrelated, and expression of certain HLA alleles has been strongly associated with long-term nonprogressor status. We therefore determined the class I HLA type of our patient by means of PCR with sequence-specific primers. The patient's haplotype (HLA-A*0205/6802, HLA-B*1510/4403, and HLA-Cw*0304/0401) did not include any of the class I alleles that have been clearly associated with delayed HIV-1 disease progression.¹⁷ Other host genetic factors explored in this subject included described polymorphisms in the genes encoding CCR5 and CCR2. Interestingly, the subject was found to be heterozygous for the 32-bp deletion in the coding region of CCR5 (Fig 2; methods and primers as previously described⁴⁵). As noted above, this mutation has been associated with a modest delay in progression to AIDS among heterozygotes. The described disease-attenuating 64I polymorphism in CCR2 was not present.

TABLE I. Cytokine production by HIV-specific T cells

Stimulus	CD4 T-cell frequency			CD8 T-cell frequency		
	IFN- γ ⁺	IL-2 ⁺	TNF ⁺	IFN- γ ⁺	IL-2 ⁺	TNF ⁺
SEB	0.510	1.240	0.310	1.340	0.420	0.091
No antigen	0.005	0.005	0.002	0.012	0.002	0.004
Gag pool	0.490	0.210	0.140	0.880	0.350	0.011
Nef pool	0.012	0.037	0.007	0.053	0.100	0.009
Pol pool	0.098	0.047	0.017	0.560	0.290	0.002
Rev/Tat/Vif/Vpr/Vpu	0.040	0.037	0.002	0.073	0.069	0.005
Env pool	0.100	0.025	0.000	0.160	0.180	0.000
Total	0.728	0.356	0.164	1.726	0.989	0.000

Positive responses, defined as greater than 3 $\times$ the response in the no-antigen control, are highlighted in bold, and the total response for each cytokine was calculated as the sum of all positive responses to individual HIV peptide pools.

SEB, Staphylococcal enterotoxin B, which was used as a positive control.

The patient's virus-specific immune response was also examined in detail. CD8⁺ and CD4⁺ T-cell responses to HIV were assessed by using intracellular staining for IFN- γ , IL-2, and TNF- α after stimulation with pooled overlapping peptides spanning all expressed HIV-1 proteins (Table I). Results are expressed as the percentage of CD4 or CD8 cells that produce the cytokine. Positive responses, defined as greater than 3 $\times$ the response in the no-antigen control, are highlighted in bold, and the total response for each cytokine was calculated as the sum of all positive responses to individual HIV peptide pools. More than 1.7% of all CD8⁺ T cells in this subject were found to be specific for HIV-1, and mapping of the recognized viral regions by means of IFN- γ Elispot with previously described methods⁴² revealed that this CD8⁺ T-cell response targeted 18 distinct epitopes (data not shown). The most striking feature of the subject's immune response to HIV-1 was the unusually robust CD4⁺ T-cell response; 0.73% of all CD4⁺ T cells were specific for HIV-1 proteins, with the majority of these cells targeting Gag (Table I). This Gag-specific response was polyfunctional, with production of IFN- γ , IL-2, and TNF- α (Fig 3, A; shown with no-antigen and staphylococcal enterotoxin B positive controls). Mapping of the Gag regions recognized by the patient's CD4⁺ T cells²³ revealed an exceptionally broad response, with 22 of 66 peptides recognized (Fig 3, B), predominantly those comprising p17 and the amino-terminal portion of p24. In addition, robust Gag-specific CD4⁺ T cell proliferation was demonstrated (Fig 3, C). This proliferation assay detects dividing cells based on progressive dilution of the intracellular fluorescent dye 5(6)-carboxyfluorescein diacetate succinimidyl ester (Molecular Probes, Eugene, Ore).⁴⁶ The undivided population stains brightly for 5(6)-carboxyfluorescein diacetate succinimidyl ester, whereas successive generations of daughter cells are progressively less bright. In this assay 20.1% of the Gag-stimulated CD4⁺ T cells present on day 6 had undergone division. Modeling based on the proliferation platform of Flowjo software (Treestar, Inc., Ashland, Ore) determined that 3.4% of the original CD4⁺ population divided. Such robust HIV-specific CD4-proliferative responses are seldom detected in

untreated children, although they have been shown to develop after durable HAART-mediated viral suppression.²³

PATHOGENESIS

Detailed laboratory analysis of this patient revealed a disease-attenuating genetic polymorphism, as well as an unusually robust HIV-specific CD4⁺ T-cell response, both of which could be contributing to the low level of viremia observed in this child. The patient was found to be heterozygous for a deletion in the *CCR5* chemokine receptor gene, which encodes the principal cellular coreceptor for HIV-1 entry into cells. Heterozygosity for this CCR5 Δ 32 polymorphism leads to diminished cell-surface expression of CCR5 and reduced infectibility of T cells *in vitro*,⁴⁵ which might act to blunt the initial burst of viral replication during acute infection with HIV-1. Enhanced early containment of HIV replication might have facilitated preservation of the unusually high frequency and breadth of HIV-specific CD4⁺ T cells observed in this subject, as well as their preserved capacity to proliferate and produce IL-2 and IFN- γ . Among adult subjects, early resolution of viremia caused by initiation of HAART during the acute phase of infection has been shown to foster the preservation of robust virus-specific CD4 T-cell responses with intact proliferative function.⁸ Thus the robust CD4⁺ T-cell response to HIV-1 observed in this child could be the consequence of diminished viral replication during early infection, a direct contributor to her ongoing viral containment, or perhaps both.

It should be noted that although heterozygosity for CCR5 Δ 32 has been associated with a statistically significant delay in progression to AIDS and death in a number of pediatric cohorts, this effect is rather modest.³⁶⁻⁴⁰ CCR5 Δ 32 heterozygosity by itself would not be expected to cause the extreme phenotype manifested by this patient. However, the *CCR5* locus is highly polymorphic, and its biology is complex.⁴¹ Multiple single nucleotide polymorphisms in the *CCR5* cis-regulatory region are inherited along with the *CCR5* gene, which, together with the tightly linked *CCR2* locus, define the *CCR5* haplotype.

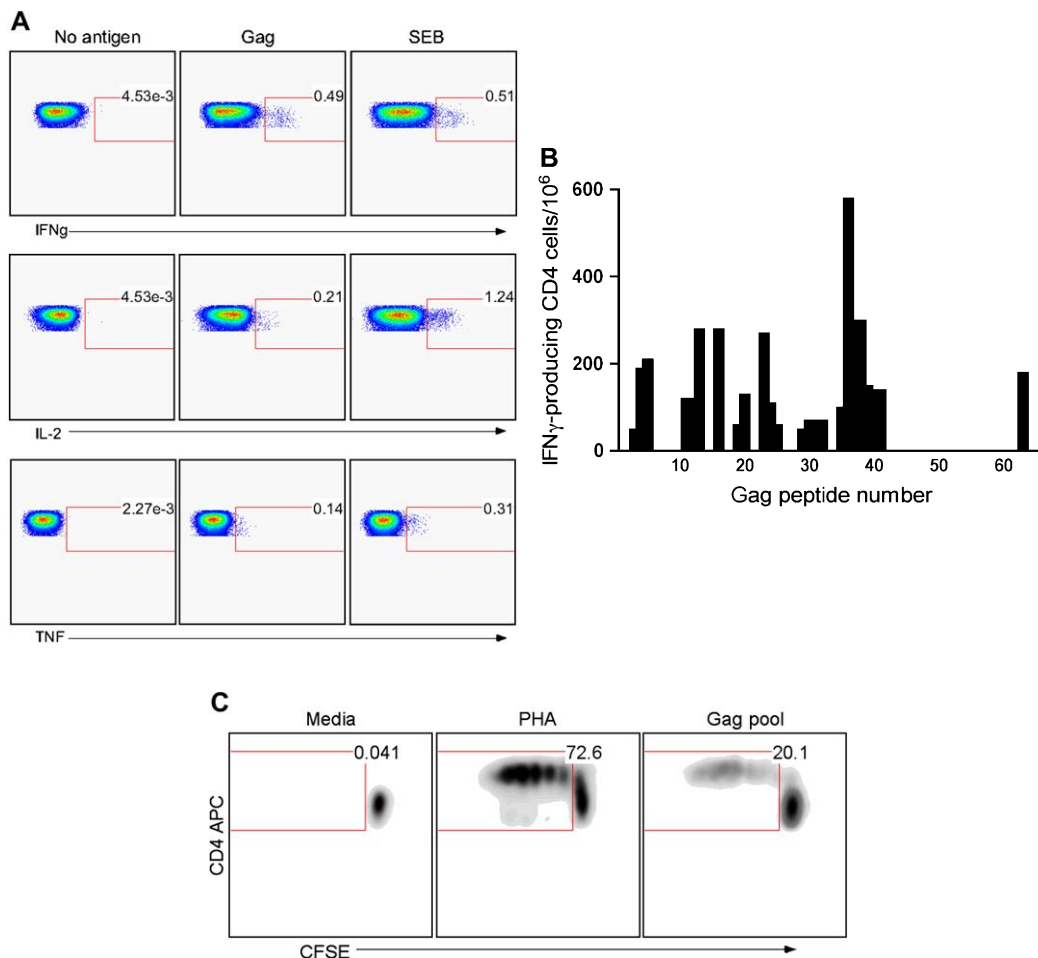


FIG 3. Broad and polyfunctional CD4⁺ T-cell response to HIV. **A**, Intracellular staining of CD4⁺ cells after stimulation with pooled Gag peptides demonstrated production of TNF, IFN- γ , and IL-2. **B**, Mapping of targeted Gag regions was performed by incubating negatively selected CD4⁺ cells with individual Gag peptides in a 24-hour IFN- γ Elispot assay. Sixty-six peptides that overlap to span Gag are displayed across the x-axis, and the y-axis displays the number of peptide-specific cells per million CD4⁺ T cells. These peptides are 18mers that overlap by 10 amino acids and correspond to the clade B HIV-1 consensus sequence. **C**, Proliferation of CD4⁺ T cells in response to stimulation with pooled Gag peptides demonstrated by 5(6)-carboxyfluorescein diacetate succinimidyl ester proliferation assay. PHA was used as a positive control.

Of the 9 human haplotypes that have been defined, some are protective and others are deleterious with respect to HIV-1 disease progression.^{41,47} Moreover, the interactions between the 2 partner alleles and their HIV-1 disease-modifying effects are complex and sometimes paradoxical. For instance, even subjects carrying human haplogroup G*2, the haplotype that contains the CCR5 Δ 32 deletion, experience accelerated disease progression when this allele is paired with the deleterious human haplotype E.⁴¹ In addition, variations in the copy number of the CC chemokine ligand 3-like 1 gene, which encodes macrophage inflammatory protein 1 α P, the most potent natural ligand for CCR5, have a marked effect on HIV-1 disease progression.¹⁸ Subjects who possess CC chemokine ligand 3-like 1 copy numbers higher than race-based norms progress to AIDS more slowly than those with low copy numbers, and this effect is enhanced in those with a

“nondetrimental” CCR5 haplotype.¹⁸ Such factors might act in synergy with the known CCR5 Δ 32 deletion in our patient to further reduce expression of CCR5 or otherwise effect this critical chemokine-receptor axis.

SUMMARY STATEMENT

This case serves as a reminder that the clinical course of HIV-1 disease is extremely heterogeneous, and multiple viral and host factors play a role in determining the rate of progression to AIDS. Although HIV infection remains, at present, an incurable illness, an increasing number of HIV-1-infected patients have been identified who remain asymptomatic well into the second or third decade of infection, including many patients who were infected perinatally. However, control of HIV viremia to undetectable

levels in an untreated perinatally infected adolescent is extremely rare, if not unprecedented. The factors that contribute to the extreme phenotype in patients such as this are likely to be multiple and complex, with viral, immunologic, and host genetic factors all influencing the balance between HIV-1 containment and disease progression.

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