Recovery of Viremic Control after Superinfection with Pathogenic HIV Type 1 in a Long-Term Elite Controller of HIV Type 1 Infection

Andrea Rachinger,1 MarjonNavis,1 Sander van Assen,2 Paul H. P. Groeneveld,3 and Hanneke Schuitemaker1

1Department of Experimental Immunology, Sanquin Research, Landsteiner Laboratory, and Center for Infection and Immunity Amsterdam at the Academic Medical Center of the University of Amsterdam, Amsterdam; 2Department of Internal Medicine, Division of Infectious Diseases, University Medical Center Groningen, Groningen; and 3Department of Internal Medicine, Isala Clinics, Zwolle, The Netherlands

A human immunodeficiency virus type 1 (HIV-1)–infected elite controller (defined as an untreated HIV-1–infected person with a plasma HIV-1 RNA level <50 copies/mL for at least 12 months) who experienced a viremic episode after superinfection regained natural viremic control, although the viral loads in the patient’s 2 partners, infected with the same viral strain, were continuously ∼30-fold higher. Thus, host mechanisms seem to be able to repeatedly control HIV-1 replication, halting disease progression.

Natural elite control of HIV-1 infection (defined as a plasma HIV-1 RNA level <50 copies/mL for at least 12 months) occurs in only a small subset (∼0.6%) of untreated HIV-1–infected persons [1]. Both viral and host factors have been implicated in the natural control of HIV-1 infection, although the exact underlying mechanisms are largely unknown [2, 3]. These mechanisms may be of interest for the design of a vaccine that aims at providing protection from disease progression and a reduction in the transmission of HIV-1 infection. Indeed, a lower plasma viral load is directly related to a lower risk of transmitting infection [4].

Here, during prospective follow-up, we identified a superinfection event in an HIV-1–infected elite controller. We compared the patient’s subsequent course of infection with the dynamics of viral load and CD4+ and CD8+ T cell counts in his superinfecting source partner and in a third partner in this relationship, who became infected with the same viral variant.

Patients and methods. We previously described a long-term HIV-1–infected elite controller (patient 1) who had well-preserved HIV-1–specific immunity despite long-term undetectable viremia [3]. This 53-year-old white man received a diagnosis of HIV-1 infection in 1991 and remained therapy naive thereafter. Beginning in 1991, the plasma HIV-1 RNA level in patient 1 was frequently measured and was always less than the limit of detection of the assay that was used (figure 1A). His CD4+ T cell count was measured routinely beginning in 1996; CD8+ T cell counts were available beginning in 2002. Patient 1 carries the HLA class I B5701 allele, which is overrepresented in long-term nonprogressors [5]. With use of an in-house ultrasensitive PCR assay, a 128–base pair integrase gene fragment and a 454–base pair gag fragment were detected in PBMCs obtained in 2004. During a 14-year period, patient 1 had unprotected sexual intercourse with his steady partner (patient 2) without transmitting HIV-1 infection—consistent with the strongly reduced risk of transmission of HIV-1 infection by individuals with low or undetectable viral loads [4]. From March 2005 onwards, patient 1 engaged in unprotected sexual intercourse with a new additional partner (patient 3), who tested positive for HIV-1 antibodies in May 2005 (figure 1A). Subsequently, in October 2006, patient 2 also had experienced seroconversion from HIV-1 antibodies (figure 1A). Multiple blood samples were obtained from patient 1 from 1991 through 2008, from patient 2 from 2006 through 2008, and from patient 3 from 2005 through 2008. Viral sequences were obtained from plasma samples and/or from replication competent clonal HIV-1 variants isolated from PBMCs. Plasma viral load was measured routinely, and CD4+ and CD8+ T cell counts were determined from 2005 onward for patient 3 and from 2006 onward for patient 2.

Phylogenetic analysis of viral gag sequences from all 3 patients was performed. In brief, gag sequences obtained at different times were aligned (BioEdit, version 7.0.5.3; Ibis Biosciences) with use of the ClustalW program (European Bioinformatics Institute); HIV-1 subtype B references were included from the Los Alamos HIV Databases of the Division of AIDS of the National Institute of Allergy and Infectious Diseases, National Institutes of Health [6]. Sequences from HIV-1–infected individuals from The Netherlands were used as local control subjects. The proper model of nucleotide substitution was chosen on the basis of Akaike information criterion with
use of Modeltest, version 3.7 [7], and a maximum-likelihood tree was generated with use of the best-fit substitution model; computations were started with a neighbor-joining tree. Bootstrapping was performed with 1000 repetitions on the neighbor-joining tree. The maximum-likelihood tree was rooted with use of the most distant B reference sequence as the out-group.

Results. Patient 1, who in the absence of antiviral therapy always had viral loads less than the lower limit of detection of the assays (50–1000 copies/mL), fulfilled the definition of elite controller. In December 2004, patient 1 had a plasma HIV-1 RNA level of 95 copies/mL (figure 1A). However, at his next visit, in February 2005, his viral load was undetectable (<50 copies/mL) again. In November 2005, patient 1 experienced an increase in his plasma HIV-1 RNA level, which peaked at 25,000 copies/mL in April 2006; his level decreased to 1700 copies/mL in November 2006 and remained low until March 2008 (2200 copies/mL) (figure 1A). Because this viremic period was preceded by engagement of patient 1 in unprotected sexual intercourse with a new additional partner (patient 3, who tested positive for HIV-1 antibodies in May 2005), we investigated whether the observed viremia in patient 1 could have been a result of superinfection. Subsequently, in October 2006, patient 2, who at that time was engaged in unprotected sexual intercourse with both patients 1 and 3, also had experienced seroconversion from HIV-1 antibodies.

A total of 26, 20, and 44 gag sequences in plasma-derived HIV-1 RNA from patients 1, 2, and 3, respectively, were generated. In addition, direct sequences were obtained from competent clonal HIV-1 variants isolated from PBMCs (5 sequences from patient 1, 7 from patient 2, and 14 from patient 3). Phylogenetic analyses revealed that all sequences belonged to HIV-1 subtype B. The phylogenetic tree of gag sequences (figure 2) revealed that viral sequences obtained from patient 1 at multiple times during his viremic period did not cluster with the previously obtained gag sequence (from 2004) but did form a monophyletic group (referred to as the “2005–2007” cluster) with HIV-1 sequences from his new partner (patient 3) and his steady partner (patient 2). This observation, which excludes that the 2005–2007 cluster descends from the sequence from 2004, combined with the fact that sequences from patient 3 are ancestral to the mixed subcluster (from patients 1, 2, and 3), indicates that patient 1 acquired HIV-1 superinfection from
Figure 2. Maximum-likelihood tree of HIV-1 gag sequences (isolated from 2004 through 2007) from patient 1, who was an elite controller, and his partners, patients 2 and 3, and of control sequences. Each tip without a symbol in the tree represents a local control sequence. Other sequences are representative of subtype B reference sequences from different countries (from the Los Alamos HIV Database [6]). Sequence DE20c was used to root the tree. *Bootstrap values (80–100; the bootstrap value of the “2005–2007” cluster is 100). Z1g2004, the sequence isolated from patient 1 in 2004.

patient 3. Whether patient 1 or 3 was the source of HIV infection in patient 2 could not be determined, because both phylogenetic analysis of gag, env, and pol sequences, as well as the monitoring of transmission of known cytotoxic T cell escape mutations in the HIV-1 genome, were inconclusive (data not shown).

Next, we compared the clinical course of HIV infection in the superinfected elite controller, his source partner for superinfection (patient 3), and his steady partner (patient 2), who became infected with the same HIV-1 variant. Until that time, all 3 patients had remained therapy naive. As mentioned previously, patient 1 had established relative control of his viremia within 2 years after the superinfection event. He consistently had CD4+ T cell counts within the normal range (median CD4+ T cell count, 655 cell/mm³; range, 480–830 cells/mm³) (figure 1B), although his CD8+ T cell count increased after HIV-1 superinfection and has remained high to date (range, 2870–3340 cells/mm³). In contrast, viral loads in the source partner (patient 3) and the steady partner (patient 2) were consistently high (figure 1A) and coincided with rapidly decreasing CD4+ T cell counts (figure 1B) and low CD8+ T cell counts (range in patient 2, 910–1050 cells/mm³; range in patient 3, 820–1930 cells/mm³). Patient 1 carries HLA-B57, which may be an important host factor for his relatively controlled superinfection. Alternatively, the progressive clinical course of infection in patients 2 and 3 may be associated with HLA class I haplotypes that were previously associated with a more progressive disease course [8]. However, analysis of HLA alleles in patients 2 and 3 did not reveal any alleles (alleles in patient 2, HLA-A*010101, A*0201, B*0801, and B*4402/4419N; alleles in patient 3, HLA-A*0201, A*2902, B*4402/4419N, and B*4403) that are known to be associated with the clinical course of HIV-1 infection.

Discussion. Here, we report an HIV-1 superinfection event in a natural elite controller of HIV-1 infection who subsequently established relative control of the superinfecting virus, although the same virus variant was associated with a high viral load and a progressive disease course in 2 other individuals. Previous studies have implied that mechanisms that may protect from disease progression after initial HIV-1 infection do not protect against HIV-1 superinfection [9, 10]. Moreover, the study by Casado et al. [10] demonstrated HIV-1 superinfection and coinfection in 2 HIV-1–infected, long-term nonprogressors, which suggests that there was repeated natural control of unrelated HIV-1 variants. However, Casado et al. [10] had no
information on the virulence of the superinfecting viruses, which hampers firm conclusions on the contribution of viral or host factors to the benign clinical outcome of HIV-1 superinfection in the long-term nonprogressors.

Our prospective study not only revealed HIV-1 superinfection in an HIV-1–infected elite controller, it also revealed the subsequent recovery of natural viremic control of the superinfecting viral variant; albeit, the viral load after superinfection remained detectable, which implies that patient 1 no longer fulfilled the definition of elite controller. Interestingly, plasma viral loads in the source partner for superinfection (patient 3) and in the steady partner (patient 2), who were infected with the same viral variant, were continuously 10–30-fold higher than those in patient 1. It could be argued that the relatively controlled viremia in patient 1 was attributable to superinfection due to an attenuated HIV-1 variant. However, the viral load in patient 1 reached 25,000 copies/mL shortly after superinfection, demonstrating the replication competence of the superinfecting virus and excluding transmission of an attenuated HIV-1 variant from the quasispecies present in the source partner. In support of this observation, sequences of multiple gene segments (env, pol, nef, and vif) of isolated clonal HIV-1 variants and HIV-1 RNA sequences from plasma samples from all 3 partners were highly similar (data not shown). Virus from patient 1 had a T242N mutation in the TW10 epitope of Gag, previously described as an escape mutation from HLA-B5701–restricted cytotoxic T cells that may cause a replication fitness cost to the virus [11]. We and others [12, 13] have demonstrated, however, that viruses with this T242N mutation can be associated with a progressive disease course, excluding that this mutation is the sole determinant for nonprogressive HIV-1 infection. We therefore conclude that the low viral load in patient 1 and the recovery of viremic control after superinfection was not attributable to infection with an attenuated virus variant. Instead, this observation strongly emphasizes the crucial role of host factors in the natural control of HIV-1 infection, which is in line with a recent study that demonstrated elite control of HIV-1 infection in an individual who was infected with an HIV-1 variant that was associated with a progressive disease course in the source partner [14]. The elite controller in our study had the protective HLA-B57 allele [2, 3, 5, 12], which may, indeed, contribute to the repeatedly controlled HIV-1 viremia [3]. However, a progressive disease course despite the presence of an HLA-B57 allele has also been described [12, 13], which indicates that, most likely, a combination of host factors determines natural control of HIV-1 infection [2].

The exact underlying mechanism for natural control of HIV-1 infection remains to be revealed. This is of utmost importance, because it could serve the development of a vaccine that aims for the induction of immune responses that will protect from HIV-1 disease progression by reducing viral load, thereby also reducing the risk of transmission of HIV-1 infection.

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