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Human Immunodeficiency Virus Type 1 Clade B Superinfection: Evidence for Differential Immune Containment of Distinct Clade B Strains

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Sequential infection with different strains of human immunodeficiency virus type 1 (HIV-1) is a rarely identified phenomenon with important implications for immunopathogenesis and vaccine development. Here, we identify an individual whose good initial control of viremia was lost in association with reduced containment of a superinfecting strain. Subject 2030 presented with acute symptoms of HIV-1 infection with high viremia and an incomplete seroconversion as shown by Western blotting. A low set point of viremia (~1,000 HIV-1 copies/ml) was initially established without drug therapy, but a new higher set point (~40,000 HIV-1 copies/ml) manifested about 5 months after infection. Drug susceptibility testing demonstrated a multidrug-resistant virus initially but a fully sensitive virus after 5 months, and an analysis of *pol* genotypes showed that these were two phylogenetically distinct strains of virus (strains A and B). Replication capacity assays suggested that the outgrowth of strain B was not due to higher fitness conferred by *pol*, and *env* sequences indicated that the two strains had the same R5 coreceptor phenotype. Delineation of $CD8^+$-T-lymphocyte responses against HIV-1 showed a striking pattern of decay of the initial cellular immune responses after superinfection, followed by some adaptation of targeting to new epitopes. An examination of targeted sequences suggested that differences in the recognized epitopes contributed to the poor immune containment of strain B. In conclusion, the rapid overgrowth of a superinfecting strain of HIV-1 of the same subtype raises major concerns for effective vaccine development.

The global distribution of circulating recombinant forms of human immunodeficiency virus type 1 (HIV-1) M group strains and the high prevalence of intersubtype recombinants in some areas where multiple subtypes are common (reviewed in reference 35) demonstrate that dual infection with different strains of HIV-1 occurs. However, specific examples of dual infection have been documented clearly in only a few cases. Most of these examples have been observations of chronically infected patients with two different subtypes in countries where more than one subtype is prevalent (4, 7, 30, 37, 38), and a few examples of intrasubtype B dual infections have been described (14, 40, 41, 51). Whether these dual infections occurred as coinfections or superinfections is unknown, and the influence of cellular immunity on coexisting viruses has been largely undefined.

A few cases of superinfection, usually involving superinfecting strains that differ in antiretroviral drug susceptibility from the initial variant, have been identified. One individual was initially infected with a strain of HIV-1 belonging to clade CRF01_AE and, after a series of therapy interruptions, became superinfected with a B subtype strain (21). Another individual was initially infected with a drug-resistant strain but became superinfected in the absence of therapy with a drug-sensitive strain of the same subtype (23). One study explored the possible role of cellular immune responses in an individual who was undergoing structured treatment interruptions and had achieved relatively stable control of viremia, subsequently becoming superinfected with another B subtype strain and suffering an acute rise in viremia (2). However, data regarding the role of cellular immunity in superinfection in the absence of drug therapy have been lacking.

We have studied a subject who was initially infected with a multiply drug-resistant strain of HIV-1 subtype B. He controlled the initial infection well in the absence of antiretroviral therapy but became superinfected 4 months later with a second, drug-sensitive strain and showed a marked rise in viremia. Detailed genetic and immunological characterization showed that cytotoxic T-lymphocyte (CTL) responses against the first strain waned with outgrowth of the second strain. The failure of immune containment of the second strain despite early infection and an initially effective HIV-specific CTL response

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exemplifies a significant challenge to the development of effective vaccines.

MATERIALS AND METHODS

The subject. Subject 2030 was a 33-year-old homosexual Asian-American male with no significant past medical history who presented after 10 days of an acute illness. He reported multiple ongoing unprotected sexual exposures as a risk factor for HIV-1 infection and was subsequently found to be seropositive. These studies were conducted with appropriate subject consent and approved by the Human Research Protections Program at the Harbor-UCLA Medical Center Research and Education Institute, University of California, Los Angeles, Calif.

HLA typing. High resolution HLA typing was performed by Pel-Freez Biologicals.

HIV-1 phenotyping. Antiretroviral susceptibility was determined by PhenoSense (ViroLogic, Inc.) as previously described (36). In this assay, a region extending from p7gag to amino acid 300 in reverse transcriptase (RT) is RT-PCR amplified from plasma viral RNA and inserted into the test vector. This construct, after transfection into the target cells, is used in a single cycle assay in the presence of drug for assaying drug susceptibility; similarly, in the absence of drug, it is used for assaying endogenous replication capacity (9). In both assays, the replication rate is compared to the replication rate of the control strain HIV-1 clone NL4-3.

HIV-1 genomic sequencing and phylogenetics. Sequencing of viral genomic regions containing CTL epitopes was performed after RT-PCR amplification of plasma viral RNA (extracted with Finnzyme one-step RT-PCR kit, MJ Research, Waltham, Mass., or RNA isolation kit, QIAGEN, Valencia, Calif.). Primers to sequence HIV-1 were those employed by Altfeld et al. (2) (except those for Env), including 737-F (GCG ACT GGT GAG TAC GCC), 2095-R (TTC CCT AAA AAA TTA GCC TG), 1232-F (ACC TAG AAC TTT AAA TGC ATG GG), 1754-R (CAA CAA GGT TTC TGT CAT CC), 1816-F (TAG AAG ACA TGA TGA CAG CAT G), 3018-R (GGT GAT CCT TTC CAT CC), and 2422-R (TCT TAC TTT GAT AAA ACC TCC) (Gag); 5040-F (ATG GAA AAC AGA TGG CAG G), 6455-R (GGG TCT GTG GGT ACA CA), and 5579-R (GGT CTT CTG GGG CTT GTT CC) (Vif); 5692-F (TAT CTA TGA AAC TTA TGG GGA TAC) and 6229-R (CTT TCA TTG CCA CTG TCT TC) (Tat/Rev); 7361-F (TTA ATT GTG GAG GAG AAT TTT T), 9403-R (ACT CCG GAT GCA GCT CTC GGG C), 8804-F (ATG GGT GGC AAG TGG TC), and 9239-R (ACT GGT ACT AGC TTG AAG CAC C) (Nef); V3-F out (CAA AGG TAT CCT TTG AGC CAA T), V3-B out (ATT ACA GTA GAA AAA TTC CCC T), V3-B in (GCG TTA AAG CTT CTG GGT CCC CTC CTG AG), and V3-F in (GAA CAG GAC CAG GAT CCA ATG TCA GCA CAG TAC AAT) (Env). DNA fragments were then gel purified (gel purification kit; QIAGEN) and cloned into the TOPO TA vector (Invitrogen Corp., Carlsbad, Calif.). For each fragment, a minimum of three individual clones were sequenced to obtain consensus sequences for each time point. PCR products were sequenced with Prism Dye terminator kits (ABI, Foster City, Calif.) on an ABI 3100 Genetic Analyzer. Sequences were compiled, aligned, and edited by using Sequencher 4.0 (Genecodes, Ann Arbor, Mich.) and BioEdit (17), and phylogenetic analysis was performed by using MEGA2 (26). Neighbor-joining phylogenetic trees for the reverse transcriptase and protease coding region were obtained from a matrix of synonymous nucleotide distances. This method is most appropriate in regions where there is strong selection on nonsynonymous changes, which otherwise can lead to incorrect trees (demonstrated at http://www.hiv.lanl.gov/content/hiv-db/CONTAM/contam_conserved.html).

ELISPOT analysis of HIV-1-specific $CD8^+$-T-cell responses. Nonspecifically expanded $CD8^+$ T cells were screened to define HIV-1-specific responses by using a standard gamma interferon enzyme-linked immunospot (ELISPOT) assay as previously described (49). Briefly, cryopreserved peripheral blood mononuclear cells from the indicated times were polyclonally expanded to yield $CD3^+$/$CD8^+$ lymphocytes by using a CD3:CD4-bispecific antibody (50) and evaluated for reactivity against overlapping HIV-1 subtype B 15-mer peptide sets (most of which are based on a consensus sequence) obtained from the NIH AIDS Research and Reference Reagent Repository (ARRRR; catalogue numbers 6869, 6208, 6451, 5189, 5138, 6445, 6446, 6447, and 6444). Initial screening was performed on pools of 16 or fewer peptides, followed by analysis of 4-by-4 matrix pools and confirmation using individual peptides. This method is reported to result in a small but quantitatively consistent bias in the expansion of antigen-specific $CD8^+$ T lymphocytes (3, 20), in agreement with our own experience comparing ELISPOT responses with fresh and expanded $CD8^+$ peripheral blood mononuclear cells ($r^2 = 0.58$ for comparisons within nine chronically infected persons; data not shown).

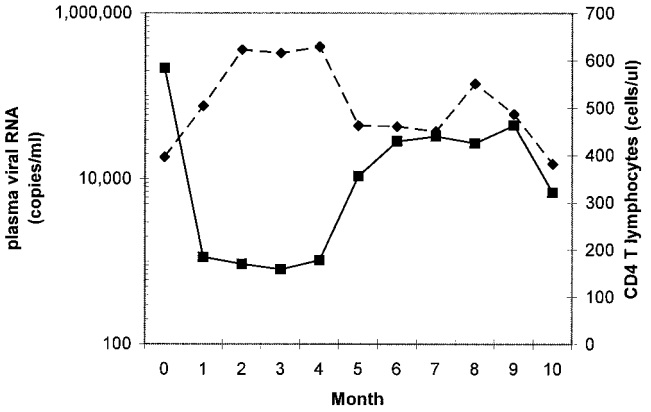

FIG. 1. Subject 2030 plasma HIV-1 and $CD4^+$-T-lymphocyte counts over the first 10 months of infection. Viremia (RNA genomes per ml of plasma; squares) and absolute $CD4^+$-T-lymphocyte concentrations (cells/μl of blood; diamonds) for subject 2030 are plotted.

RESULTS

Clinical presentation and course of subject 2030. Subject 2030 presented with an acute syndrome of fever, rash, myalgia, cervical lymphadenopathy, oral ulcers, and headache of 10 days duration. Upon presentation, he had a positive serum ELISA for HIV-1 antibodies, a borderline Western blot for HIV-1 (bands for gp160 and p24 only), a peripheral blood $CD4^+$-T-lymphocyte count of 396 cells/μl, and viremia of approximately 300,000 HIV-1 copies of HIV RNA/ml of plasma. Subsequently, the viremia decreased to an initial set point of approximately 1,000 copies/ml, and the $CD4^+$-T-lymphocyte count rose to over 600 cells/μl (Fig. 1). However, after this period of relative stability following acute infection, plasma viremia rose sharply to a new plateau of approximately 30,000 to 40,000 copies/ml at 5 months after presentation, with a concomitant decline in the $CD4^+$-T-lymphocyte count to about 450 cells/μl. Subject 2030 reported ongoing high-risk sexual exposures, raising the possibility that this acute change in set point could be due to HIV-1 superinfection.

Dramatic shift in HIV-1 phenotype and genotype indicating infection with two distinct strains. Phenotypic testing revealed that the initial infecting virus was resistant to multiple drugs (Fig. 2). The 50% inhibitory concentration of drug (IC_{50}) of the baseline sample was 50-fold higher than the wild-type control HIV-1_{NL4-3} for zidovudine, approximately 160-fold higher for delavirdine, and 13-fold higher for nelfinavir. The *pol* genotype revealed multiple resistance-associated mutations in both protease (PR) and RT: V77I and L90 M in PR; and M41L, K103N, Y181C, T215F, and an insertion of two serine amino acids at position 69.

When viremia rose at month 5 (Fig. 1), this initially drug-resistant phenotype abruptly shifted to a phenotype that was susceptible to all tested drugs (Fig. 2). Genotyping at this point revealed none of the prior drug resistance mutations and showed distinct variations at polymorphic sites other than those associated with drug resistance. A phylogenetic analysis of protease and reverse transcriptase for this subject, and for control subjects in the same area with acute HIV-1 infection within the year, confirmed that the initial virus strain (A) and

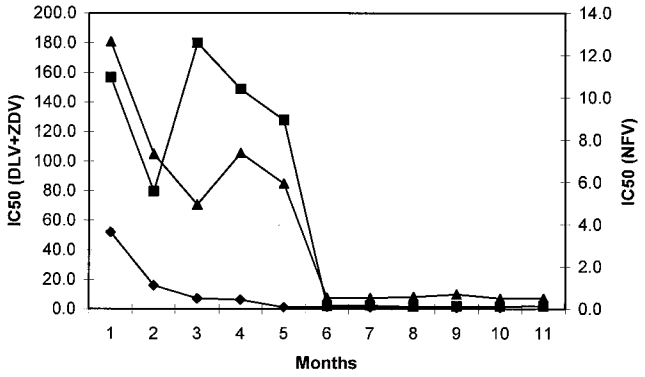

FIG. 2. Subject 2030 plasma virus phenotypic susceptibility to delavirdine, zidovudine, and nelfinavir over the first 12 months of infection. Changes (*n*-fold) in the 50% inhibitory concentration (IC_{50}) are plotted for delavirdine (DLV; squares), and zidovudine (ZDV; diamonds) using the *y* axis on the left and for nelfinavir (NFV; triangles) using the *y* axis on the right.

the subsequent strain (B) were phylogenetically distinct (Fig. 3). The genetic distance between strains A and B at synonymous sites within the sequenced *gag-pol* region was 15.42 ± 2.0%. This genetic distance was greater than that seen in 95% of comparisons among local acute HIV-1 infections involving subtype B (mean, 11.98%; maximum, 16.8%). The *nef* genes of the strains also were clearly distinct, although not as divergent as *gag-pol*. Within *nef*, nonsynonymous sites differed more, on average, between the two than did synonymous sites (d_N = 5.7% and d_S = 4.5%, as estimated by the modified Nei-Gojobori method with Jukes-Cantor correction for multiple hits). In the C2V3 region of *env*, however, there is no difference between d_N and d_S[43], and a single overall rate was estimated. On the basis of 12 clonal sequences 409 bp in length from each of the two strains (strain A, month 3; strain B, month 7), the mean genetic distance was 19.2 ± 0.039% (Tamura-Nei model with γ-distributed rates; α value, 0.4). Overall, sequence data from across the genome clearly demonstrated infection of this individual with two distinct HIV-1 strains.

Recombination between strains A and B. Very little viral sequence diversity accumulated in the first three months of infection, with the exception of amino acid position 215 in RT. At month 4, the consensus sequence contained a series of mixtures at both synonymous and nonsynonymous positions, clustered between amino acids 190 and 300 in RT. These mixtures generally contained one nucleotide identical to the consensus sequence from the first 3 months (strain A) and another nucleotide corresponding to the subsequently distinct consensus sequence at 6 months (strain B; Fig. 4). The most parsimonious reconstruction of the month 4 *pol* genotype therefore was a mixture of two distinct sequences, one from strain A and a second recombinant of strains A and B. Only at amino acid 215 in RT, where one of the parental alternatives (T) was not detected, was this reconstruction ambiguous. This was the first evidence of the presence of strain B in Subject 2030 and the only evidence of recombination between the two infecting strains detected in this study.

Reduced replicative capacity of *pol* in strain A compared to strain B. The phenotypes of the *pol* genes from the two strains were compared also by using the ViroLogic replication capacity assay (Fig. 5). This assay correlates significantly with in vitro fitness and usually, in the context of drug resistance, with plasma viremia (9). The assay compares the number of infective particles produced (in the absence of drug) by recombinant viruses containing subject-derived gagp7-RT segments in an NL4-3 backbone. Normalized to the average of subject-derived viruses, the mean replicative capacity for strain A (i.e., before month 4) was 35% of wild-type subject-derived virus isolates (range, 64 to 12%), while that for strain B (after month 4) was 8% (12 to 1.5%) of wild-type subject-derived virus isolates. The generally lower value for strain B was consistent with the observation of protease hypersusceptibility of this strain (data not shown), which has been associated with low replicative capacity (29). These data show that the in vivo overgrowth of strain A by strain B was not a result of lower intrinsic replicative ability of strain A associated with drug resistance mutations in *pol*, raising the question of differential immune containment of the two viruses.

Coreceptor usage of strains A and B. Because the coreceptor usage of HIV-1 determines the cells that can serve as targets of infection and is associated with disease pathogenesis, the usages of strains A and B were determined by sequencing of the C2V3 region of Env. Clonal sequences obtained from the plasma were assessed for amino acids at positions 11 and 25 of V3, because CXCR4 coreceptor usage is determined by specific amino acid substitutions in the V3 region of *env*, notably basic amino acid substitutions (16). Although the V3 loop sequences of the strains were distinct (12 clonal sequences each from the month 3 and month 12 time points, data not shown), 12 of 12 clones from strain A (month 3) and 11 of 12 clones from strain B (month 12) had serine at position 11. Similarly, 12 of 12 strain A and 11 of 12 strain B sequences had an acidic residue at position 25 (glutamic acid in strain A and aspartic acid in strain B). One strain B clone had asparagine at position 11 and another had asparagine at position 25, but no clone had negatively charged amino acids at both positions. Thus, there was no significant difference between the two strains with respect to predicted coreceptor usage, with both strains being the R5 phenotype.

Evolution of $CD8^+$ cellular immune responses suggesting differential recognition of the viruses. The initially low set point of viremia followed by the subsequent sharp increase (in the absence of antiretroviral therapy) suggested a change in immune control over time. We therefore analyzed the HIV-1-specific $CD8^+$-T-lymphocyte responses by gamma interferon ELISPOT assays (Table 1). Recognition of epitopes in Gag, Nef, and Vif was observed as early as 17 days after the onset of symptoms of acute infection. These early responses generally persisted during the subsequent 3 months, accompanied by broadening to recognize additional epitopes in Gag and Tat. However, after the fifth month, the early responses waned, and new responses were noted in Nef and Rev by the ninth month. Classifying CTL responses as those detected at the first time point ("early responses") or only afterwards ("late responses") demonstrated a clear pattern of evolution (Fig. 6), with the magnitude of early responses declining consistently starting in the third month and the magnitude of late responses rising sharply starting in the fifth month. In the context of superinfection and overgrowth of strain A by strain B, these data strongly suggest that the initial CTL responses were specific to

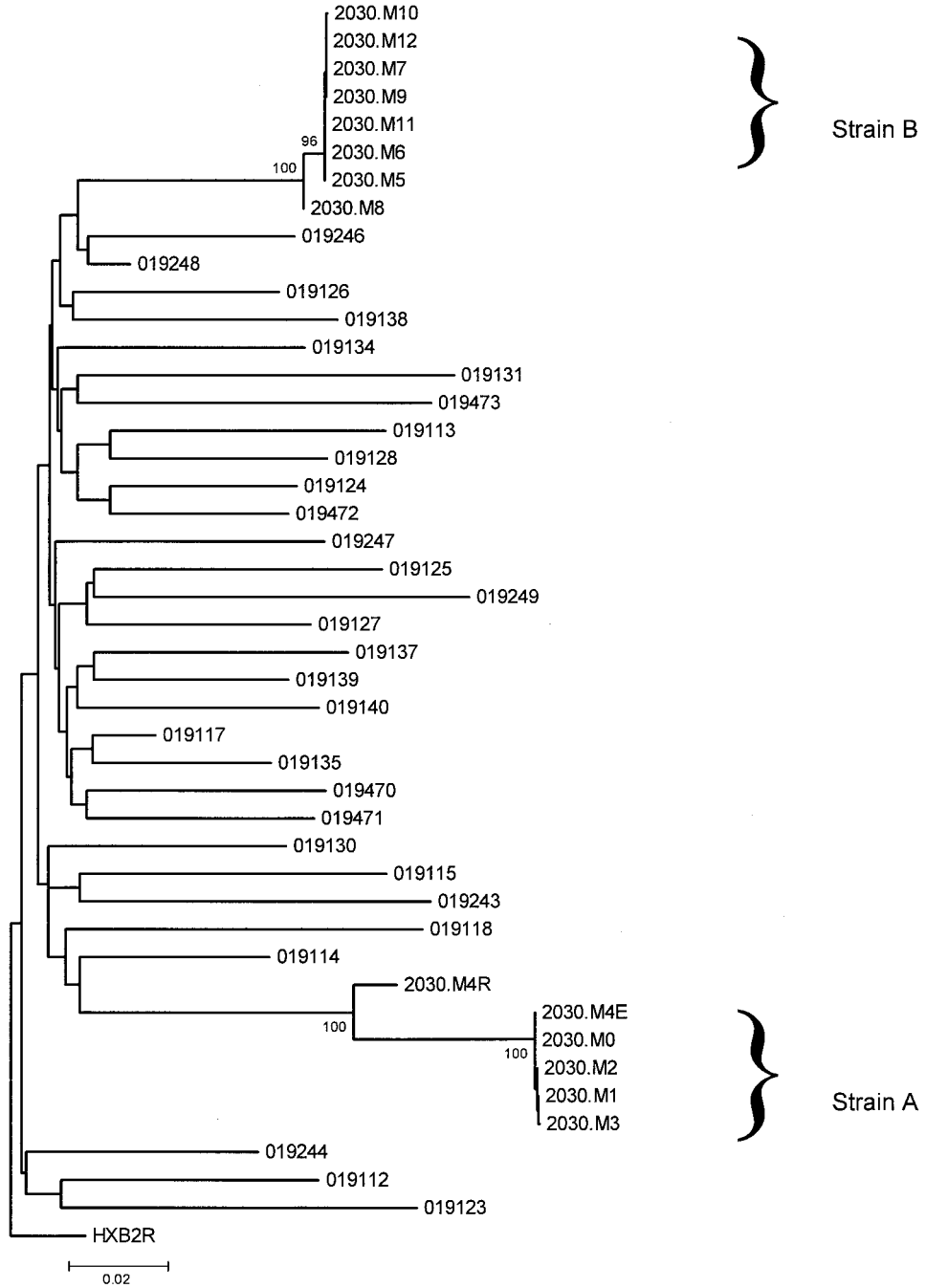

FIG. 3. Phylogeny of *pol* sequences for subject 2030 and other HIV-1 infected persons in the Los Angeles area. Serial *pol* sequences from subject 2030 were compared to those of other HIV-1-infected persons presenting with acute infection in the Los Angeles area from 1998 to 2000. Consensus sequences of PR and 315 codons of RT were used to construct a neighbor-joining tree on synonymous sites by using a modified Nei-Gojobori model, rooted on HIV-1_{HXB2R} (25). The percentage of bootstrap resamples (out of 1,000) in which a clade was identified are shown when above 70%. Sequences from subject 2030 are distinguished by month (2030.M0 to 2030.M12) and identified as strain A and strain B.

strain A and poorly recognized strain B; while there was adaptive retargeting of responses, this retargeting was inadequate to contain strain B to the degree that the initial responses had contained strain A.

Differences in strain A and strain B CTL epitopes. To examine whether sequence differences might account for differential immune control of the two viruses, regions targeted by CTL were sequenced from strains A and B (Table 2). The in vivo viral sequences corresponding to the recognized screening peptides were compared in the context of the HLA type of the subject (A*03, A*24, B*35, and B*40). Of responses that exceeded 100 spot-forming cells (SFC)/10^6 $CD8^+$ T lymphocytes, the majority that waned after superinfection contained potentially significant differences in strain B compared to strain A.

```
                61        70  90        100
                ....|...  .|  |....|....|....|...
Baseline        FAIKKKDSSSTK  VQLGIPHPAGLKKNRSVTV
Month 4 #A      ............  ....................
Month 4 #R      .........*...  ....................
Month 6         .....**.--..  *...*..*.....KK.*..

                210       220       230
                .|....|... .|....|... .|....|...
Baseline        LLKWGFFTPD KKHQKEPPFR WMGYELHPDK
Month 4 #A      .......... .......... ..........
Month 4 #B      ..*.**L... ......*..L .....*....
Month 6         ..*.**T..* ......*..L ..*..*....
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FIG. 4. Recombinant RT sequence detected in month 4 sample. ., same amino acid as in baseline sequence; *, difference from baseline at synonymous nucleotide site in that codon; –, codon deletion; #A, reconstructed month 4 sequence of strain A type; #R, reconstructed month 4 sequence of recombinant type. The month 4 sequence showed a series of ambiguity codes at sites towards the carboxy-terminal end of the sequenced region, which when resolved always included the nucleotide observed with strain A. This result is most readily interpreted as indicating that a recombinant sequence reached significant frequencies at this time point. No recombinant sequence was observed at any other time point.

These differences included an insertion of four amino acids flanking a predicted epitope (Table 2, row 4) and changes within the TCR-binding region (Table 2, rows 6 and 8) or HLA-binding motif (Table 2, row 9) of the predicted epitopes in four of six examples. Two of these six examples (Table 2, rows 1 and 2) showed no difference between the strains. However, it is clear that this waning of the initial responses was not due to generalized immune deficiency, because a vigorous new response against Rev was generated after superinfection (Table 2, row 8). The recognized region of Rev contained two amino acid differences in the TCR-binding region of the predicted epitope in strain A compared to strain B, and the screening peptide was identical to strain B in this sequence. Furthermore, the persistence of an early response recognizing a predicted epitope that was identical in strains A and B (Table 2, row 5) indicated that the decay of the early responses was selective. Overall, these findings are consistent with significant epitope differences in the two viruses having a central role in differential immune containment by CTL and persistence of the CTL response.

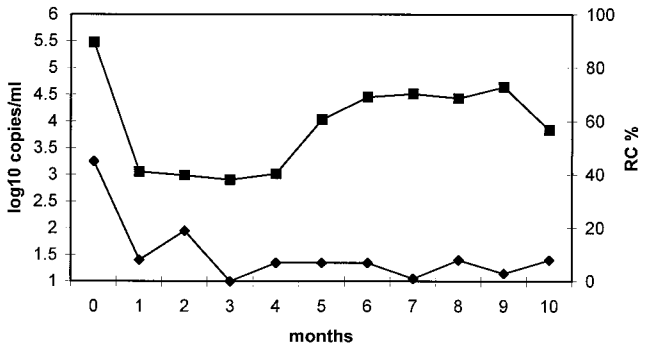

FIG. 5. Replicative capacity of subject 2030 *pol* and plasma viremia over time. Replication capacity (as determined by the PhenoSense assay of *pol*) and plasma viremia are shown over the period of shift from viremia with strain A (months 0 to 3) to that with strain B (months 5 to 10). Squares, viremia (RNA genomes per ml of plasma); diamonds, replicative capacity (RC; percentage of median wild-type value).

DISCUSSION

Previous studies of the evolution of the HIV-1 quasispecies within infected individuals led to the widespread belief that superinfection is a rare event (5, 10–13, 18, 27, 28, 31, 33, 34, 39, 42–48). While dual infection was demonstrated to be possible (51), only recently have clear examples of superinfection emerged (2, 21). These two cases demonstrated superinfection in subjects who underwent structured treatment interruptions with partial control of viremia, presumed to be immunologically mediated. Other recent studies, including a third case in which coinfection or superinfection occurred in the absence of therapy (23), suggest that it can occur in the setting of natural infection without alteration of immunity by pharmacologic intervention. The case described here extends these novel findings and clearly documents superinfection after an initial infection reaching a stable set point in the absence of antiretroviral treatment as a confounding factor.

It is impossible to entirely exclude the possibility that a superinfecting strain was present as an initial coinfection. In the case of subject 2030, however, overgrowth by strain B occurred rapidly and completely once it was detected, showing that its overall growth advantage in vivo was great. If present during acute infection, this overgrowth should have occurred much earlier than 4 months after infection. This example is striking because superinfection was clearly documented after a stable viremia set point was established, in contrast to most published reports. The subject had developed a broad and stable CTL response to the first virus and reached an equilibrium of viremia, suggesting efficient immune suppression of viral replication. Strain B, which was highly genetically distinct from strain A, was detectable only at 4 months after primary infection, but it rapidly overgrew strain A with a substantial increase in viremia set point. A fitness assessment of the *pol* genes showed a lower replication capacity for strain B than strain A, suggesting that substantially greater intrinsic replicative fitness due to differences in *pol* was not the mechanism of this overgrowth. Although fitness differences due to genes other than *pol* have not been excluded, the high level of viremia during primary infection suggested that strain A was not generally defective in replicative capacity. Furthermore, an evaluation of coreceptor usage revealed that both strains were predominately the R5 phenotype, making it unlikely that a difference in tropism could account for differences in replication in vivo. This finding indicates that differential immune containment of strain B versus strain A could be the key factor.

Consistent with the report on immune responses of a subject who had superinfection after structured treatment interruption (2), we found that our subject had developed a stable and broadening repertoire of CTL responses against the initial virus, which failed to prevent or contain infection with the second virus. Sampling of epitope sequences revealed significant differences between the viruses, suggesting that the mechanism of immune failure was nonrecognition of the second

TABLE 1. CTL responses over time[a]

Peptide (ARRRR no.)	Location	SFC count on month indicated			
		0	3	5	9
LKETINEEAAEWDRV (5035)	Gag 201–215 (p24)	0	143	0	0
INEEAAEWDRVHPVH (5036)	Gag 205–219 (p24)	321	253	138	0
AAEWDRVHPVHAGPI (5037)	Gag 209–223 (p24)	899	341	328	0
DRVHPVHAGPIAPGQ (5038)	Gag 213–227 (p24)	45	0	0	0
GPIAPGQMREPRGSD (5040)	Gag 221–235 (p24)	42	0	0	0
PGQMREPRGSDIAGT (5041)	Gag 225–239 (p24)	238	0	0	0
EPIDKELYPLTSLRS (5104)	Gag 477–491 (p6)	0	270	208	0
KIATESIVIWGKTPK (5593)	Pol 529–543 (RT)	0	0	65	0
WPTVRERMRRAEPAA (5142)	Nef 13–27	0	0	0	60
EVGFPVRPQVPLRPM (5155)	Nef 65–79	0	255	0	448
PGPGIRYPLTFGWCF (5171)	Nef 129–143	231	927	463	401
IRYPLTFGWCFKLVP (5172)	Nef 133–147	704	1,185	559	0
KCCFHCQVCFTTKGL (5120)	Tat 29–43	0	148	0	0
DEELLKTVRLIKFLY (5993)	Rev 9–23	0	0	0	764
LKTVRLIKFLYQSNP (5994)	Rev 13–27	0	0	0	789
YWGLHTGERDWHLGQ (6035)	Vif 69–83	704	215	268	43
HTGERDWHLGQGVSI (6036)	Vif 73–87	831	361	376	62

[a] CTL responses identified by ELISPOT screening against overlapping peptides (obtained from the NIH ARRRR) are shown. The locations of the peptides (in relationship to the HXB2 sequence) are given. Values are SFC per 10^6 $CD8^+$ T lymphocytes (means of results of duplicate experiments).

strain. Strikingly, the majority of CTL responses decayed rapidly after superinfection, similar to the observed loss of CTL after epitope mutation and escape occurring in chronically infected persons (19). Furthermore, while new CTL responses were detected after superinfection, these were fewer and failed to contain the second virus. This result could have been a consequence of the divergent sequence of the superinfecting strain or might imply a role for a mechanism, such as original antigenic sin (22), limiting the adaptability of CTL responses after initial infection.

The marked rise in viremia after superinfection is consistent with another detailed study of superinfection in the setting of immune control achieved by structured treatment interruption therapy in early infection (2). This phenomenon is also reminiscent of the increased viremia that has been observed with escape occurring during natural infection (15, 32) and reinfusion of an ex vivo expanded CTL clone (24) and associated with SIV vaccine failure in macaques (6). The ability of CTL to recognize the challenging viral sequences thus appears to be a crucial determinant of immune control.

Superinfection and loss of immune control in this subject, despite apparently effective CTL responses to the initial strain, may have serious implications for vaccine design. The determinants of an effective CTL response against HIV-1 remain unknown, and attempts to correlate the magnitude or breadth of the response (8) to control of viremia generally have been disappointing (1). Our data, in agreement with those of Altfeld et al. (2), indicate that protective CTL targeting against HIV-1 may vary not only by subtype of virus but even by individual strains. Given the great variability of HIV-1, this phenomenon may pose a significant obstacle to generating protective immunity with a fixed vaccine sequence.

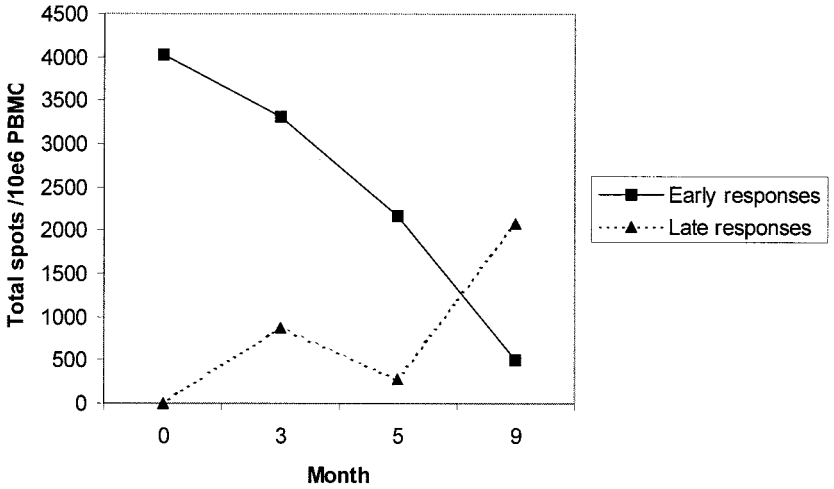

FIG. 6. Dynamics of early and subsequent CTL responses for subject 2030. The sum of CTL frequencies for responses detected upon initial testing (month 0) are plotted over time, in comparison to the sum of frequencies of CTL responses detected only after initial testing. PBMC, peripheral blood mononuclear cells.

TABLE 2. Epitope sequence comparisons of strain A and strain B[a]

Recognized peptide	Presumed epitope (HLA)	Epitope location	Sequence for strain A and strain B	ΔCTL
LKETINEEAAEWDRV	EEAAEWDRV (B*40)[c]	Gag 207–215 (p24)	EEAAEWDR**L** EEAAEWDR**L**	↓
INEEAAEWDRVHPVH ....AAEWDRVHPVHAGPI	AEWDRVHPV (B*40)[b]	Gag 210–218 (p24)	AEWDR**L**HPV AEWDR**L**HPV	↓
PGQMREPRGSDIAGT	Unknown	(Gag 225–239 [p24])	(PGQMREPRGSDIAGT) (PGQMREPRGSDIAGT)	±
EPIDKELYPLTSLRS	KELYPLTSL (B*40)[b]	Gag 481–489 (p6)	KELYPL**A**SL **RGID**KELYPL**A**SL[d]	↓
EVGFPVRPQVPLRPM	FPVRPQVPL (B*35)[b]	Nef 68–76	FPVRPQVPL FPVRPQVPL	↔
PGPGIRYPLTFGWCF ....IRYPLTFGWCFKLVP	YPLTFGWCF (B*35)[b] or RYPLTFGWCF (A*24)[b]	Nef 135–143 or 134–143	YPLTFGWCF or RYPLTFGWCF YPL**C**FGWCF or RYPL**C**FGWCF	↓
KCCFHCQVCFTTKGL	Unknown	(Tat 29–43)	(**O**CCFHCQVCF**I**TKGL) (KCC**L**HCQVCFT**R**KGL)	↓
DEELLKTVRLIKFLY ....LKTVRLIKFLYQSNP	TVRLIKFLY (A*03)[c]	Rev 15–23	TV**KI**IKFLY TVRLIKFLY	↑
YWGLHTGERDWHLGQ ....HTGERDWHLGQGVSI	HTGERDWHL (B*35)[c]	Vif 73–81	H**P**GERDWHL HTGERDWHL	↓

[a] For peptides recognized in the ELISPOT assay at a frequency of at least 100 SFC/10^6 $CD8^+$ T lymphocytes, the following are listed: the presumed epitope and its location, the sequence of the epitope in strain A (top) and strain B (bottom; differences from the screening sequence are shown in underlined boldface type), and the change of the CTL response following superinfection. Putative locations and sequences are shown in parentheses. ↓, Present before superinfection and waned after superinfection; ↑, present only after superinfection; ↔, persisting before and after superinfection; ±, present early but waned before superinfection.

[b] Previously reported epitope consistent with subject's known HLA type (A*03, A*24, B*35, B*40).

[c] Inferred from the HLA binding motifs of the subject's known HLA type.

[d] Insertion of four amino acids.

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