Highly pathogenic adapted HIV-1 strains limit host immunity and dictate rapid disease progression

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Objective: The study of HIV-1 rapid progressors has been limited to specific case reports. Nevertheless, identification and characterization of the viral and host factors involved in rapid progression are crucial when attempting to uncover the correlates of rapid disease outcome.

Design: We carried out comparative functional analyses in rapid progressors ($n = 46$) and standard progressors ($n = 46$) early after HIV-1 seroconversion (≤ 1 year). The viral traits tested were viral replicative capacity, co-receptor usage, and genomic variation. Host CD8^{+} T-cell responses, humoral activity, and HLA immunogenetic markers were also determined.

Results: Our data demonstrate an unusual convergence of highly pathogenic HIV-1 strains in rapid progressors. Compared with standard progressors, rapid progressor viral strains show higher in-vitro replicative capacity (81.5 vs. 67.9%; $P = 0.025$) and greater X4/DM co-receptor usage (26.3 vs. 2.8%; $P = 0.006$) in early infection. Limited or absent functional HIV-1 CD8^{+} T-cell responses and neutralizing activity were measured in rapid progressors. Moreover, the increase in common HLA allele-restricted CD8^{+} T-cell escape mutations in rapid progressors acts as a signature of uncontrolled HIV-1 replication and early impairment of adaptive cellular responses.

Conclusion: Our data support a dominant role for viral factors in rapid progressors. Robust HIV-1 replication and intrinsic viral properties limit host adaptive immune responses, thus driving rapid disease progression.

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Introduction

The clinical outcome of HIV-1-infected patients varies considerably. In the absence of antiretroviral therapy, the median time to development of AIDS has been estimated to vary from 7.7 to 11.0 years, depending on age at seroconversion [1]. Nevertheless, some individuals are able to control HIV-1 replication for longer periods (HIV-1 controllers and elite controllers), whereas others progress quickly to AIDS or meet the criteria for initiation of antiretroviral treatment within the first 3 years after seroconversion (rapid progressors). Thus, research into extreme phenotypes can provide us with invaluable data on the contribution of viral and host factors to HIV-1 disease outcome [2–4].

Most studies on extreme HIV-1 phenotypes have focused on HIV-1 controllers and elite controllers as models for defining the correlates of protective immunity and control of viral replication [3,5]. The study of rapid progressors, on the contrary, has proved particularly challenging, because identification of this phenotype requires a reliable estimation of the seroconversion date within a small observation window [4]. This limitation is even more remarkable if we consider that multiple biological samples need to be taken for analysis. Consequently, very few core studies have analysed rapid progressors, and the data reported thus far are heterogeneous [6–8]. Nevertheless, the factors contributing to rapid disease progression must be uncovered to improve the clinical management of these individuals.

The present study is based on a previous case report study analysing extremely severe progression of HIV-1 infection [7] and attempts to broadly characterize rapid progressors in functional terms by assessing viral and host factors. Our objective was to provide new insights into the causes of rapid disease progression. We recruited an unprecedentedly large group of rapid progressors and systematically compared them to HIV-1-infected patients with average disease progression. Our selection criteria for rapid progressors were based on the reduction in CD4$^+$ T-cell counts to less than 350 cells/μl within 3 years after documented seroconversion in the absence of antiretroviral treatment, as previously validated by our genomic studies [4].

Our findings reveal a profile of HIV-1-specific traits (e.g. replicative capacity, co-receptor usage, and virus variability) that is common to rapid progressors. Additional differences between groups were observed in cellular and humoral immune profiles and in HLA class I immunogenetics.

Material and methods

Cohort description and study groups

We established two progression cohorts: one from IrsiCaixa (Barcelona, Spain) and one from the Swiss HIV Cohort (Lausanne, Switzerland). The cohorts were defined based on an HIV-1 seroconversion window of less than 1 year and documented negative and positive serology result. Disease progression stage was established during the 3 years after seroconversion (Fig S1, http://links.lww.com/QAD/A517). Patients in the first cohort (CoRP, $n = 106$) had two or more CD4$^+$ T-cell counts below 350 cells/μl with no subsequent increase in the absence of antiretroviral treatment. Individuals with average progression or standard progressors (CoSP, $n = 84$) maintained CD4$^+$ T-cell counts above 350 cells/μl over the same period in the absence of antiretroviral treatment. From these two cohorts, we selected two study groups: the rapid progressors ($n = 46$) and the standard progressors ($n = 46$). These groups were selected based on clinical data and sample availability early after HIV-1 seroconversion at baseline (median ≤1 year) and a median of 1.5 years from initial sampling post-baseline. Samples were distributed and prioritized according to functional analysis (for details, see Fig S1, http://links.lww.com/QAD/A517). The characteristics of both groups are summarized in Table 1. Differences in the mode of HIV-1 transmission between the groups were due to an increase in the number of intravenous drug users (IVDUs) in rapid progressors. Consistency of the results was demonstrated by sensitivity analyses in all data sets regardless of the presence of IVDUs in rapid progressors. The project was approved by the institutional review boards of Hospital Germans Trias i Pujol and the University Hospital of Lausanne. Participants gave their written informed consent to participate.

HIV-1 isolation, replicative capacity, and co-receptor usage

Viral isolates were obtained from plasma samples using anti-CD44 beads (Miltenyi Biotec, Germany [7,9]. Viral isolates from cryopreserved PBMCs were obtained by co-culture as described in reference [7]. Virus replicative capacity was determined for all isolates. Viral growth was monitored by p24 ELISA in supernatants (Perkin Elmer, Spain), and replicative capacity was calculated by the slope of p24 production in the exponential growth phase [10]. Tropism from viral isolates was measured in U87-immortalized cell lines expressing CCR5 or CXCR4 [11]. Virus tropism was assessed in parallel in plasma samples using the enhanced sensitivity tropism assay (ESTA).

HLA typing and assessment of HIV-1-specific CD8$^+$ T-cell responses

High-resolution HLA class I typing for alleles A, B, and Cw was performed by sequence-based typing methods. Comprehensive HIV-1 epitope screening of optimal responses was carried out using the interferon-γ (IFN-γ) ELISpot assay in a subset of patients, as previously described [12] (Table S1, http://links.lww.com/QAD/A517). Wells were considered positive if they contained at least 50 spot-forming cells per 10^6 PBMCs above the

Table 1. Characteristics of the rapid and standard progressors included in the study.

Male sex [n (%)]	35 (76.1)	43 (93.5)	0.02
Age at diagnosis of HIV-1 infection, median (IQR) (years)	31 (26–37)	30 (26–35)	0.48
White ethnicity [n (%)]	39 (84.8)	32 (71.1)	0.08
Mode of transmission [n (%)]			0.002
Heterosexual	12 (26.1)	5 (10.9)	
MSM[a]	20 (43.5)	37 (80.4)	
IVDU[b]	10 (21.7)	1 (2.2)	
Other and NA	4 (8.7)	3 (6.5)	
Viral subtype B [n (%)]	38 (86.4)	43 (93.5)	0.11
Baseline CD4^{+} T-cell count[c] [median (IQR)]	385 (245–508)	658 (442–804)	<0.0001
Baseline viral load[d] [log$_{10}$ copies/ml, median (IQR)]	4.88 (4.48–5.51)	4.35 (3.75–4.99)	0.02
Viral load set point[e] [log$_{10}$ copies/ml, median (IQR)]	4.73 (4.28–5.21)	4.10 (3.22–4.37)	<0.0001
Mean CD4^{+} T-cell count in the absence of ART[f] (IQR)	317.3 (216.1–393.7)	615.3 (518.8–713.6)	<0.0001

IQR, inter-quartile range; IVDU, intravenous drug user; NA, not available.
[a]MSM.
[b]Intravenous drug users.
[c]Baseline CD4^{+} T-cell count was available for 37 rapid progressors and 30 standard progressors.
[d]Baseline viral load was available for 34 rapid progressors and 28 standard progressors.
[e]Viral load set point was defined by the level of circulating virus in plasma during the non-symptomatic phase preceding progression to AIDS (steady stage). Data were available for 35 rapid progressors and 37 standard progressors.
[f]Mean CD4^{+} T-cell count was defined by the mean of the levels of CD4^{+} cell counts during the non-symptomatic phase preceding progression to AIDS (steady stage). Data were available for 43 rapid progressors and 39 standard progressors.

background level ($2\times$ mean $+ 3\times$ STD). The frequency of HIV-1-specific responses was calculated as follows: [number of positive responses/number of optimal peptides tested] $\times$ 100. The frequency of response was corrected by the number of optimal peptides tested per sample.

Evaluation of viral susceptibility to neutralization and plasma HIV-1-neutralizing activity

Neutralization experiments were performed using the PhenoSense HIV-1 neutralization assay. To determine virus sensitivity to neutralization in rapid progressors and standard progressors, Env-pseudotyped viruses from both groups were tested against a panel of broadly neutralizing antibodies (bNAbs) (b12, 4E10, 2F5, 2G12). Neutralization values are expressed as the concentration of IgG that reduces infectivity by 50% (IC$_{50}$). Heterologous neutralizing activity of plasma was tested against a standard panel of HIV-1 isolates. The reference panel viruses NL4-3, SF162, and JR-CSF were included as positive controls and aMLV was used as specificity control. Neutralizing activity was also tested in a panel of autologous and heterologous Env-pseudotyped recombinant viruses obtained from plasma samples [13]. Neutralizing antibody titres in plasma samples were calculated as described in reference [14].

HIV-1 sequencing and variation at optimal CD8^{+} T-cell epitopes

Partial HIV-1 genome sequencing was carried out for Gag and Pol genes in plasma samples from 40 patients (22 rapid progressors and 18 standard progressors). Gag and Pol genes were amplified from viral RNA by one-step RT-PCR and nested PCR. Virus subtype was assigned using the REGA HIV-1 subtyping tool in Gag and Pol sequences. Deep sequencing of Gag covering the TW10 region in RP52 was carried out in total viral RNA from

plasma or primary viral isolates, as previously described [7].

Optimal HIV-1 CD8^{+} T-cell epitopes were tested based on the best-defined CD8^{+} epitope summary from the Los Alamos Molecular Immunology Database. HIV-1 variation at the optimal CD8^{+} T-cell epitopes was determined in Gag and Pol viral sequences matched to patients' HLA class I alleles with four-digit resolution. HIV-1 variation at optimal epitopes was defined using the EPIMAP tool (available upon request), which allowed fuzzy matching with up to three substitutions per HIV-1$_{HXB2}$ epitope and the HLA-I restriction element. The frequency of virus variation was defined as the ratio of total mismatches found at optimal epitopes to the number of total optimal epitopes found per sequence.

Statistical analysis

For the clinical group comparison, *P*-values were calculated using the Mann–Whitney and Fisher's exact test. The relationship between CD4^{+} T-cell count and replicative capacity was assessed using the Spearman's correlation coefficient. The statistical significance of the HLA class allelic distribution was reported after correcting for multiple comparisons using the Benjamin and Hochberg procedure. All analyses were performed using SAS 9.3.

Results

HIV-1 strains from rapid progressors are highly replicative and have greater X4/DM co-receptor usage

Virus replicative capacity and co-receptor usage are essential for establishing a productive HIV-1 infection

when transmission bottlenecks occur and for determining the speed of CD4+ T-cell depletion during early stages. These viral traits have been widely associated with the pathogenesis and outcome of HIV-1 infection [15–18] and are crucial for evaluation in the context of rapid progression of HIV-1. Thus, we assessed viral replicative capacity and co-receptor usage in rapid progressors and compared them with those of standard progressors in samples obtained early after seroconversion (median ≤1 year).

We measured replicative capacity *in vitro* in primary isolates from rapid progressors ($n = 27$) and standard

progressors ($n = 20$). Our results revealed a significantly higher viral replicative capacity in isolates from rapid progressors than in those from standard progressors in early infection (median 81.5 vs. 67.9%; $P = 0.025$; Fig. 1a). We then studied the correlation between replicative capacity and clinical parameters as predictors of rapid progression. Our data demonstrate an inverse correlation between replicative capacity and baseline CD4+ T-cell counts in the overall study group [19,20]. In addition, a complementary analysis by disease status revealed the specific contribution of the highest replicative virus to the significant association between replicative capacity and baseline CD4+ T-cell count
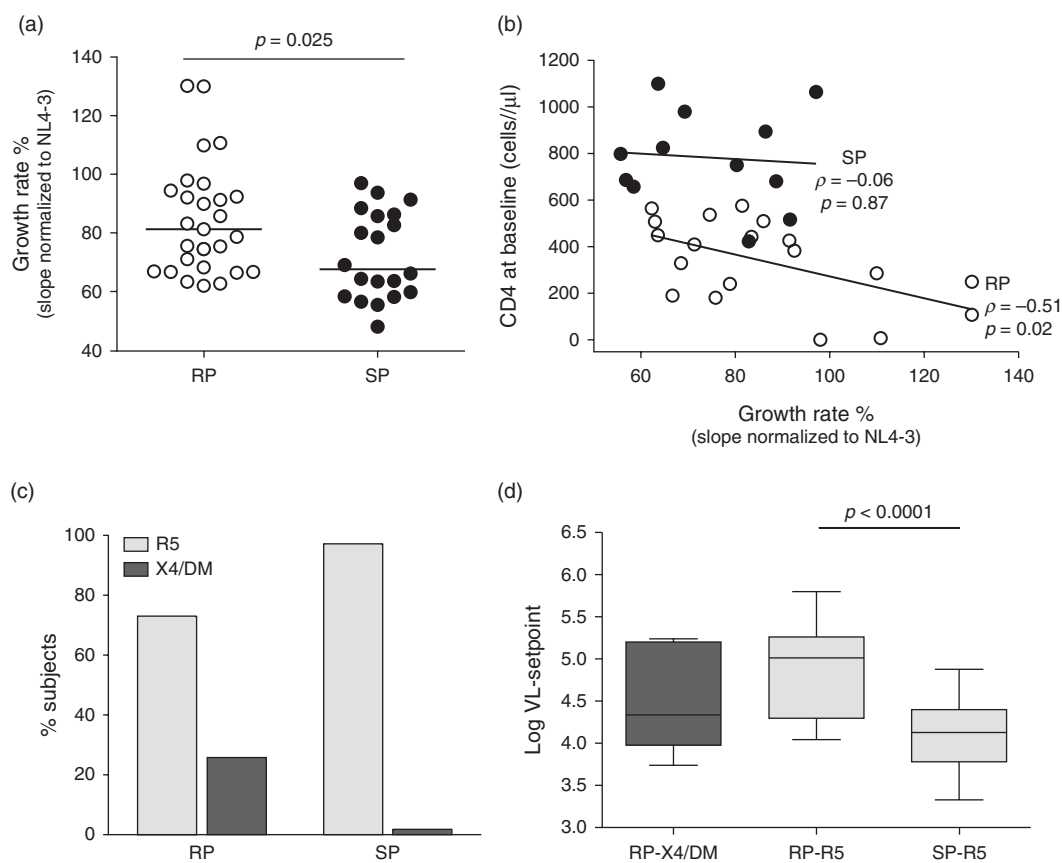

Fig. 1. HIV-1 co-receptor usage and replicative capacity in rapid progressors and standard progressors. (a) Viral replicative capacity from primary isolates in rapid progressors (RPs) and standard progressors (SPs). Replicative capacity was measured in 27 RP and 20 SP at baseline. Experiments were carried out in stimulated PBMC pooled from three seronegative individuals. PBMCs were then infected in duplicate to a multiplicity of infection of 0.001 of each primary isolate in independent cultures for 10 days. Variation between replicates was low. Viral growth was measured every 3 days based on p24 production, and replicative capacity was calculated as the slope in the exponential growth phase for each isolate. Values were normalized to the slope of HIV-1$_{NL4-3}$ as 100%. The horizontal line represents median values (the *P*-value was calculated using the Mann–Whitney test). (b) Correlation between baseline CD4+ T-cell counts and replicative capacity. Lines indicate the fit of the data to linear regressions in 27 RP and 20 SP. The Spearman correlation coefficient (ρ) and the associated two-tailed *P*-value were calculated per group. (c) Total frequency of R5 and X4/DM virus in RP and SP. Viral tropism was evaluated in 38 RP and 36 SP by Trofile and U87. Inter-assay concordance was 96%. Discordant results were recorded in only three samples, all of which were R5 by ESTA and X4/DM by U87. We assigned 2 as R5 by ESTA owing to the very low levels of p24 in U87-X4 culture. The other was defined as X4/DM based on U87-X4 positivity and clonal sequences reporting X4/DM usage. Grey bars represent R5 virus and black bars X4/DM tropic virus. (d) Viral load set point stratified by virus co-receptor usage. The box and whiskers plot shows median values and 10th–90th percentiles in 33 RP and 30 SP. The *P*-values were calculated using the Mann–Whitney test. Only significant *P*-values ($P < 0.05$) are shown. ESTA, enhanced sensitivity tropism assay.

(Fig. 1b, rapid progressors; Spearman's $\rho = -0.51$, $P = 0.002$).

We also analysed virus co-receptor usage in 74 samples (38 rapid progressors and 36 standard progressors) using the ESTA and the U87 phenotypic assay; inter-assay concordance was 96%. We found a previously unreported increase in X4/DM co-receptor usage in association with rapid progression (26.3% rapid progressors vs. 2.8% standard progressors; $P = 0.006$) (Fig. 1c). These results indicate the contribution of X4/DM virus to the superior replicative capacity in a subset of rapid progressors. However, despite the increase in X4/DM distribution, the remaining 73.7% of rapid progressors carried R5 variants. These R5 variants were associated with higher plasma viraemia ($P < 0.001$; Fig. 1d), thus supporting the pathogenic properties of the R5 virus in rapid progressors.

Taken together, these results demonstrate the contribution of virus replicative capacity and X4/DM co-receptor usage to CD4$^+$ T-cell depletion and rapid disease progression.

Rapid progressors have weak or absent anti-HIV-1 cellular and humoral adaptive responses

Once the viral traits that prevail in HIV-1 rapid progressors were established, we investigated the contribution of host immune factors to rapid disease outcome. Therefore, we extended our comparative analyses to cellular and humoral functional markers in our study groups. We focused on virus-specific CD8$^+$ T-cell responses, virus susceptibility to neutralization, and plasma–neutralizing activity as key components of the initial adaptive immune responses against HIV-1.

We measured HIV-1-specific CD8$^+$ T-cell responses in a subgroup of 17 rapid progressors and 13 standard progressors in a comprehensive panel of optimally defined HIV-1 CD8$^+$ T-cell epitopes. Our results demonstrated weak or even absent HIV-1-specific CD8$^+$ T-cell responses in rapid progressors when compared to standard progressors at baseline (mean 2.58% rapid progressors vs. 10.45% standard progressors; $P = 0.004$) (Fig. 2a). These differences in early infection were maintained when responses were tested later in infection (post-baseline: mean 1.34% rapid progressors vs. 8.29% standard progressors; $P = 0.005$) (Fig. 2a), with no improvement or recovery despite normalization of CD4$^+$ T-cell levels after initiation of treatment in rapid progressors.

We also evaluated humoral responses by measuring virus susceptibility to neutralization and plasma–neutralizing activities across groups. No differences between rapid progressors and standard progressors were observed regarding the sensitivity of HIV-1 Env-pseudotyped virus against a panel of bNAbs (Fig. 2b). Moreover, plasma-neutralizing activity in 12 rapid progressors and 32 standard progressors was evaluated against a panel of viral isolates with a wide range of susceptibilities to neutralization (Table S2, http://links.lww.com/QAD/A517). Even though a consistent trend towards lower IC$_{50}$ values was observed in rapid progressors, the results did not reach statistical significance (Fig. 2c). Similarly, no differences were found between groups in total heterologous plasma-neutralizing activity (Fig. 2d and Table S3, http://links.lww.com/QAD/A517). Furthermore, autologous plasma neutralization was generally low against contemporaneous virus and only increased in standard progressors over time (rapid progressors vs. standard progressors; $P = 0.019$) (Fig. 2e). By contrast, autologous neutralizing titres against ancestral viruses were higher in both groups [rapid progressors ($P = 0.024$) and standard progressors ($P < 0.0001$)], but with a lower increase in the rapid progressors within the same period (Fig. 2e).

These data demonstrate the lack of early anti-HIV-1 adaptive host immune response in rapid disease progression. Weak or absent specific CD8$^+$ T-cell responses, together with limited neutralizing activity, define a common dysfunctional immune phenotype in rapid progressors.

Presence of HIV-1 variants adapted to common HLA class I alleles is associated with rapid disease progression

The high rate of initial virus adaptation to or escape from HLA class I molecules is a major limitation for the early control of HIV-1 by CD8$^+$ T-cell responses [21]. Thus, transmission of HLA-adapted variants has been associated with rapid progression [2], even in individuals with protective HLA alleles [22,23]. Therefore, in order to understand the lack of early anti-HIV-1 CD8$^+$ T-cell responses in rapid progressors, it is critical to identify the contribution of HIV-1 adaptation to or escape from HLA class I immunogenetics.

Initially, to define the contribution of HLA-adapted strains to rapid progression, we focused on patients with protective HLA class I alleles (HLA-B*58 and B*27) and rapid progression. We identified one HLA-B*27 rapid progressors and three HLA-B*58 rapid progressors. We evaluated the existence of adapted virus to protective KK10 and TW10 Gag epitopes. As a result, we found immune escape variants at positions K264R in KK10 and T242N in TW10 in all rapid progressors. By contrast, no immune escape was observed in two HLA-B*27 standard progressors within the same period of infection (Fig. 3a). Additionally, Gag K264R and T242N mutations known to cause viral replication defects [24,25] appeared alongside compensatory changes in replication at positions S173 for K264R and I223/G248 for T242N in rapid progressors (Fig. 3a). Development of such a
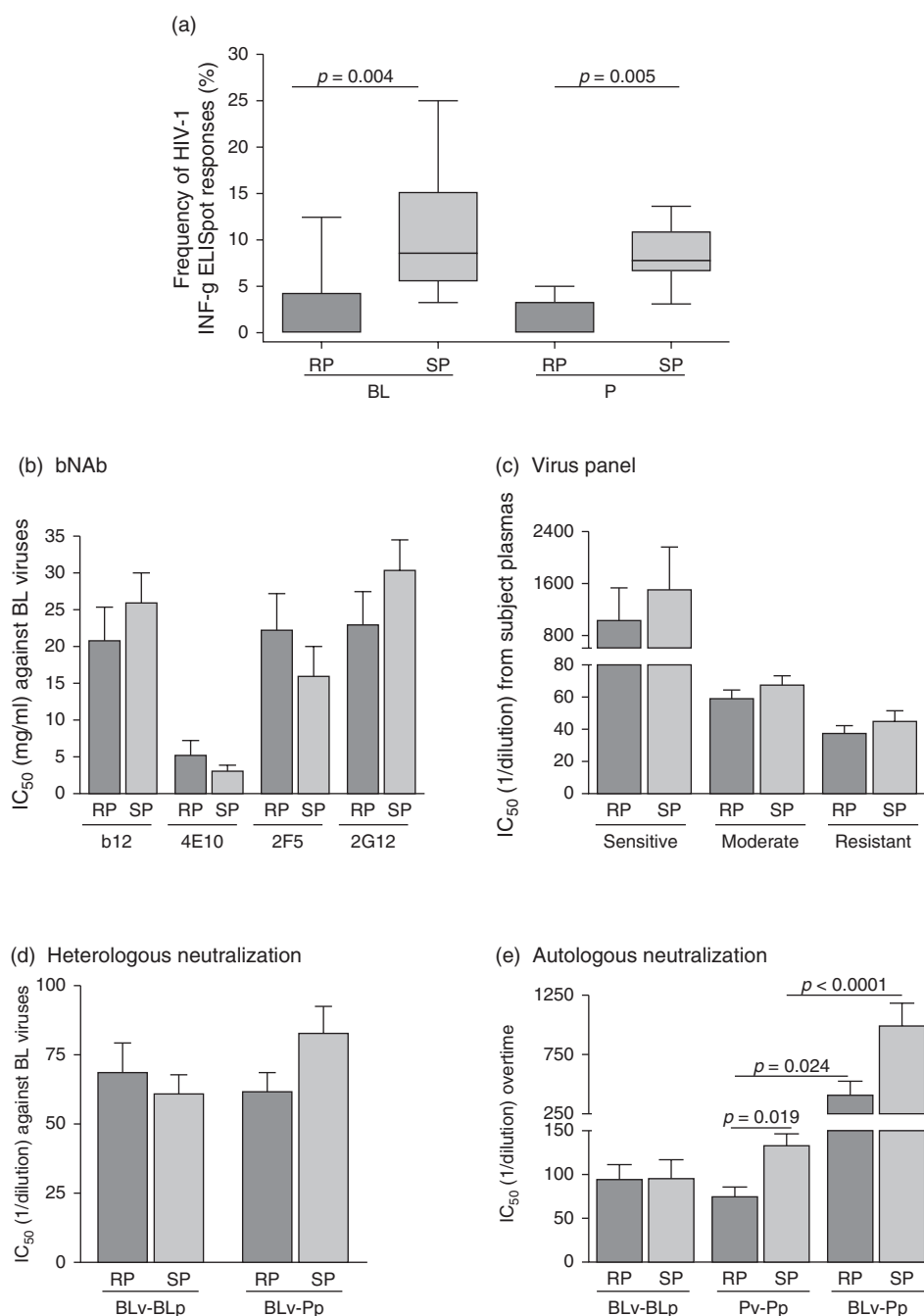
Fig. 2. HIV-1-specific CD8$^+$ T-cell responses, virus sensitivity to neutralization and plasma-neutralizing activity in rapid progressors and standard progressors. (a) Frequency of IFN-γ HIV-1-specific CD8$^+$ T-cell responses at baseline (BL) and post-baseline (P) were measured in PBMCs against a panel of optimally defined HLA class I HIV-1 epitopes in 17 rapid progressors (RPs) and 13 standard progressors (SPs). The box and whiskers plot shows median values and 10th and 90th percentiles. The *P*-values were calculated using the Mann–Whitney test. Only significant *P*-values ($P < 0.05$) are shown. (b) Sensitivity to neutralization of Env-pseudotyped virus derived from the plasma of 24 RP and 31 SP against a panel of broadly neutralizing antibodies (bNAbs) including b12, 4E10, 2F5, and 2G12. (c) Heterologous plasma-neutralizing activity at baseline against a standard panel of viral isolates from various subtypes and with various ranges of susceptibilities to neutralization (sensitive, moderate, and resistant). (d) Heterologous cross-reactive virus-neutralizing activity in plasma samples, at baseline (BLp) and post-baseline (Pp), against a panel of baseline patient-derived Env-pseudotyped viruses (BLv). (e) Autologous neutralizing activity in plasma and Env-pseudotyped virus pairs. All *P*-values were calculated using the Mann–Whitney test. Only significant *P*-values ($P < 0.05$) are represented. BLp, baseline plasma; BLv, baseline virus; Pp, post-baseline plasma; Pv, post-baseline virus.
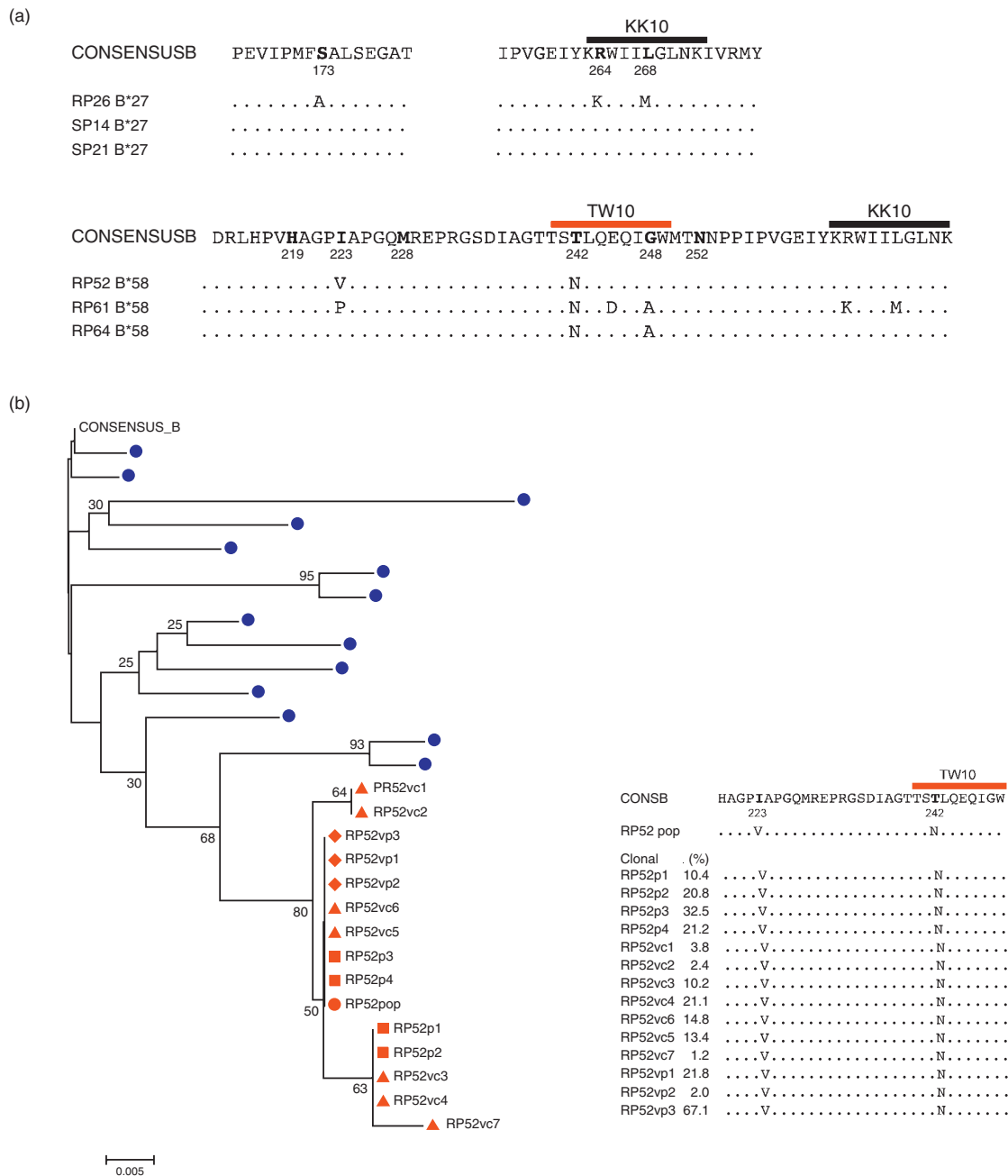
(a)

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                                                    KK10
                                              _____
CONSENSUSB   PEVIPMFSALSEGAT        IPVGEIYKRWIILGLNKIVRMY
                    173                     264  268

RP26 B*27    .......A.......        .......K...M.........
SP14 B*27    ..............         .....................
SP21 B*27    ..............         .....................
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                                            TW10                          KK10
                                        _____                    _____
CONSENSUSB   DRLHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNK
                  219   223   228           242    248  252

RP52 B*58    ..........V.................N................................
RP61 B*58    ..........P.................N..D..A...............K...M....
RP64 B*58    ...........................N.....A..........................
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(b)


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                                        TW10
                                   _____
CONSB   HAGPIAPGQMREPRGSDIAGTTSTLQEQIGW
           223                  242
RP52 pop    ....V.................N.......

Clonal    (%)
RP52p1   10.4   ....V.................N.......
RP52p2   20.8   ....V.................N.......
RP52p3   32.5   ....V.................N.......
RP52p4   21.2   ....V.................N.......
RP52vc1   3.8   ....V.................N.......
RP52vc2   2.4   ....V.................N.......
RP52vc3  10.2   ....V.................N.......
RP52vc4  21.1   ....V.................N.......
RP52vc6  14.8   ....V.................N.......
RP52vc5  13.4   ....V.................N.......
RP52vc7   1.2   ....V.................N.......
RP52vp1  21.8   ....V.................N.......
RP52vp2   2.0   ....V.................N.......
RP52vp3  67.1   ....V.................N.......
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Fig. 3. HIV-1 strains adapted to HLA-B∗58 and B∗27 alleles in rapid progressors at Gag TW10 and KK10 protective epitopes. Gag viral sequences were obtained from plasma samples in an HLA-B*27 RP (RP26) and 3 HLA-B*58 RP (RP52, RP61 and RP64). For comparative purposes, Gag sequences were obtained from two HLA-B*27 SP (SP14, SP21). (a) Comparison of HIV-1 Gag amino acid sequences at KK10 and TW10 epitopes. Epitopes are marked at the top of the sequence with a black line (KK10) or red line (TW10). Letters in bold indicate residues associated with escape in KK10 (264, 268) and replicative capacity compensatory sites (173). Similarly, bold letters indicate escape in TW10 (242) and replicative capacity compensatory sites (219, 223, 248, 252). Amino acid numbering is based on the HXB2 sequence. (b) Gag phylogenetic tree in HLA-B*58 RP52 covering the TW10 epitope and the 223 compensatory site at 12 months after seroconversion. The tree was built by the neighbour-joining method based on over 1000 bootstrap replicates. The tree was rooted to the consensus B Gag sequence and edited with MEGA.5. The number at the nodes indicates bootstrap support. Blue dots indicate Gag population sequences from additional RP. Red symbols indicate RP52 Gag sequences. The red dot represents the population sequence (RP52pop). Squares, diamonds, and triangles represent clonal viral sequences. Red triangles and diamonds are clonal viral sequences obtained from primary viral isolates from cells (RP52vc) or plasma (RP52vp). Red squares are clonal viral sequences obtained directly from total plasma (RP52p). The alignment next to the tree shows the clonal populations of TW10 escape variants in RP52. The frequency of the variant in the total population is expressed as a percentage next to each clonal sequence. RP, rapid progressors; SP, standard progressors.

complex mutational pattern is rare in early infection [23,26,27] and likely represents a marker of HIV-1 transmission events. This observation is reinforced by the presence of KK10 escape mutants in non-B*27 rapid progressors (Fig. 3a) and by the homogeneous clonal populations of TW10 escape mutants found in an HLA-B*58 rapid progressors (Fig. 3b). Overall, these findings are consistent with the low level of viral genetic variability described in recent infection and probably identify transmission events in rapid progression.

With the aim of generalizing previous observations to specific immunogenetic profiles in rapid progressors, we performed an exploratory analysis of HLA class I distribution in 122 HIV-1-infected individuals (74 rapid progressors and 48 standard progressors). Our data demonstrate an increase in the frequency of common Caucasian HLA haplotypes, including HLA-A*02:01, B*07:02, and B*08:01 in rapid progressors (75.6% rapid progressors vs. 50% standard progressors) to the detriment of protective ones (6.76% rapid progressors vs. 22.9% standard progressors) Fig. 4a. In addition, a modest increase was observed in risk alleles. Similar trends across data analyses were supported by individual HLA type analyses (Table S4, http://links.lww.com/QAD/A517).

In order to evaluate the contribution of virus adaptation or escape to the immunogenetics of rapid progressors, we focused on HLA-A*02:01 carriers because of their higher prevalence in the study population. Thus, we determined HIV-1 variation at the immunodominant A*02:01-restricted SL9-Gag epitope. We compared HIV-1 SL9-Gag variation in HLA-A*02:01-positive individuals (A*02:02+ve) early after seroconversion (Fig. 4b). Our data demonstrated preferential variation at position 3 (Y3F), but not position 8, of the SL9–Gag epitope in A*02:01+ve rapid progressors when compared to A*02:01+ve standard progressors (62.5% rapid progressors vs. 0% standard progressors; $P = 0.031$) (Fig. 4b). These differences revealed a specific increase in SL9 escape variants in rapid progressors. Furthermore, to extend these findings to complete viral proteins, we measured total variation in Gag and Pol regions and limited the analyses to those epitopes recognized by each patient's HLA. Our data indicated a significant increase in total Gag epitope variation in viral strains from rapid progressors when compared to standard progressors (1 rapid progressors vs. 0.63 standard progressors; $P = 0.008$) (Fig. 4c), but not in Pol. Moreover, these differences in epitope variation were not equally distributed across functional domains in Gag, but significantly accumulated in the p24 region (0.87 rapid progressors vs. 0.35 standard progressors; $P = 0.0084$) (Fig. 4d). These results support the association between HLA class I adaptation or escape of HIV-1 variants and rapid disease progression. This association is marked by a high prevalence of common Caucasian haplotypes and higher mutational changes against p24 epitopes.

Discussion

The time required for the development of AIDS after HIV-1 infection is associated with the temporal equilibrium between viral and host factors. This dynamic interplay can be disrupted and present as extreme disease that either contributes to prolonged periods of HIV-1 control or drives rapid disease progression. In this context, rapid progression remains one of the least understood extreme HIV-1 phenotypes, yet it must be understood if we are to tackle the epidemic. Consequently, the present study overcomes previous study limitations through the identification and functional characterization of the largest group of rapid progressors to date. For the first time, we provide evidence of the contribution of intrinsic viral properties to rapid disease progression in a study group.

Our data demonstrate how viral factors, including robust HIV-1 replication, higher X4/DM co-receptor usage, and an increased number of common HLA footprints, could lead to uncontrolled virus replication. Such a robust replicative profile will likely limit the generation of adaptive responses and precipitate rapid disease progression. The pathogenic HIV-1 strains found in rapid progressors are rare at the onset of infection, and discrepancies in viral factors are most apparent with regard to viral tropism. Thus, the reported prevalence of X4/DM virus ranged from 5 to 10% in primary infection [17,28], compared with above 25% in rapid progressors. However, differences in the selection criteria according to rapid disease progression might partially account for the divergence between studies. Although X4/DM viruses are more pathogenic than R5 viruses [29], R5 variants in rapid progressors were equally associated with high replicative capacity and viral load set point. Therefore, these data highlight a previously unnoticed contribution of R5 strains to disease progression.

Intrinsic viral properties may also contribute to the limited adaptive immunity found in rapid progressors, which is key to controlling HIV-1 replication upon transmission. We observed defective HIV-1-specific CD8$^+$ cellular responses and no major role of neutralizing antibodies, as previously reported [30,31]. However, the generalized static immune phenotype in rapid progressors does not reflect a pre-existing immunodeficiency [2], but rather an outstanding loss of CD4$^+$ T cells in primary infection. The dramatic loss of CD4$^+$ T cells will irreversibly affect CD4$^+$ homeostasis and thus compromise CD4$^+$ T-cell helper responses and CD8$^+$ T-cell [32] and B-cell function [33,34]. In addition, subsequent limited anti-HIV-1-adaptive immunity established early on could add to the lack of control of viral replication and disease progression.

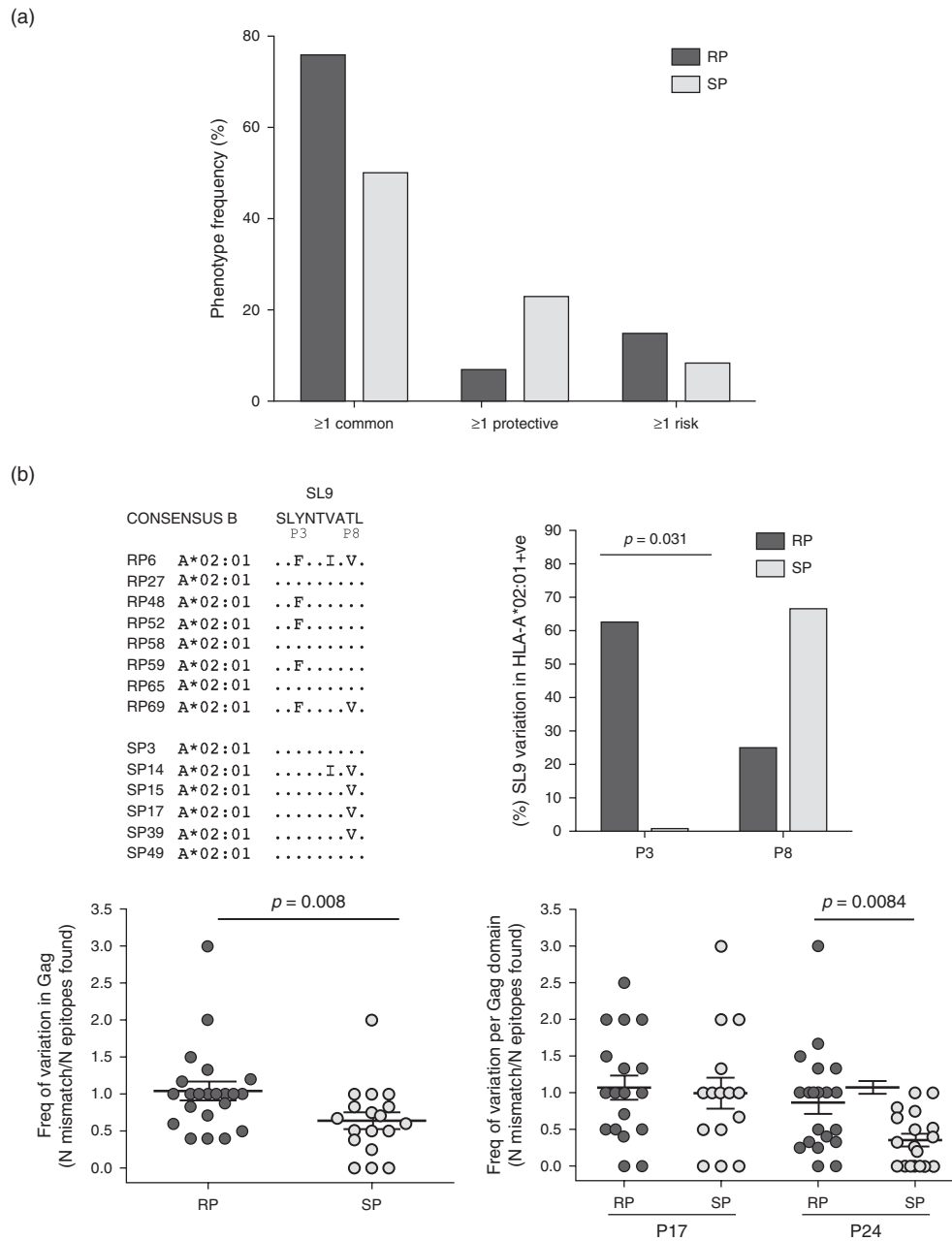In this study, we observed early disruption of host immunity in favour of viral replication. This phenomenon

Fig. 4. HLA class I immunogenetics and HIV-1 variation at HLA-matched epitopes in rapid progressors and standard progressors. (a) Phenotypic frequency (number of patients with a defined haplotype/number of total individuals in the group × 100) in pooled analyses for HLA class I alleles. Patients in groups (74 RP and 48 SP) were included if they had at least one common allele (A*02:01, B*07:02 and B*08:01), a protective allele (B*57:01, B*14:01 and B*27:05), or a risk allele (B*35:02, B*35:03, B*35:04 and B*53:01). Only those alleles with a frequency above 3% in the overall population were taken into account for data representation. (b) HIV-1 SL9 epitope alignment in HLA-A*02:01+ve RP and SP; P3 and P8 indicate amino acid sites in SL9. The bar graph shows the frequency of epitope variation at P3 and P8 sites in HLA-A*02:01+ve SP and RP. (c) Frequency of epitope variation in HIV-1 Gag. Partial sequencing was carried out in a total of 40 patients (22 RP and 18 SP). (d) Frequency of HLA-matched epitope variation in HIV-1 Gag per functional domains p17 and p24. Values in (c) and (d) are expressed as mean and SEM. The *P*-values were calculated using the Mann–Whitney test. Only significant *P*-values ($P < 0.05$) are represented. RP, rapid progressors; SP, standard progressors.

seems to be associated with the increase in HLA class I virus adaptation or escape. HIV-1 adapts to the most frequent alleles in a population [21]. Therefore, the probability of transmission of adapted viruses will increase among common HLA carriers, thus reducing the chances for a potent anti-HIV-1 CD8+ T-cell response [21,35]. Consistent with this hypothesis, our data identified the accumulation of common Caucasian alleles, including

A*02:01, B*07:02, and B*08:01, in more than 75% of rapid progressors. Our results confirm and extend those of previous studies showing a disadvantage of A2 and B7 supertypes in control of HIV-1 infection [35,36]. According to this immunogenetics profile, the likelihood of having adapted or escaped variants should increase in rapid progression of HIV-1. This observation is consistent with the presence of well adapted and well compensated viruses in HLA-B*58 and HLA-B*27 patients and reinforced by the spread of SL9 escaped variants in HLA-A*02:01-positive rapid progressors. In addition, the overall increase in HIV-1 Gag-p24 mutations matched to HLA class I epitopes in rapid progressors, but not in standard progressors, points to the existence of adapted or escape variants during the first year after seroconversion. Despite the fact that we cannot directly associate specific amino acid changes in Gag-p24 epitopes with HIV-1 escape, multiple intra-epitope variation might interfere with the generation of particularly protective Gag CD8$^+$ T-cell responses [3,37−39]. Although our study demonstrated an increase in HLA class I-adapted HIV-1 variants with rapid progression, we were unable to determine whether viruses were transmitted as homogeneous adapted founder strains or whether escape mutations quickly emerge through the robust replicative profile in acute infection. Nevertheless, the presence of viruses with complex mutational patterns found only at late stages of disease [23,26,40] and homogeneous populations of escape mutants in early infection seem to indicate that transmission events are the origin of these pathogenic strains. Thus, rapid progression could originate from acquisition of HIV-1 from chronically infected untreated individuals [41]. These source patients increase the probability of transmission of X4/DM or pathogenic R5 variants [42], which are fully replicative and adapted to host HLA through long-term viral replication.

The limitations of the present study include the lack of transmission pairs to clarify the contribution of founder viruses to rapid progression. Moreover, ideally, comparisons between groups should be made during acute stages, which might minimize differences at baseline. However, this would reduce the number of study participants and thus make it difficult to draw conclusions. Additionally, availability of biological material was limited owing to the size and heterogeneity of the samples included in some sub-analyses.

In conclusion, our findings suggest that rapid progression results from the acquisition of highly pathogenic strains already adapted to HLA class I alleles. These strains avoid the initial control of HIV-1 replication mediated by adaptive CD8$^+$ T-cell responses, leading to rapid progression, and might help to explain the increased virulence of HIV-1 observed over the course of the epidemic [43]. Therefore, it would be advisable to develop strategies for early identification of rapid progressors, specific clinical guidelines, and robust prevention policies in order to provide adequate care and to prevent the spread of highly pathogenic viral strains in the population.

Acknowledgements

Conflicts of interest

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