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Rapid HIV Progression Is Associated with Extensive Ongoing Somatic Hypermutation

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The Ab response to HIV is of great interest, particularly in the context of a protective vaccine and broadly neutralizing Abs, but research is typically geared toward elite controllers because of their ability to successfully control the virus. In this study, we studied the evolution of the Ab repertoire over the first year of HIV infection in people classified as rapid progressors (RP) compared with typical progressors. HIV RPs are an important yet understudied group of HIV patients classified by a rapid decline in CD4 counts and accelerated development of AIDS. We found that the global IgG somatic hypermutation load negatively correlated with disease progression, possibly because of exaggerated isotype switching of unmutated sequences in patients with low CD4 counts. We measured Ab sequence evolution over time using longitudinal samples taken during the early stages of infection and 1 year postinfection. Within clonal lineages spanning both timepoints, visit 2–derived sequences harbored considerably more mutations than their visit 1 relatives. Despite extensive ongoing somatic hypermutation, the initially strong signs of Ag selection pressure observed in visit 1–derived sequences decayed by visit 2. These data suggest that excessive immune activation in RPs leads to a hyperactive B cell response that fails to confer protection. ***The Journal of Immunology*****, 2020, 205: 587–594.**

Usually, HIV infects and slowly destroys the $CD4^+$ T cell compartment of the adaptive immune system. These $CD4^+$ T cells carry out a wide range of effector functions to help coordinate an immune response, including activating macrophages to digest intracellular bacteria, signaling B cells to go through somatic hypermutation (SHM), class-switching, affinity maturation, and secreting a host of cytokines and chemokines (1). The deterioration of the $CD4^+$ T cell population severely hampers the immune system's ability to ward off infection. As such, HIV^+ individuals exhibit reduced efficacy in response to vaccination and are prone to opportunistic infections that healthy individuals typically keep at bay.

HIV infection is known to perturb the B cell compartment (2, 3). During primary infection, B cells display an altered phenotype characterized by general activation (a rise in plasma cells and activated memory B cells, hypergammaglobulinemia, and secretion of autoantibodies) (4, 5). Chronic HIV infection is associated with a depletion of classical memory B cells, and this effect is directly correlated with peripheral CD4 counts (6). $CD4^+$ T cell lymphopenia eventually gives rise to the expansion of immature/transitional B cells during the late stages of HIV (7, 8).

Despite the expansion of HIV-specific follicular helper T (T_{FH}) cells during chronic infection, neutralizing Abs can take years to develop (9, 10). Follicular regulatory T cells and PD-L1 expression on germinal center B cells have been implicated in the impairment of T_{FH} cell function, and highly functional circulating T_{FH} cells have been shown to correlate with broadly neutralizing Ab production (11–13).

Without treatment, HIV infection ordinarily progresses to AIDS over the course of 5–10 years. "Elite controllers" have been studied extensively because of their extraordinary ability to combat the notoriously evasive virus and maintain a competent immune system. However, less is known about another unique class of HIV responders: rapid progressors (RP). RPs experience a rapid decline

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B.S.W. conducted experiments, acquired and analyzed data, and wrote the manuscript with contributions from all authors. Y.F., Z.Z., Y.J., and X.H. performed clinical donor recruitment, donor evaluation, blood purification, and cell lysate preparation. Y.F. worked on the sample transportation approval and shipping. M.Q. worked on sample processing. C.H. performed BASELINe analysis. S.M.H. helped prepare sequencing libraries. J.X., H.D., and Y.F. performed clinical data collection and data analyses. N.J. designed the research study and analyzed data. H.S. designed, directed, and supervised the clinical data collection and analysis.

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Abbreviations used in this article: ART, antiretroviral therapy; FWR, framework region; MID, molecular identifier; MIDCIRS, molecular identifier clustering-based immune repertoire sequencing; pdf, probability density function; RP, rapid progressor; SHM, somatic hypermutation; T_{FH}, follicular helper T; TP, typical progressor; VL, viral load.

in their peripheral CD4 counts, dropping to <350 cells/μl within 1 year of infection, whereas typical progressors (TP) maintain a CD4 count of >500 cells/μl for at least 2 years. In a rhesus macaque SIV infection model, RPs were associated with acute depletion of PD-1–expressing activated memory B cells, and PD-L1 blockade is currently being explored as an HIV treatment option (14–16).

In this study, we study the Ab repertoire in HIV RPs undergoing the early stages of disease. Using PBMCs drawn within an average of two months of infection and once again 1 year postinfection, we find that the SHM load, defined as the average number of SHMs for a set of Ab sequences, in IgG is negatively correlated with disease progression. Analysis of B cell clonal lineages spanning both blood draws reveals that RPs continue to dramatically mutate their Ab sequences. Although the visit 1 sequences initially show strong signs of Ag selection, they are abrogated in the corresponding visit 2 sequences, implying a defect in the Ag selection and T cell help as the disease progresses. These analyses shed light onto how HIV disrupts the immune system to avoid neutralization.

Materials and Methods

Study design and cohort

Approval for this study was obtained from the Medical Research Ethics Committee of First Affiliated Hospital of China Medical University, and written informed consent for participation was obtained from all study participants.

HIV$^-$ men who have sex with men were enrolled and checked every 6–10 wk for at least 2 y from 2009 to 2013 in Dr. Hong Shang's laboratory at the First Affiliated Hospital of China Medical University. At each visit, the presence of HIV Ab and viral RNA were tested. HIV infection was identified using a third-generation ELISA (bioMérieux, Amersfoort, the Netherlands). A rapid test for HIV Ab screening and the Western blot (Genelabs Diagnostics, Singapore, Singapore) were used for validation. Ab-negative specimens were pooled and tested further for HIV RNA. Specimens with positive ELISA but negative or inconclusive Western blot results were tested for HIV RNA individually without pooling. The infection date was estimated by self-reported exposure and verified via Fiebig classification (17, 18). For patients with no or multiple exposures, the midpoint between the last seronegative test and the first seropositive test was used. To mitigate age-associated effects on the Ab repertoire, age restriction was 17–27 y old. During recruitment, five RPs were identified, and five age-matched TPs were selected (Table I) in this study. Whole blood was obtained from these five RP and five TP patients 1–3 mo (visit 1) and 1 y postinfection (visit 2). Coded de-identified samples were sent to Dr. Ning Jiang's laboratory at the University of Texas at Austin with approval from the Human Genetic Resources Management Office, Ministry of Science and Technology of China.

During recruitment, the recommendation for antiretroviral therapy (ART) initiation was when the CD4 count ≤200 (2009–2010) or ≤350 cells/μl (2011–2013) by both the World Health Organization and the Chinese government. All patients were recommended to receive therapy when their CD4 T cell counts dropped below the recommended threshold. All patients were treatment naive at visit 1 and began treatment after visit 2 unless they refused ART medication.

CD4 and CD8 counts were determined by BD FACSCalibur (Becton Dickinson). HIV RNA viral load (VL) was determined by COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche) with a detection limit of 40 copies/ml in plasma.

Ab repertoire sequencing

Ab repertoire sequencing was performed as previously described (19). Briefly, up to 5 million PBMCs were lysed in RLT lysis buffer supplemented with 1% 2-ME. RNA purification was performed using QIAGEN AllPrep DNA/RNA Mini Kit following the manufacturer's protocol. Thirty percent of total RNA was used for reverse transcription using a 12-N molecular identifier (MID) fused to isotype-specific primers followed by two sequential PCR amplification steps. Eleven pooled primers were used during the first PCR to amplify all Ig H chain V region families, then sequencing adapters were ligated during the second PCR. PCR products were gel purified and quantified via Agilent Tapestation 2000. Pooled libraries were sequenced via MiSeq 2x250PE.

Raw sequencing reads were processed through molecular identifier clustering-based immune repertoire sequencing (MIDCIRS) (19) to first group sequences with the same MID together. MID groups were further clustered with an 85% sequence similarity threshold to form subgroups. As detailed in our original method development (19), it is possible for different Ab transcripts to be tagged with MIDs of the same sequence. Thus, this clustering step separates PCR products derived from these different Ab transcripts that stochastically have the same MID. Next, consensus sequences (equivalent to RNA molecules) were generated within subgroups to mitigate sequencing errors. The use of consensus or RNA molecules instead of the raw reads in following analysis also corrects amplification bias. Finally, identical consensus sequences were merged to yield unique consensus sequences or unique RNA molecules.

Unique RNA molecules were aligned to ImMunoGeneTics database set of human V, D, and J gene alleles, and mismatches between the template and sequence of interest, omitting the CDR3, were tallied as SHMs. Sequencing read statistics are summarized in Table II.

Selection strength analysis

BASELINe (20) was used to assess the strength of Ag selection pressure applied upon the Ab repertoire. As amino acid–replacing mutations are necessary to grant higher binding affinity, positive selection during affinity maturation leads to an enrichment of replacement mutations. BASELINe relates the observed replacement mutation frequency to that expected for a random mutation. A higher-than-expected frequency of replacement mutations indicates positive selection, as expected in the CDRs, whereas a lower-than-expected frequency indicates negative selection, as expected in the framework region (FWR), where replacement mutations can disrupt proper Ab folding.

To compare between progressor groups, probability density functions (pdf) for each subject were initially calculated, CDR and FWR separately. Then, the pdfs for the subjects belonging to the same group (RP or TP) were convoluted. To compare between sequences from lineages lowly mutated at visit 1 that increase in SHM load by visit 2, lineages with a visit 1 average SHM load of 10 or fewer that increased by five or more SHMs at visit 2 were isolated. Visit 1– and visit 2–derived sequences were segregated. Selection strength pdfs for each unique sequence within each lineage of the corresponding visit were first convoluted, and then the resulting pdfs for each lineage for each subject were convoluted, and then finally, the pdfs for subjects belonging to the same group were convoluted.

Clonal lineage formation and two-timepoint analysis

Unique sequences were clustered into clonal lineages as previously described (19) with some modifications. Sequences from both visits were pooled together, and sequences with the same V and J gene alleles and 90% similarity on the CDR3 nucleotide sequence were clustered into clonal lineages. Lineages containing sequences derived from both visits were isolated to track the evolution of the Ab sequences over time.

Statistics

Significance tests were used as indicated in the figure legends. A two-tailed paired *t* test was used to determine significance for parameters compared between visits for matched subjects. A two-tailed Mann–Whitney *U* test was used when comparing between progressor groups. Spearman ρ was used to test correlations with disease progression. Selection strength significance was calculated as previously described (20). Briefly, the *p* value was determined by the probability that a random value from the pdf is higher than a random value from another pdf.

Results

RPs are defined by a rapid decline in CD4 count

We isolated PBMCs from RPs and TPs at visit 1 and visit 2 (Fig. 1A, Table I). RPs experience a dramatic reduction in peripheral CD4 counts, dropping below 350 cells/μl within the first year of infection, whereas TPs maintain normal CD4 counts of greater than 500 cells/μl for at least 2 y. Between visit 1 and visit 2, RPs exhibited uniform depletion of peripheral CD4$^+$ T cells, whereas TPs' CD4 counts remain unchanged or even increased (Fig. 1B). The RP group was associated with a higher VL at the early timepoint, but the decreasing CD4 count was not accompanied by an increasing VL (Fig. 1C). RPs have lower CD4 [CD8 ratios, a measure that is associated with T cell activation and poor

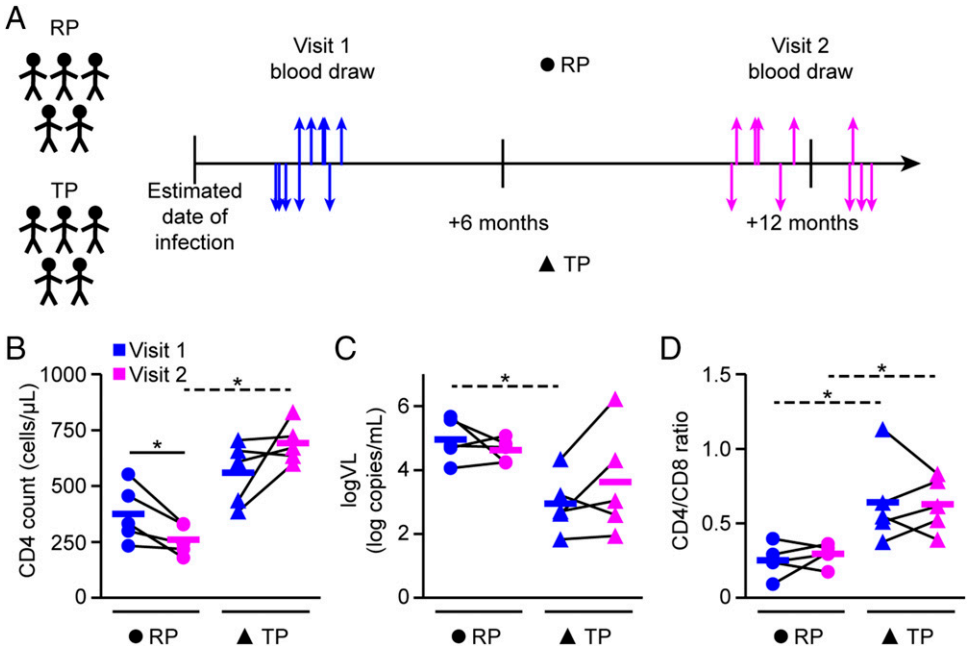

FIGURE 1. RPs undergo distinct CD4 count decline within 1 y of infection. (**A**) Study design and sample collection timeline. (**B**–**D**) CD4 count (B), VL (C), and CD4/CD8 ratio (D) comparison for RP (circles, $n = 5$) and TP (triangles, $n = 5$) between visit 1 (blue) and visit 2 (magenta). Bars indicate means. $^*p < 0.05$, two-tailed paired t test (solid lines) or two-tailed Mann–Whitney U test (dashed lines).

prognosis in ART-treated HIV patients (21, 22)], than TPs across both timepoints (Fig. 1D).

Disease progression correlates with diminished IgG SHM load

Despite the increased initial VL and rapid loss of $CD4^+$ T cells, RPs do not differ from TPs in overall SHM loads, defined as the average number of SHMs for a set of Ab sequences, in the three major isotypes (Fig. 2A). In fact, on the bulk level, SHM loads within the RPs are not significantly altered between the two timepoints. Only IgG in TPs displays significantly more SHMs upon visit 2 (Fig. 2A, middle panel). The SHM load of IgG Abs but not IgM or IgA is inversely correlated with disease progression (Fig. 2B, Supplemental Fig. 1). Higher CD4 count (Fig. 2B, middle panel) and lower VL (Supplemental Fig. 1) both correlate with higher average IgG mutations. For the subset of subjects with available data ($n = 2$ RPs and 2 TPs, eight total samples), these IgG mutations were inversely correlated with the percentage of $CD8^+$ T cells expressing the activation marker CD38 (Supplemental Fig. 1), suggesting that general immune activation could be linked to the reduced IgG SHM load observed in patients with further disease progression.

Chronic immune activation is a key factor in HIV infection (23, 24). There is evidence that hyperactive naive B cells and/or $CD27^-$ atypical memory B cells contribute to the increased secretion of IgG Abs in HIV patients (5). These subsets of B cells have undergone fewer divisions and harbor fewer SHMs than classical memory B cells in these patients (25). Class-switching of these lowly mutated classes of B cells upon aberrant activation and/or defective germinal center T cell help could contribute to the overall lower IgG SHM load with further disease progression. To test the first possibility, we compared the percentage of unmutated sequences to the CD4 counts within the cohort. Consistent with the hypothesis that recently activated and class-switched naive B cells contribute to the observed reduction of IgG SHM load with disease progression, the fraction of unmutated IgG but not IgM or IgA correlated with decreasing CD4 count (Fig. 2C) and increasing VL (Supplemental Fig. 1C). However, these unmutated sequences do not fully account for the trend, as the average number of mutations in IgG but not IgM or IgA still negatively correlated with disease progression after excluding unmutated sequences (Supplemental Fig. 1D, 1E). Normalized Shannon entropy (26–28), a measure of repertoire diversity with bounds between 0 (all sequences are the same) and 1 (all sequences are different), was significantly lower in TP than RP at visit 2 in IgG sequences but not IgM or IgA (Fig. 2D). A typical adaptive immune response involves the activation and expansion of certain Ag-specific B cell clones, which leads to lower entropy. A higher entropy implies a defect in Ag-driven clonal expansion or the

Table I. Cohort summary

Individual	Group	Sex	Visit 1, Age (y)	Visit 1, Days Postinfection	Visit 2, Days Postinfection
R1	RP	Male	27	76	332
R2	RP	Male	23	87	321
R3	RP	Male	22	69	335
R4	RP	Male	26	77	390
R5	RP	Male	17	62	334
T1	TP	Male	22	80	347
T2	TP	Male	22	50	395
T3	TP	Male	25	48	388
T4	TP	Male	22	54	401
T5	TP	Male	18	52	318

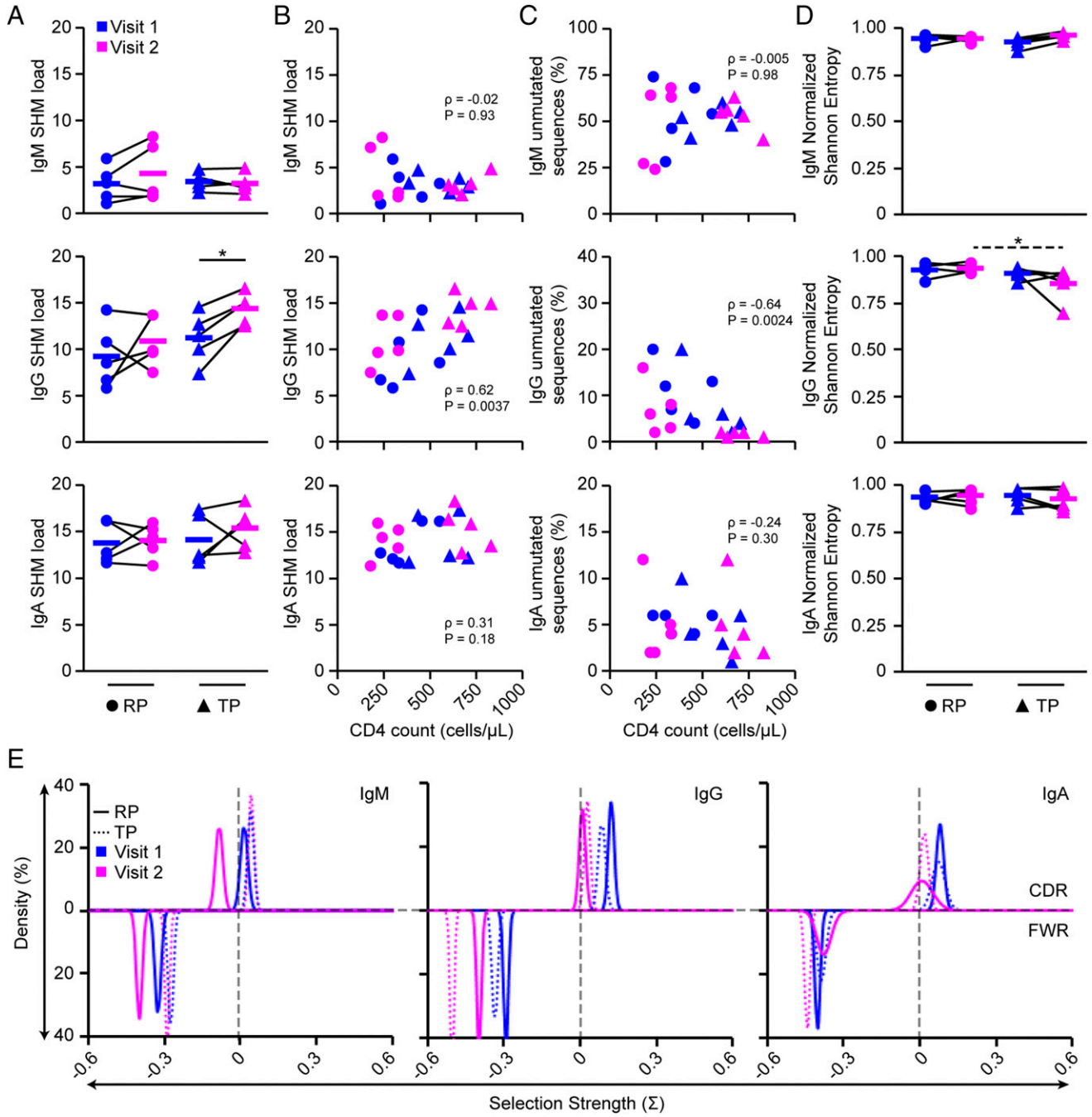

FIGURE 2. Global IgG SHM reduces with declining CD4 count. (**A**) Average SHM load comparisons for RP (circles, $n = 5$) and TP (triangles, $n = 5$) between visit 1 (blue) and visit 2 (magenta) split by isotype: IgM (top), IgG (middle), and IgA (bottom). Bars indicate means. $^*p < 0.05$, two-tailed paired t test. (**B** and **C**) Average SHM load (B) and unmutated percentage of unique sequence (C) correlations with CD4 count, split by isotype: IgM (top), IgG (middle), and IgA (bottom). Spearman ρ and corresponding p value indicated in each panel for all timepoints from all subjects. For IgG, the correlations remain significant for each timepoint separately. (B) Visit 1: ρ = 0.648, $p = 0.0425$; visit 2: ρ = 0.673, $p = 0.033$. (C) Visit 1: ρ = 0.648, $p = 0.0425$; visit 2: ρ = 0.684, $p = 0.0289$. (**D**) Normalized Shannon entropy comparisons for RP and TP between visit 1 and visit 2, split by isotype: IgM (top), IgG (middle), and IgA (bottom). Bars indicate means. $^*p < 0.05$, two-tailed Mann–Whitney U test. (**E**) BASELINe (20) selection strength comparisons for RP (solid curves) and TP (dotted curves) for visit 1 (blue) and visit 2 (magenta), split by isotype: IgM (left), IgG (middle), and IgA (right). Selection strength for CDR (top half of each panel) and FWR (bottom half of each panel) were calculated separately. See Supplemental Table I for p values for pairwise comparisons. For IgG, the most discussed isotype in this figure, all comparisons for the FWR are statistically significant, and all comparisons but one (RP visit 2 versus TP visit 2) for the CDR are statistically significant. See also Supplemental Fig. 1 and Supplemental Table I.

recruitment of new, unexpanded clones, in line with aberrant activation and/or defective germinal center T cell help in RP. It is possible that a large, diverse $CD4^+$ TCR repertoire contributes to efficiently inducing SHM in the global Ab repertoire.

To test the second part of the hypothesis, we performed BASELINe (20) analysis to assess the degree of Ag selection pressure as a measure of germinal center $CD4^+$ T cell help (Fig. 2E). Replacement mutations in the FWR can disrupt proper Ab folding, so negative selection strength is expected and observed in the FWR of Abs of all isotypes (Fig. 2E, bottom half of each panel; Supplemental Table I). The CDR governs Ab binding properties. We observed slight positive selection in the IgG Abs

during the first visit that was reduced upon visit 2 for both groups (Fig. 2E, top half of middle panel; Supplemental Table I). To put this selection into perspective, recent studies found strong selection strength ($\Sigma > 0.5$) in the CDRs of B cells from the CNSs of multiple sclerosis patients (29) and neutral or negative ($\Sigma \leq 0$) selection strength in the CDRs of B cells from donors up to 4 wk after receiving influenza vaccination (30). Thus, this average level of $\Sigma = 0.1$ in the IgG Abs at visit 1 represents weak but significant selection. Indeed, HIV-specific IgG Abs have been detected just 2 wk postinfection and steadily rise over the next month (31). Despite the reduced CD4 count in RPs, we did not detect any major differences in selection strength between the two groups on the global level.

Longitudinally tracked clonal lineages mutate dramatically in RPs with impaired selection

We next sought to track the evolution of Ab sequences over time. We isolated clonal lineages that contain sequences derived from both visits and compared the SHM properties of the visit 1 sequences to their visit 2 relatives. An average of 636 two-timepoint lineages were identified per patient (Table II, range: 199–1547). These two-timepoint lineages were composed of, on average, 5190 unique sequences (1475–11,013) from 13,974 RNA molecules (2886–35,204). This represents ~7.1% of all observed sequences (1.9–14.7%). Both RPs and TPs harbor significantly more SHMs in their visit 2 sequences (Fig. 3A). These two-timepoint lineages, which already contain over 10 SHMs on average at the first visit, continue to mutate further. Surprisingly, despite fewer peripheral $CD4^+$ T cells, RPs induce significantly more SHMs over this time period (Fig. 3B). This increase in SHM within these two-timepoint lineages counterintuitively correlated with disease progression (Fig. 3C, Supplemental Fig. 2), although this could possibly be linked to the expansion of HIV-specific T_{FH} cells in chronically infected lymph nodes (9).

BASELINe analysis revealed that the initial mutations at visit 1 were strongly selected in RPs but only weakly selected in TPs (Fig. 3D, blue curves in top half; Supplemental Table II). Unlike the influenza vaccination experiment that did not detect positive selection, the consistent availability of Ag and ongoing infection, particularly in the case of RPs with high VL at visit 1 (Fig. 1C), could contribute to this stronger selection strength. However, the positive Ag selection strength completely disappeared by visit 2 (Fig. 3D, pink curves in top half). The de novo mutations that arise in visit 2, particularly in RPs, occur in the absence of Ag selection. These mutations may result from polyclonal activation in an extrafollicular T–independent manner, or they could be affected by dysfunctional T_{FH} cells.

The differential mutation increase observed between RPs and TPs within these two-timepoint lineages stems from RP lineages with few mutations at visit 1 (≤10 SHMs) undergoing a burst of SHM upon visit 2, increasing by upwards of 5–20 mutations (Fig. 3E). Further analyzing these actively mutating lineages revealed that the visit 1 sequences in these lineages were especially strongly selected, particularly in RPs (Fig. 3F, blue curves). Analyzing lineages spanning the two timepoints allowed us to dissect the selection at the early stages of disease and after the infection has been established. B cells that have not had time to accumulate many mutations are initially well selected, but by visit 2, when the SHMs have increased, the selection is attenuated (Fig. 3F, magenta curves). However, most broadly neutralizing HIV Abs are highly mutated and take years to develop (32). If multiple specific mutations must accumulate before an appreciable effect can be made on binding affinity, it is unlikely that these have occurred in the first year of infection. It is possible that these initial mutations reach a local energy minimum such that most replacement mutations reduce binding affinity, leading to an accumulation of silent mutations and reduction of the positive selection signal. Another possibility involves viral escape mutations disrupting affinity maturation. Additionally, the disruption of germinal center formation during early-stage infection has been reported and could contribute to diminished Ag selection (33). Our data suggest that RPs experience not only accelerated disease progression but also an accelerated immune response. However, without outside intervention, the RP immune system ultimately loses this arms race.

Discussion

Since the identification of HIV as the causal agent behind AIDS fewer than 40 years ago, great strides have been made in both our understanding of the virus and how we treat it. Like the virus itself, the best practices of treatment are constantly evolving with every new insight. Recent advancements in ART have opened the doors for most HIV^+ patients with adequate access to healthcare to manage the infection to below detectable limits; however, the latent nature

Table II. Next generation sequencing reads statistics

Individual	Visit	Cell Count (1×10^6)	Raw Reads	Merged Reads	Ig Reads	Consensus Subsample	Total Lineages	Nonsingleton Lineages	Shared Lineages
R1	1	5.0	3,497,431	3,327,523	3,225,666	120,000	23,544	4,390	1,547
	2	5.0	3,918,688	3,721,911	3,471,494	120,000	34,153	9,580	
R2	1	4.0	969,863	926,237	916,434	96,000	21,501	2,299	345
	2	1.8	491,245	466,031	460,883	43,200	20,919	827	
R3	1	5.0	3,879,575	3,658,460	3,365,368	120,000	25,445	9,057	789
	2	5.0	3,982,331	3,701,255	3,364,865	120,000	13,991	5,717	
R4	1	2.4	1,274,967	1,203,843	1,191,395	57,600	24,468	1,102	318
	2	2.4	1,142,053	1,080,390	1,069,095	57,600	26,521	1,233	
R5	1	5.0	2,703,157	2,550,971	2,491,135	120,000	38,967	2,475	199
	2	5.0	1,438,263	1,361,716	1,326,860	120,000	17,819	1,759	
T1	1	1.0	760,839	712,978	705,940	24,000	8,377	503	267
	2	2.0	1,096,552	1,031,565	1,020,496	48,000	25,291	913	
T2	1	4.0	2,430,943	2,324,087	2,299,914	96,000	45,303	1,964	1,201
	2	3.4	2,114,302	1,988,585	1,964,583	81,600	40,636	2,086	
T3	1	1.4	315,379	299,300	295,543	33,600	9,309	700	479
	2	5.0	5,514,582	5,348,820	5,297,239	120,000	27,395	2,855	
T4	1	5.0	4,807,447	4,334,591	4,227,113	120,000	24,098	3,774	510
	2	5.0	531,238	1,402,512	1,345,652	120,000	30,568	1,042	
T5	1	5.0	1,656,253	1,568,802	1,515,912	120,000	27,104	2,305	701
	2	5.0	3,019,719	2,873,554	2,748,430	120,000	23,313	2,287	

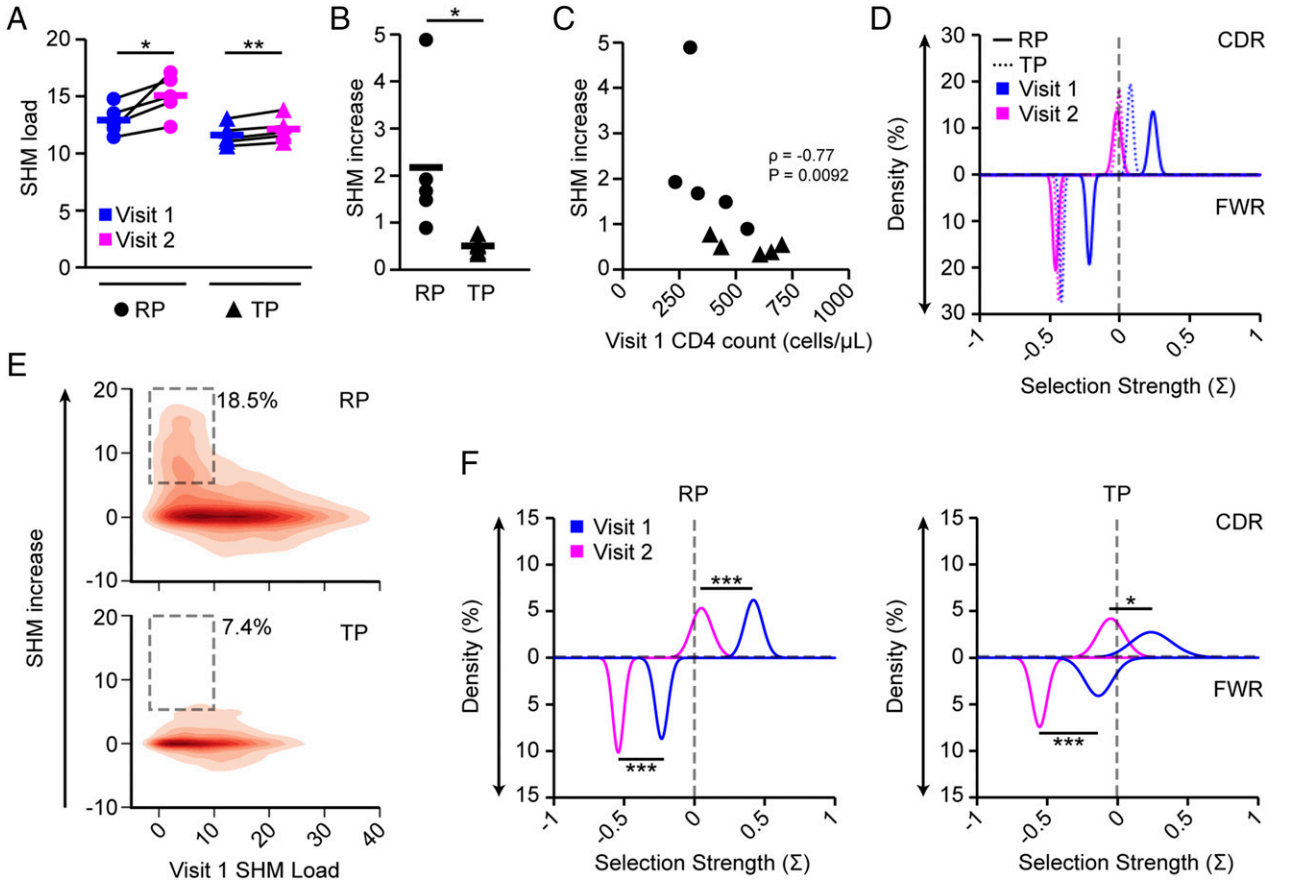

FIGURE 3. Ab lineage tracking within 1 y reveals strong ongoing SHM in RP and to a lesser extent TP, with decreased Ag selection strength in both groups. (**A**) SHM load comparison for RP (circles, $n = 5$) and TP (triangles, $n = 5$) between visit 1 (blue) and visit 2 (magenta) sequences within the same lineages. Bars indicate means. $*p < 0.05$, $**p < 0.01$, two-tailed paired t test. (**B**) Average SHM increase between visit 1 and visit 2 sequences within the same lineages. Bars indicate means. $*p < 0.05$, two-tailed Mann–Whitney U test. (**C**) Correlations between SHM increase and CD4 count at visit 1. Spearman ρ and the corresponding p value are indicated in the panel. (**D**) BASELINe (20) selection strength comparisons for RP (solid curves) and TP (dotted curves) for visit 1 (blue) and visit 2 (magenta) sequences from two-timepoint lineages. Selection strength for CDR (top half) and FWR (bottom half) calculated separately. See Supplemental Table II for p values for pairwise comparisons. All comparisons but two (RP visit 1 versus TP visit 2 and TP visit 1 versus TP visit 2) are significant for the FWR, and all comparisons but one (RP visit 2 versus TP visit 2) are significant for the CDR. (**E**) Density contour plot of SHM increase for two-timepoint lineages by visit 1 average SHM load for RP (top) and TP (bottom). Gray dashed box indicates lineages lowly mutated at visit 1 (≤10 SHMs) that increase by visit 2 (≥five SHMs increase) analyzed in (**F**); number indicates percentage of lineages falling within the box. (F) BASELINe selection strength analysis of lineages lowly mutated at visit 1 (blue) that increase by visit 2 (magenta) for RP (left) and TP (right). $*p < 0.05$, $***p < 0.0005$, calculated as previously described (20). See also Supplemental Fig. 2 and Supplemental Table II.

of the symptoms often leads to a significant delay before diagnosis. Typically, AIDS-like symptoms take several years to develop, yielding a large window to identify the infection before major complications arise. In contrast, rapidly progressing HIV patients develop AIDS within 1–2 y. In addition to the shortened window for intervention, the implications of this acute decline in CD4 count have not been fully elucidated. As such, HIV RPs remain an understudied group. In this study, we analyzed the Ab repertoire response to HIV infection in RPs compared with that of TPs to shed light on one aspect of the complex interaction between HIV and the immune system.

On the global repertoire level, the notable differences arise in IgG Abs: TP SHM loads increase by 1 year postinfection, whereas RP SHM loads do not, and the overall IgG SHM load is inversely correlated with disease progression. Considering the occurrence of hypergammaglobulinemia in HIV patients and the dominance of the IgG1 subclass in HIV-specific Abs (34), it is likely that this overall increase in IgG SHMs is HIV-driven. The relatively intact CD4 T cell compartment in TPs may contribute to ongoing SHM of IgG^+ Abs, whereas patients with depleted CD4 counts experience impaired B cell help and disproportionate extrafollicular activation of B cells. Indeed, we observed a significant negative correlation between the percentage of IgG Abs harboring zero mutations and the CD4 count. Indeed, during normal B cell development, B cells expressing high-affinity poly/autoreactive BCRs are removed from the repertoire at different developmental checkpoints to establish B cell tolerance. Those Abs produced by hyperactive naive B cells and/or $CD27^-$ atypical memory B cells lacking B cell tolerance checkpoints may suffer more risks of autoreactivity. Our study provides new information regarding why Abs produced in HIV-infected patients are polyreactive or autoreactive against common self-antigens (35). However, we still do not know whether those disruptions on B cell tolerance are necessary and sufficient to produce broad neutralizing Abs against HIV.

MIDCIRS, a second generation immune repertoire sequencing technique, reduces the error rate of Ab sequencing to allow for accurate clonal lineage analysis and interrogation of the quality of mutations. We tracked clonal lineages observed across both visits to directly assess the ongoing evolution of Ab sequences over the first year of infection. Although both RPs and TPs continued to induce SHMs between the two visits, RPs underwent a larger SHM increase. This SHM burst was largely attributed to a subset of Abs

that initially harbored few mutations that mutated dramatically by visit 2. The initial mutations showed signs of strong Ag selection, but they were lost by visit 2, coinciding with the decline in CD4 count. The ablation of the CD4 T cell compartment in RPs may render the rampant activation of the immune system ineffective. In addition, even for those RP patients with low CD4 counts, their B cell repertoire still have ongoing mutation likely driven by HIV. Such a finding is in agreement with an early study demonstrating that individuals with advanced HIV disease progression were more likely to generate broadly neutralizing Abs (36). Although sample size is limited, our results may provide insights for practical and feasible vaccine designs.

In conclusion, we used MIDCIRS to elucidate the Ab response to HIV infection in an underappreciated class of HIV responders: RPs. On the global repertoire level, RPs are similar to TPs, although more severe disease progression was associated with a reduction in IgG SHM load, possibly because of a combination of polyclonal activation and class-switching of activated naive B cells and poor SHM induction. Global IgG Abs show signs of weak Ag selection at visit 1, but these signs disappear 1 year postinfection. Two-timepoint lineage analysis enabled direct detection of clonal lineage evolution between the two visits. These lineages continued to readily mutate in RPs, but the initial signs of strong Ag selection in the visit 1–derived sequences were lost by visit 2. Despite strong initial selection and the ability to further mutate, RPs fail to generate protective Abs and experience a rapid decline in CD4 counts. Understanding the mechanism behind the loss of Ag selection pressure could be instrumental for the design of an effective HIV vaccine.

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Disclosures

N.J. is a Scientific Advisor and holds equity interest in ImmuDX, LLC and Immune Arch, Inc., companies that are developing products related to the research reported. C.H. was a paid consultant of Immune Arch, Inc. The other authors have no financial conflicts of interest.

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