Germinal Center Function in the Spleen during Simian HIV Infection in Rhesus Monkeys

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Germinal Center Function in the Spleen during Simian HIV Infection in Rhesus Monkeys

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Infection with HIV-1, SIV, or simian HIV is associated with abnormalities in the number, size, and structure of germinal centers (GCs). To determine whether these histopathologic abnormalities are associated with abnormalities in Ab development, we analyzed nucleotide sequences of Igs from splenic GCs of simian HIV-infected macaques. Virus-specific GCs were identified in frozen splenic tissue sections by inverse immunohistochemistry using rHIV-1 gp120 as a probe. B cells from envelope-specific GCs were isolated from these sections using laser capture microdissection. Their Igs were amplified from cDNA using nested PCR, then cloned and sequenced. Nucleotide sequences were recovered from nine multimember clonal lineages. Within each lineage, sequences had similar V-D-J or V-J junctions but differed by somatic mutations distributed throughout the variable domain. The clones were highly mutated, similar to that previously reported for HIV-1-specific human IgG Abs. The average clone had 37 mutations in the V region, for a frequency of 0.11 mutations/base. The mutational pattern was strikingly nonrandom, with somatic mutations occurring preferentially at RGYW/WRCY hotspots. Transition mutations were favored over transversions, with C→T and G→A replacements together accounting for almost one-third of all mutations. Analysis of replacement and silent mutations in the framework and CDRs suggests that the Igs were subjected to affinity selection. These data demonstrate that the process of Ab maturation is not seriously disrupted in GCs during the early stages of immunodeficiency virus infection, and that Env-specific Igs developing in GCs are subject to extensive somatic mutation and profound selection pressures. The Journal of Immunology, 2006, 177: 1108–1119.

Striking alterations in B lymphocyte populations occur in secondary lymphoid tissues of HIV-1-infected humans (1) and SIV-infected monkeys (2). B cells in lymph nodes and spleens become activated early in the course of infection, resulting in increases in the size and number of secondary lymphoid follicles and in hyperplasia of germinal centers (GCs)6. As immunodeficiency progresses, the GCs are reduced in size and number. Because lymphoid follicles are a crucial site for the clonal expansion and terminal differentiation of Ag-specific B cells, virus-induced abnormalities in secondary lymphoid tissues could have important consequences for the immune responses of these cells.

Critical processes occur in lymphoid follicles that lead to the generation of mature Ab responses. An iterative process of selection, expansion, mutation, and further selection culminates in the generation of high-affinity memory B lymphocytes and plasma cells. Specific features of the somatic mutations observed in expressed Igs have been well-characterized (3, 4) and are similar in humans and mice. Some disease states are associated with atypical patterns of somatic mutation in expressed Igs (5–9). In light of the follicular abnormalities seen in the setting of HIV/SIV infections, the processes that transpire in GCs may also show abnormalities in HIV/SIV-infected individuals.

The Ab response to the envelope glycoproteins (Env) of HIV-1 is of particular importance because of the ability of some anti-Env Abs to neutralize viral infectivity, protect against infection (10–14), exert selective pressure on HIV-1 and SIV following infection in vivo (15–23), and contribute to the control of viremia (24–26). Importantly, neutralizing Abs effective against a broad range of HIV-1 or SIV isolates are produced upon virus exposure only after a substantial delay, months or years following infection (16, 27–32), suggesting that maturation of the immune response is necessary for the development of such broadly reactive Abs. Changes in the specificity of circulating neutralizing Abs continue to occur throughout at least the first 5 years of HIV-1 infection (33).

Abbreviations used in this paper: GC, germinal center; SHIV, simian HIV; p.i.d., postinfection day; FR, framework.
molecular mechanisms that underlie this evolution in virus-specific Abs have not been identified, but it has been suggested that GC processes may be critical for development and maintenance of virus-neutralizing Abs (34).

Macaques infected with recombinant viruses that express HIV-1 Env on an SIV backbone (simian HIVs (SHIVs)) have proven valuable for studying humoral immunity to HIV-1 (11, 12, 24, 35). A high degree of conservation of Ig sequences between humans and these monkeys increases their value as animal models for studying human Ab responses. Studies have shown that homologous human and macaque H (36 – 40) and L chain (41) V regions studied in primate GCs are plentiful (42). Pieces of spleen were frozen in Tissue-Tek cryomedium (Sakura Finetechnical), and frozen sections were cut (10-μm thickness, thaw-mounted onto Superfrost Plus slides (Fisher Scientific), immediately refrozen, and then stored at −80°C.

**Materials and Methods**

**Abs and recombinant proteins**

Recombinant HIV-1 89.6 gp120 (rgp120-89.6) was prepared by Dr. R. Doms (University of Pennsylvania, Philadelphia, PA) as previously described (43, 44) from supernatants of cells infected with recombinant vaccinia viruses expressing HIV envelope glycoproteins, and purified by heparin-affinity chromatography. The rgp120-89.6 was biotinylated in 100-μg batches using the FluoroReporter Minibiotin-XX protein labeling kit (Molecular Probes) according to the manufacturer’s instructions. Unreacted biotin was removed by filtration over a ChromaSpin LC-30 column (BD Clontech). Preservation of conformation-dependent epitopes in the biotinylated Env was documented as described (42).

**SHIV clones, animal infections, and sample handling**

The SHIVs used for this study are described elsewhere (45). In brief, the env, tat, and rev genes of HIV-1 HXB2 were introduced into an infectious molecular clone of SIVmac239. The env gene of the resulting virus was replaced with the env gene of the HIV-1 clone 89.6 to generate SHIV-89.6 (46). Serial passage of SHIV-89.6 in monkeys generated the highly pathogenic SHIV-89.6P quasispecies, from which SHIV-KB9 was cloned. SHIV-KB9 containing the parental 89.6 and more pathogenic KB9 clones. These viruses were selected for the present study because they expressed a gp120 identical with or cross-reactive with that of the rgp120-89.6 used as an inverse immunohistochemical probe and showed increased pathogenicity compared with the parental SHIV-89.6 (45, 47).

The rhesus monkeys used in this study were maintained at the Oregon National Primate Research Center in accordance with the guidelines of the committee on Animals for the Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (48). Healthy rhesus monkeys were inoculated i.v. with cell-free SHIV. Subject Mm18284 was infected with SHIV-KB9ct, and subject Mm19941 was infected with SHIV-KB9 (45, 47). Some immunologic and virologic data from these monkeys have been previously reported (42, 45, 47). Infection of both animals was confirmed by serologic and virologic assays (45, 47). Before infection, subject Mm18284 had a CD4+ cell count of 1200/μL. Following infection the CD4+ cell count fell to 831 by p.i.d. 21 and rose gradually to stabilize at 80°C.

**Inverse immunohistochemistry**

Slide-mounted tissue sections of spleen from SHIV-infected monkeys were fixed in cold acetone for 10 min, air dried, and rehydrated in TBS (pH 8). Slides were then processed using the Sequenza immunostaining workstation (Shandon Lipshaw). Inverse immunostaining was enhanced using catalyzed signal amplification with the Renaissance TSA-Indirect kit (PerkinElmer). Each slide was first treated with Tris–NaCl buffer with a proprietary blocking reagent (Renaissance kit) to reduce nonspecific staining, and then incubated for 1 h at room temperature with biotinylated rHIV-1 envelope gp120-89.6 at a concentration of 0.2 μg/mL (1.67 nM). Slides were rinsed three times with TBS containing 0.05% Tween 20 (TBST) after each subsequent staining step. To catalyze amplification of the biotin signal, the slides were incubated with streptavidin-HRP (1/100 dilution, Renaissance kit) for 30 min at room temperature and subsequently incubated with biotinyl tyramide (1/50 dilution) for 10 min. The amplified biotin signal, indicating bound biotinylated rgp120, was detected histochemically using alkaline phosphatase-conjugated streptavidin-biotin complex (DakoCytomation) and Vector Blue chromogenic substrate (Vector Laboratories) together with levamisole (DakoCytomation) to suppress endogenous alkaline phosphatase activity. After dehydration and nuclear fast red (Vector Laboratories) or Gill’s hematoxylin (Fisher Scientific), slides were covered with Crystal/Mount permanent mounting medium (Fisher Scientific) and air dried.

**Microdissection of Env-binding GCs**

Slide-mounted spleen frozen sections stored at −80°C were fixed in cold acetone for 10 min and air dried. Sections were rehydrated in nuclelease-free water, stained in nuclear fast red (Vector Laboratories), dehydrated through graded alcohols and xylenes, and air dried. The targeted GCs were microdissected using an Arcturus PixCell II laser microdissection workstation (Arcturus Engineering) and the following method. After Env-binding GCs were identified by inverse immunohistochemical staining, the same GCs were located on adjacent tissue sections using landmarks such as position within the section, orientation relative to other GCs, and distinctive features such as GC contour or the presence of vessels within the structure. Cells from each targeted GC were isolated from the surrounding tissue using laser capture microdissection (49) with CapSure HS membranes (Arcturus Engineering). Multiple overlapping laser pulses of 30-μm diameter spot size were used to capture substantial portions of each GC from the slide. Care was taken to avoid capturing cells from the surrounding mantle zones and other regions outside the targeted zone. Digitized images of immunostained tissue sections and the microdissection process were prepared using the Arcturus Engineering workstation and formatted using Adobe Photoshop version 4 software.

**Cloning and sequence analysis of Ig genes from Env-binding GCs**

mRNA present in the captured cells was purified using the RNeasy kit (Qiagen) according to the manufacturer’s instructions. cDNA was prepared using the AMV Reverse Transcription System (Promega). The resulting cDNA (1 μl/reaction) was amplified using nested or seminested PCR catalyzed by proofreading enzyme, PfuTurbo DNA Polymerase (Stratagene) to provide high copying fidelity and optimal yield from the minute samples obtained using microdissection. Each round of the PCR was performed in 50 μL using oligonucleotide primers designed to selectively amplify rearranged Ig H or L chains (Table 1) for 30 cycles using the following parameters: 20 s at 94°C, 30 s at 55°C, 120 s at 72°C. Each PCR was concluded with an extension step at 72°C for 10 min. Products of the second-round PCRs were size selected by electrophoresis in 1.5% agarose, visualized by ethidium bromide staining and cut from the gel. The PCR products were purified using the Qiia I Gel Extraction System (Qiagen), and sequencing was performed using the DyeDeoxy Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Life Technologies). Plasmids containing inserts were isolated from expanded cultures using Qiaprep Spin Minipur kit (Qiagen), and sequenced on both strands using an Applied Biosystems Prism 377XL Automated DNA Sequencer and primers that bound outside the insert.

Nucleotide sequence data from both strands were reconciled using the Sequence analysis module of the Lasergene2000 software package (DNASTAR). Sequences of all clones were submitted to GenBank (accession numbers AJ452533-AJ452600). The set of individual sequences was analyzed to identify clusters of sequences with similar overall length, CDR3 length,
and junctional nucleotide sequence; each cluster might represent descendents of a common ancestor clone.

Somatic mutations in expressed Ig V regions are usually identified with reference to the sequence of their probable precursor in the germline. Because the macaque Ig loci have not been comprehensively catalogued, macaque Ig cDNA sequences were compared with their nearest homologs among the human germline variable (V), diversity (D), and joining (J) region minigenes, which are closely related to those of macaques (36–39, 41). The nearest homolog minigenes were identified from the complete database of proteins of immunological interest, National Institutes of Health, (www.kabatdatabase.com/), summarized online at (www.rubic.rdg.ac.uk/abeng).

Nucleotide mutations that result in amino acid substitutions (replacement mutations, R) or do not change the amino acid sequence (silent mutations, S) were counted separately for FR1, 2, and 3 and CDR1, 2, and 3 of each macaque clone. Enumeration of R and S mutations was performed using an automated algorithm that incorporates the methods of Nei (51, 52), implemented online at (http://hiv-web.lanl.gov/content/hiv-db/SNAP/SNAP.html) (B. T. Korber, Synonymous Non-Synonymous Analysis Program, Los Alamos National Laboratory, 53). The excess or paucity of replacement mutations in the FRs or CDRs of each sequence was compared with that expected by chance using a multinomial distribution model, implemented online at (www.stat.stanford.edu/Ig) (54).

For analysis of purine or pyrimidine transition or transversion mutations, the test for whether the rates of mutation from T to C, from A to T, and from G to C were all equal given that T was mutated (and similarly for mutations from C, from A, and from G) was an exact Poisson test (i.e., the multinomial test of ratios after conditioning on the sum of the three mutations). Comparisons between pairs of mutation rates used the exact Poisson test (i.e., the binomial test of ratios after conditioning on the sum). The tests of differences between lineages within chain (H or L) and animal used the exact Zelen test for homogeneity of odds ratios. An adjustment was made for multiple comparisons using Holm’s method. That is, when there were n relevant comparisons, the smallest of the n p values was compared with 0.05/n, the next smallest p value was compared with 0.05/(n-1), and so on until one of the p values was not smaller than its bound (i.e., 0.05/n), in which case that comparison and all comparisons with larger p values were

Nucleotide differences between the clones were tallied for the entire V region excluding positions that overlap the PCR primers, which correspond with the initial 22 nt of the IgH framework region 1 (FR1) region or 18 nt of the IgA and Igκ FR1. FR and CDR in the cloned Igs were identified based on standard Kabat criteria (E. A. Kabat, Kabat database of sequences of proteins of immunological interest, National Institutes of Health, (www.kabatdatabase.com/)), summarized online at (www.stat.stanford.edu/Ig) (54).
declared nonsignificant. The multiple comparison adjustment was done separately for each different initial nucleotide.

Within the motif RGYW and its reverse complement WRGY, only the underlined G or C was considered a potential hot spot preferentially targeted for mutation (55, 56). Comparison of mutation frequency for hot spot and non-hot spot sites was made using the Fisher exact text.

Results

Identification and microdissection of GCs containing Env-binding cells, and cloning of their Ig sequences

Splenic tissue frozen sections derived from two rhesus monkeys chronically infected with moderately pathogenic variants of SHIV-89.6 were stained by inverse immunohistochemistry using biotinylated recombinant HIV-1 envelope gp120 (Env) as an Ag probe. Splenic tissues from both monkeys demonstrated numerous GCs containing cells that bound Env. For Mm19941, 35% of >1500 examined GCs contained Env-binding cells, while for Mm18284, 3% of >400 evaluated GCs contained such cells. Both intensely stained and entirely unstained GCs were frequently apparent on a single tissue section in close proximity (data not shown), indicating that the Ag probe was bound to B cells or Abs produced locally rather than to serum Abs nonspecifically bound to GC follicular dendritic cells. One Env-binding GC from each animal, shown in Fig. 1, A and B, was selected for further evaluation.

Cells from each targeted GC were isolated from multiple, serial sections using laser capture microdissection. The capture of tissue from the Env-binding GC shown in Fig. 1B is depicted in the panels of Fig. 1C. The upper row presents the same GC as it appeared in several adjacent sections. The next row shows the same tissue sections after microdissection. The bottom row shows the GC cells isolated by microdissection.

Samples from each tissue section were processed separately. mRNA present in the captured cells was purified, reverse transcribed into cDNA, and amplified using nested PCR with oligonucleotide primers designed to selectively amplify rearranged Ig H or L chains (Table I). Products of the second-round PCRs were size selected, cloned, and both strands were sequenced.

Sequence analysis of Igs cloned from microdissected GCs

The recovered nucleotide sequences from each GC could be grouped on the basis of sequence similarity into multimer clonal lineages (Table II). Within each lineage, all sequences have similar V-D-J or V-J junctions but differ by somatic mutations distributed throughout the variable domain. The V-D-J-C and V-J-C joins were all “in frame” (i.e., productive rearrangements), and no premature termination codons were found in any of the clones. Data from nine clonal lineages were combined to create an aggregate data set that was analyzed statistically.

Ig clones from GC2

H chain. cDNA from GC2 was amplified using oligonucleotide primers designed to amplify Ig H chain V regions encoding IgG or IgM Abs. The IgG-primed reactions revealed an amplicon of the expected size only for the VH3-primed reaction. Nucleic acid sequencing of VH3-IgG clones yielded a clonal lineage, designated 2S1H3, with nine distinct members (Fig. 2A).

The IgM-primed reactions also yielded an amplicon of the expected size only for VH3-primed products. The VH3-IgM PCR product was ligated and cloned, and 30 clones were sequenced. They were all identical, with no intrachromat variation. This IgM may represent a B cell resident in the mantle zone outside GC2, inadvertently collected during laser capture microdissection.

L chain. cDNA from GC 2 was amplified using oligonucleotide primers designed to amplify Ig L chain V regions of either κ or λ isotype. Electrophoresis of PCR products revealed an amplicon of the expected size only among the VJ5-primed reaction. Nucleic acid sequencing of clones from GC2 yielded one multimeric clonal lineage, designated 2S2L5, with six distinct members (Fig. 2B).

Ig clones from GC4

H chains. cDNA from GC4 was amplified using oligonucleotide primers designed to amplify Ig H chain V regions encoding IgA, IgD, IgE, IgG, or IgM Abs. Only IgG-specific PCRs produced an amplicon of the expected size. Sequencing of clones from the IgG-specific PCR revealed three multimeric clonal IgG lineages, designated 4S1H3, 4S2H3, and 4S3H3, whose sequences are presented in Fig. 2, C–E, respectively.

L chains. cDNA from GC4 was amplified using oligonucleotide primers designed to amplify Ig L chain V regions of either κ or λ isotype. A L chain PCRs yielded Ig sequences that belonged to three distinct multimeric clonal lineages, designated 4S4L5, 4S5L2, and 4S6L8, whose sequences are presented in Fig. 2, F–H. κ L chain PCRs yielded Ig sequences that could be assigned to a single multimeric clonal lineage.4S7K2, presented in Fig. 2J.
Frequency and rate of somatic mutations in V regions of the Ig clones

The somatic mutation rate was calculated based upon two different assumptions, as described in Materials and Methods. Under assumption 1, the 66 Ig sequences cloned from microdissected GCs of SHIV-infected monkeys have an average of 37.1 apparent somatic mutations per sequence, with a range of 24.3–56.8. This corresponds to an average mutation frequency of 0.11 mutations/nucleotide. No significant difference was found between H and L chain clones in this regard. Under assumption 2, the Ig sequences have an average of 16.5 apparent somatic mutations per sequence, with a range of 2.0–37.9. The corresponding mutation frequency is 0.05 mutations/nucleotide. Under assumption 2, the mutation frequency of H chains (0.015) was significantly less than that of L chains (0.07; \( p < 0.02 \)).

Bias in somatic mutation of Ig V regions

Some disease states are associated with atypical patterns of somatic mutation in expressed Igs (5–8). We therefore analyzed the pattern of mutations observed in GC B cells from these SHIV-infected monkeys to determine whether they are indicative of abnormalities.

The frequency with which mutations were targeted to nucleotides within the previously described RGYW/WRCY “hot spot” motif was statistically analyzed for the 66 Ig clones obtained from the SHIV-infected monkey spleens. Within and across animals, for both H and L chains, there was a higher probability of mutation at hot spots than elsewhere (\( p < 0.0001 \)). Preferential targeting of hot spots was also evident within each lineage (Table III), despite the smaller sample size. Overall, the percentage of hot spot sites that were mutated was more than twice as large as that seen in non-hot spots.

As noted earlier, transversion mutations should be twice as common as transition mutations in a purely stochastic process. To assess the substitution pattern in the macaque Igs, the frequency of mutation from each of the four nucleotide species to each of the other three nucleotides. After pairwise comparisons, it was found that there were 28 (of a possible 93) significant differences occurred within a nucleotide group (e.g., from T to C vs T to A). Such comparisons were only performed for lineages where, given that a nucleotide of a particular type (T, C, A, or G) mutates, it was not equally likely to mutate to each of the other three nucleotides. After pairwise comparisons, it was found that there were 28 (of a possible 93) significant differences, and 20 of these favored transition mutations. The other eight significant differences occurred within a nucleotide group (e.g., from T to A vs T to G). In all comparisons between transition and transversion mutations showing a statistically significant difference, transition mutations were favored. Overall, 58% of mutations were transitions (Table IV), similar to the normal human pattern but contrasting with the stochastic prediction of 33% transitions.

Similarly, under the more conservative assumption 2, it was found that there were 23 (of a possible 42) significant differences, and transition mutations were favored for 18 of them, while the other 5 occurred within a nucleotide group. Altogether, transition mutations were determined by comparing the R and S values for the CDR and FR regions using a multinomial distribution model, as implemented by the website ([http://hiv-web.lanl.gov/SNAP/WEBSNAP/SNAP.html]). The overall average was weighted based on the number of clones in each lineage.
Figure 2. Continued
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mutations comprised half of observed mutations, rather than one-third as predicted for a random process.

Mutational bias attributable to cytosine deaminase (57) was specifically examined. Under assumption 1, for eight of the nine lineages, the nucleotide C when mutated was most often replaced by T. Mutational bias in mutations of C was statistically significant for seven of nine lineages (range of \(p\) values, 0.0008–0.0001). Similarly, eight of nine lineages showed preferential replacement of G by A, and the deviation from randomness was statistically significant for all of these (range of \(p\) values, 0.021–0.0001).

Combining the results for all lineages, the C→T and G→A replacements together constituted almost one-third of all mutations (Table IV). Under assumption 2 also, the nucleotide C when mutated was most often replaced by T, and G was most often replaced by A. Thus, calculations based on either assumption support the conclusion that somatic mutational processes are operating normally in these GCs.

Selection pressures on somatic mutations in Ig V regions

To assess the influence of positive and negative selection on the Ig sequences cloned from the Env-binding GCs of SHIV-infected monkeys, the ratio of replacement to silent mutations was determined for the FRs and CDRs of each V(D)J region by comparison with the nearest human germline homologs (Table II). The excess or paucity of replacement mutations in the FRs or CDRs of each sequence, tallied both under assumptions 1 and 2, was compared with that expected by chance using a multinomial distribution model (54). The fraction of clones from each lineage that showed statistically significant evidence for a selection bias under assumption 1 is shown in Table II. Remarkably, 63 of 66 clones had a statistically significant (\(p < 0.05\)) paucity of R mutations in the FRs. This indicates that nearly all the clones had survived substantial negative selection pressures in vivo. The absence of premature termination codons, out-of-frame V(D)J junctions and other inactivating mutations among the cloned sequences provides additional evidence of negative selection. A majority of the clones (37 of 66) also had a statistically significant excess of R mutations in the CDRs, reflecting positive selection pressures in vivo.

We repeated the analysis under assumption 2 (data not shown). Although this assumption drastically reduced the total number of apparent somatic mutations, 35 of 66 clones still showed a statistically significant paucity of R mutations in their FRs, and 27 clones had a statistically significant excess of R mutations in the CDRs. Therefore, calculations based on either assumption support the conclusion that positive and negative selection processes are operating normally in these GCs.

Discussion

Abnormalities in the number, size, and structure of lymphoid GCs are common in individuals infected with HIV-1, SIV, or SHIV. Because GCs are an important anatomic site for the maturation of Ab responses, these abnormalities could have important consequences for the development of virus-specific Abs. To determine whether immunodeficiency virus infection is associated with abnormalities in Ab development in GCs, we analyzed the nucleotide sequences of Igs in B cells obtained from the HIV-1 Env-binding splenic GCs of SHIV-infected monkeys. The types of observed nucleotide mutations and the targeting of base substitutions in both

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H and L chain clones support the conclusion that the same Ag-driven GC processes observed in normal humans and mice are also operating in the GCs of SHIV-infected monkeys. The Ig sequences from SHIV-infected monkeys had an average of 16.5–37.1 apparent somatic mutations per sequence. This is close to the mutation frequency previously reported for human IgG Abs recognizing HIV-1 gp120 (58–64), and substantially higher than the average of 9.5 somatic mutations in IgG produced by unselected human tonsillar GC B cells (65).

The abundance of mutations in the Igs indicates that the B cells were subjected to a profound degree of antigenic stimulation in the GC microenvironment. Although it was not formally demonstrated that the studied Igs actually bind Env or another SHIV protein, the abundance of Env-binding cells in the studied GCs provides direct evidence that the viral envelope is a potent immunogen for B cells in fully functional GCs.

Somatic mutation among GC B cells typically occurs at a rate of \(10^{-3}\) mutations per base pair per cell per generation (66, 67). The cell cycle of GC B cells has been calculated at between 6 (68) and 12 h (69). Assuming the same rate of mutation applies to the tissues from which the clones were derived, calculations show that the average clone in our dataset underwent between 50 and 113 cycles of replication. Based on the 6–12 hour cycling time, this number of generations could arise in 2–8 wk of continuous antigenic stimulation. Because the tissues were obtained at least 13 wk after SHIV infection, it cannot be determined whether diversification of the clones we studied occurred rapidly during acute infection or accumulated more gradually over subsequent weeks.

Using the lower estimate of 50 generations, this number of cell divisions is sufficient to produce nearly 800 trillion cells, if all survived. However, the largest GCs contain a few million cells at most. The difference between these values highlights the stringency of negative selection enforced during clonal expansion in these GCs.

In the present study, we found that GC function was preserved in two monkeys in the steady state stage of SHIV infection who had \(\sim 50\%\) reductions in CD4\(^+\) T cell counts. This corresponds with the protracted lymphadenopathy stage of HIV-1 infection in the early years following infection, although the subset of CD4\(^+\) T lymphocytes lost in these monkeys differs from those targeted for destruction in HIV-1-infected humans (70). However, GC function likely declines with more advanced immunodeficiency. HIV-1-infected individuals with very low numbers of CD4\(^+\) cells often develop follicular involution, with GCs depleted of lymphocytes and follicular dendritic cells.

The analysis of data in this study was subject to several limitations. First, any characterization of GC B cell lineages will necessarily be incomplete because the iterative process of mutation and selection will delete intermediate ancestors, branches, and entire lineages as an indispensable component of the process leading to affinity maturation. Other B cells present in the GC will be operating in the GCs of the SHIV-infected monkeys was supported under either assumption. There also is little published information regarding the normal pattern of somatic mutation in macaques. The lack of a formal baseline or comparator would be of greater consequence had our results suggested that macaque GCs introduce somatic mutations according to some novel pattern. However, because the pattern was the same in normal humans and rodents as in the SHIV-infected macaques we studied, it is likely that the pattern in normal macaques is also the same.

There are few published studies that address the role of GCs during immune responses to viral infection in humans or nonhuman primates. Analyses of somatic mutations in mAbs derived from individuals exposed to viruses suggest that the processes of somatic mutation and Ag-affinity selection can play a major role in shaping the Ab response to viruses, including HIV-1 (58, 59, 71–73). The present study directly demonstrates that GCs are a major site for somatic mutation and selection of antiviral Abs in primates. Additional studies are needed to determine the contribution of somatic mutation outside of GCs (74–76) to shaping the Ab response to an infecting virus.

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Disclosures
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References


