Lack of B Cell Dysfunction Is Associated with Functional, gp120-Dominant Antibody Responses in Breast Milk of Simian Immunodeficiency Virus-Infected African Green Monkeys

Joshua D. Amos,a Andrew B. Wilks,a Genevieve G. Fouda,a Shannon D. Smith,b Lisa Colvin,b Tatenda Mahlokozera,a Carrie Ho,a Krista Beck,b R. Glenn Overman,a C. Todd DeMarco,a Terry L. Hodge,a Celia C. LaBranche,d David C. Montefiori,d Thomas N. Denny,a Hua-Xin Liao,a Georgia D. Tomaras,a,c,d,e,f M. Anthony Moody,a,b,c Sallie R. Permar,a,c,d,e,f Duke Human Vaccine Institute,a Division of Laboratory Animal Resources,a,c and Departments of Pediatrics,a,c Surgery,a,c Immunology,a,c and Molecular Genetics and Microbiology,c Duke University Medical Center, Durham, North Carolina, USA; Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USAa

The design of an effective vaccine to reduce the incidence of mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) via breastfeeding will require identification of protective immune responses that block postnatal virus acquisition. Natural hosts of simian immunodeficiency virus (SIV) sustain nonpathogenic infection and rarely transmit the virus to their infants despite high milk virus RNA loads. This is in contrast to HIV-infected women and SIV-infected rhesus macaques (RhMs), nonnatural hosts which exhibit higher rates of postnatal virus transmission. In this study, we compared the systemic and mucosal B cell responses of lactating, SIV-infected African green monkeys (AGMs), a natural host species, to that of SIV-infected RhMs and HIV-infected women. AGMs did not demonstrate hypergammaglobulinemia or accumulate circulating memory B cells during chronic SIV infection. Moreover, the milk of SIV-infected AGMs contained higher proportions of naive B cells than RhMs. Interestingly, AGMs exhibited robust milk and plasma Env binding antibody responses that were one to two logs higher than those in RhMs and humans and demonstrated autologous neutralizing responses in milk at 1 year postinfection. Furthermore, the plasma and milk Env gp120-binding antibody responses were equivalent to or predominant over Env gp140-binding antibody responses in AGMs, in contrast to that in RhMs and humans. The strong gp120-specific, functional antibody responses in the milk of SIV-infected AGMs may contribute to the rarity of postnatal transmission observed in natural SIV hosts.

Breastfeeding remains a significant mode of mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) in resource-limited countries, where replacement feeding is associated with higher rates of morbidity and mortality (1). Approximately half of the 330,000 new infant HIV infections occurring annually can be attributed to transmission of the virus through breastfeeding (1). Administration of antiretroviral prophylaxis to the mother and/or infant during the period of breastfeeding has effectively reduced the incidence of new infant HIV infections (2–5). However, little is known about the long-term effects of antiretroviral therapy (ART) prophylaxis on maternal health or childhood development, including the emergence of ART-resistant HIV strains. In addition, limited access to the most effective ART regimens in resource-challenged areas of the world provide further impediments to long-term ART prophylaxis as a successful strategy to eliminate MTCT. Therefore, the development of immunologic strategies, such as maternal or infant vaccination, to reduce the incidence of new HIV infections transmitted via breastfeeding remains an important scientific and public health pursuit.

Interestingly, in the absence of ART prophylaxis, only 10% of HIV-infected mothers transmit the virus to their breastfed infants despite chronic daily low-dose exposure (1, 6–8), suggesting the presence of protective immune factors in milk that impede virus acquisition. As breast milk contains a large amount of IgG and IgA antibodies, it has been suggested that protection is mediated by mucosal IgG and IgA responses that are effective in blocking mucosal HIV transmission. HIV Envelope (Env)-specific antibodies have been identified in the milk of HIV-infected, lactating women, but the magnitude of these responses is similar in postnatal transmitting and nontransmitting HIV-infected mothers (9–11). Likewise, simian immunodeficiency virus (SIV) Env-specific antibody responses in the milk of SIV-infected, lactating rhesus macaques (RhMs) are similar in magnitude between animals that did and did not transmit the virus through breastfeeding (12). We have previously reported a limited ability of breast milk HIV or SIV Env-specific antibodies of chronically HIV-infected women or SIV-infected RhMs to mediate autologous and heterologous virus neutralization (13, 14). Furthermore, as the mucosal HIV/SIV Env-specific IgA response in milk of chronically infected women and RhMs is generally of low magnitude and nonneutralizing (13, 14), the role of mucosal IgA in mediating protection against viral transmission is an area of ongoing study. Despite these limitations of the Env-binding and neutralizing antibody responses against HIV/SIV in breast milk of humans and RhMs, there are data which suggest that antibody-dependent cell cytotoxicity (ADCC) responses are of higher magnitude in milk of women who do not transmit the virus to their infant (15). Moreover, the passive transfer of HIV-specific broadly neutralizing antibodies to neonatal monkeys orally challenged with simian-hu-
man immunodeficiency virus (SHIV) can prevent infants from virus acquisition through breastfeeding (16–18). Therefore, it is conceivable that a successful maternal or infant HIV vaccine that elicits these types of functional antiviral humoral immune responses could protect against postnatal HIV transmission.

African-origin primates that are natural hosts of SIV have evolved to sustain nonpathogenic lentiviral infections that do not typically progress to an AIDS-like illness (19–21). Interestingly, these animals only rarely transmit the virus to their infants (22–25), despite repeated daily exposure to high milk virus RNA loads (26, 27). This phenomenon is in contrast to pathogenically SIV-infected, Asian-origin nonnatural SIV hosts and HIV-infected humans, which exhibit higher rates of postnatal virus transmission through breastfeeding (28). African green monkeys (AGMs), a natural host species of SIV, are an excellent model in which to study the potentially protective immune responses in milk of natural SIV hosts. Strikingly, we previously identified strong autologous virus neutralizing antibody responses in the milk of chronically SIV-infected AGMs (27), in contrast to the lack of detectable autologous neutralizing response in milk of HIV-infected women (13) or SIV-infected RhMs (14). Thus, we sought to characterize the mucosal and systemic SIV-specific B cell responses in lactating AGMs that arise during acute infection and may contribute to this robust mucosal antibody response. In this study, we compared the kinetics of systemic and mucosal B cell populations and associated antibody responses in lactating AGMs and RhMs inoculated with species-specific SIV strains. Comparing the SIV-specific B cell responses in the milk and blood of SIV-infected AGMs to that of pathogenically SIV-infected RhMs could elucidate the potentially protective immune responses that have evolved to prevent postnatal SIV acquisition in natural hosts.

MATERIALS AND METHODS

Study population and sample collection. Six female AGMs and four female RhMs were hormonally induced into lactation as described previously (29), followed by intravenous inoculation with 7.9 × 10^6 copies of cloned SIVab92018ivTF (500 ng of Gag p24) (30) and 2.1 × 10^5 copies of SIVmac251 virus stock (29), respectively. The RhMs all carried the Mamu-A*01 allele, a major histocompatibility complex (MHC) allele associated with long-term control of SIV replication. Blood and milk were collected one to two times per week, respectively, during acute infection, at 16 to 26 weeks postinfection and again at 1 year postinfection. Plasma and peripheral blood mononuclear cells (PBMCs) were isolated from the blood by density gradient centrifugation using Ficoll-Paque plus (GE Healthcare, Waukesha, WI). Milk was separated into cellular, supernatant, and lipid fractions by low-speed centrifugation as described previously (30). A virus pellet was isolated from the milk supernatant by high-speed centrifugation as described previously (29). Pinch biopsy samples from the colon and vaginal tract were obtained from AGMs at 11 to 12 months postinfection and digested with collagenase, and lymphocytes were isolated over a Percoll gradient within 6 h (14). Vaginal, rectal, and saliva secretions were collected from AGMs by a modified wick method using Weck–cel sponges as described previously (31) at 26 weeks postinfection. Animals were maintained according to the Guide for the Care and Use of Laboratory Animals (32).

Human milk and plasma samples used in this study were obtained from the CHAVI009 cohort of chronically HIV-infected, lactating Malawian women at 4 to 6 weeks after delivery, as described previously (13). The HIV-infected women included in this assessment were previously untreated with antiretrovirals and were provided with single-dose nevirapine at delivery for prevention of MTCT according to the national policy at the time (2008 to 2009).

SVImac251 and SIVab92018ivTF quantitative real-time PCR (RT-PCR). Determination of SIVmac251 RNA load in milk and plasma was performed by quantitative real-time PCR as described previously (29). To quantitatively SIVab92018ivTF viral load, RNA from 500 μl of plasma was isolated into 60 μl of elution buffer using a QiAamp viral RNA kit (Qiagen, Hilden, Germany). RNA from milk virus pellets was isolated manually via a QiAamp viral RNA kit (Qiagen, Hilden, Germany). All subsequent reactions were set up using an automated PCR setup platform, the QIAbility (Qiagen, Hilden, Germany). Twenty-five μl of viral RNA was annealed to a target-specific reverse primer, 5′-CTT ACC AGA GAA CCA CCA G-3′, and then reverse transcribed using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) and PCR nucleotides (Roche Applied Science, Indianapolis, IN), along with RNase Out (Invitrogen, Carlsbad, CA), using an optimized version of the manufacturer’s protocol. Resulting cDNA was treated with RNase H (Applied Biosystems, Carlsbad, CA) according to the manufacturer’s protocol. Ten μl of cDNA was then used for a real-time PCR using TaqMan gene expression master mix (Applied Biosystems, Carlsbad, CA) along with target-specific labeled probe (5′-6-carboxyfluorescein–GGTGG AGG CTC GCA CAT GAT T–black hole quencher 1-3′) and forward (5′-CCC GGT GCT GCT CAAAT AGG-3′) and reverse (5′-CTT ACC AGA GAA CCA CCA G-3′) primers (custom synthesis by Integrated DNA Technologies, Coralville, IA). Real-time PCR was performed on a StepOne Plus platform (Applied Biosystems, Carlsbad, CA) using the standard curve protocol. The RNA standard was transcribed from a plasmid containing the SIVab92018ivTF long terminal repeat gene using the Megascript T7 kit (Ambion, Grand Island, NY), quantitated by optical density (OD), and serially diluted to generate a standard curve. The sensitivity of this assay is 250 copies/ml.

Flow cytometry analysis of mucosal and systemic B cells and calculation of absolute B cell numbers. Mononuclear cells isolated from mucosal and systemic samples were stained with a panel of fluorescently labeled monoclonal antibodies reactive with the following cell surface markers: CD20 peridinin chlorophyll protein (PerCP)-Cy5.5 (L27), CD3 Alexa Fluor 700 (SP34-2), surface IgM fluorescein isothiocyanate (FITC) (G20-127), CD21 allophycocyanin (APC) (Bly4), streptavidin phycoerythrin (PE)-Cy7 (all from BD Biosciences, San Jose, CA), CD27 brilliant violet 421 (O323) (iCyt, Champaign, IL), surface IgD (sIgD), PE, or biotinylated sIgD (both polyclonal; Southern Biotech, Birmingham, AL). Aqua vital dye (Invitrogen, Carlsbad, CA) was used to discriminate live cells. Stained cells were acquired on an LSRII flow cytometer (BD Biosciences, San Jose, CA), and flow cytometry data were analyzed using FlowJo software (TreeStar, Ashland, OR). The gating strategy used for the selection of total B cells included gating of the lymphocyte population on forward scatter versus side scatter dot plot, selection of live, Aqua vital dye-negative lymphocytes on side scatter versus Aqua vital dye dot plot, and selection of CD3−/CD20−/CD27− lymphocytes on CD3 versus CD20 dot plots. Automated complete cell counts are skewed by the fat droplets present in milk and cannot be used to quantitate lymphocyte numbers in milk. In order to calculate absolute lymphocytes per microliter of milk, the absolute number of lymphocytes determined by flow-cytometric analysis of each sample type was normalized to the ratio of fluorospheres (AccuCheck counting beads; Invitrogen, Carlsbad, CA) added to the original sample and quantitated by flow cytometry at the end of each staining procedure, as described previously (27). The normalized number of lymphocytes was then divided by the volume of the original milk sample volume. Automated complete blood counts (CBCs) were used to quantitate the absolute number of lymphocytes per milliliter of blood. The absolute numbers of B cell subsets per ml of blood or milk was calculated by multiplying the percentage of the population by the normalized absolute lymphocyte count.

Purification of IgG and non-IgG fractions. IgG and non-IgG fractions were isolated using protein G HP MultiTrap 96-well depletion plates (GE Healthcare, Waukesha, WI) as described previously (13) from breast
milk and plasma of SIV-infected AGMs and RhMs with adequate milk volumes available to perform the purifications (500 µl).

SIV/HIV Envelope protein production. SIVmac251 and SIVsab92018ivTF gp120 and gp140 env genes (30) were coxidized by employing the coxodon usage of highly expressed human housekeeping genes, synthesized de novo (Blue Heron Biotechnology, Bothell, WA, and Genezwiz, South Plainfield, NJ), and cloned into pcDNA3.1/Hygro or pcDNA3.1 expression plasmids (Invitrogen, Carlsbad, CA) using standard molecular technology. Recombinant Env proteins were produced in 293F cells by transient transfection and purified by Galanthus nivalis lectin-agarose (Vector Laboratories, Burlingame, CA) column chromatography (33).

Quantification of total and Env-specific IgG and IgA in mucosal and systemic compartments. Total IgG and IgA contents from plasma, milk supernatant, and mucosal secretions were measured in duplicate using commercially available monkey-specific enzyme-linked immunosorbent assay (ELISA) kits and standards according to the manufacturer’s instructions (Alpha Diagnostic International, Inc., San Antonio, TX). Env-specific IgG and IgA were measured by incubation of serial 3-fold dilutions of IgG or IgA at which RLU are reduced by 50% compared to RLU in virus control wells after subtraction of background RLU in cell control wells.

SIV neutralizing antibody assays. Neutralizing antibody activities in plasma, milk, and the IgG and non-IgG fractions were measured in 96-well culture plates by using Tat-regulated Luc reporter gene expression to quantify reductions in virus infection in TZM-bl cells with SIV variants, including SIVmac2018ivTF infectious molecular clone and tier 1A T cell line-adapted (TCLA) or difficult-to-neutralize wild-type SIVmac251 Env pseudovirus (clone 30) as described previously (14, 34). Briefly, neutralization assays were performed with serial dilutions of heat-inactivated (56°C, 1 h) samples. Diluted samples were preincubated with virus (−150,000 relative light unit [RLU] equivalents) for 1 h at 37°C before addition of cells. Following 48 h of incubation, cells were lysed and Luciferase activity determined using a microtiter plate, luminometer, and BriteLuc plus reagent (PerkinElmer, Waltham, MA). Neutralization titers are the sample dilution or concentration (for sCD4 and purified IgG and IgA) at which RLU are reduced by 50% compared to RLU in virus control wells after subtraction of background RLU in cell control wells.

SIV Western blotting. SIV Western blotting was performed with RhM plasma samples using an SIV Western blotting assay kit (ZepetoMetrix, Buffalo, NY) per the manufacturer’s instructions. For AGMs, SIVsab92018ivTF viral lysate was prepared, transferred to a polyvinylidene difluoride (PVDF) membrane, and probed using plasma from uninfected or acutely SIV-infected AGMs. For detection, an alkaline phosphatase-conjugated, polyclonal goat anti-human IgG antibody (Sigma, St. Louis, MO) was used as the secondary antibody and developed using Western Blue stabilized substrate for alkaline phosphatase (Pierce, Waltham, MA) and Ultra-Blue BC100 (Sigma, St. Louis, MO) as the secondary antibody and developed using Western Blue stabilized substrate for alkaline phosphatase (Pierce, Waltham, MA).

Statistical analyses. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). Virus loads, antibody content, and SIV-specific antibody titers between mucosal and systemic compartments in the same animals were compared by the Wilcoxon signed-rank test, whereas comparisons between groups were performed with the paired nonparametric Mann-Whitney U test. Due to small animal numbers in this cohort of RhMs, all observations were reported only when fluctuations in circulating B cell subsets occurred in all RhMs, reaching a P value of 0.12 and indicating a trend toward statistical significance using the paired Wilcoxon signed-rank test.

RESULTS

Hypergammaglobulinemia is associated with chronic SIV infection in RhMs but not AGMs. Intravenous species-specific SIV inoculation of AGMs and RhMs resulted in similar viral kinetics yet lower viral load in AGMs, with peak SIV RNA loads at 14 days after infection ranging from 2.9 × 10^5 to 7.8 × 10^5 copies/ml in AGMs and 2.9 × 10^6 to 1.0 × 10^6 copies/ml in RhMs. However, the true peak of viremia may have occurred at unsampled time points around day 10 of infection, as previously indicated (35, 36). Viral set point at 50 days after infection ranged from 1.2 × 10^3 to 8.5 × 10^5 copies/ml in AGMs and 6.1 × 10^3 to 9.4 × 10^5 copies/ml in RhMs.

Hypergammaglobulinemia has been characterized as a marker of disease progression and B cell dysfunction in HIV-infected individuals and SIV-infected RhMs (37, 38). Therefore, we first investigated the kinetics of total plasma IgG levels during SIV infection in AGMs and compared them to those of RhMs. Total plasma IgG levels remained similar to preinfection levels through 1 year postinfection (median change, 0.7; range, −11.2 to 7.6 mg/ml) in SIV-infected AGMs, whereas total plasma IgG levels in SIV-infected RhMs exhibited a greater than 2-fold increase above preinfection levels by 1 year postinfection in all animals (median change, 30; range, 26.5 to 42.1 mg/ml) (Fig. 1A). Given this difference in total plasma IgG levels, we next sought to compare the kinetics of the autologous SIV gp120 Env binding titers in plasma of AGMs and RhMs. Autologous SIV Env gp120 IgG titers developed with similar magnitude and kinetics between the two species (Fig. 1B). Western blot analysis supported these findings, revealing that autologous SIV-specific antibody responses against gp120 and gp41 were present by 4 weeks postinfection in both species (data not shown).

Transient total and memory B cell loss occurs in the peripheral blood during acute SIV infection in AGMs. To further investigate the underlying mechanism responsible for differences in total plasma IgG regulation between chronically SIV-infected AGMs and RhMs, we next compared the effect of SIV infection on the kinetics of circulating B cell populations during the acute and chronic stages of infection. Depletion of the number of circulating total and memory B cells during acute HIV/SIV infections has been described in pathogenic infection of nonnatural hosts (39–42). Thus, we first compared the kinetics of the total systemic B cell populations during SIV infection of AGMs and RhMs. Interestingly, we observed a small transient decline in the proportion (median decline from baseline, 3.5%; range, 0.4 to 6.9%) (Fig. 2A) of total B cells by 2 weeks postinfection (P = 0.03) in all AGMs (35) and a concurrent decline in the absolute number (median decline, 136; range, 8 to 186 CD20+ lymphocytes/µl) (Fig. 2B) of total B cells in five of six SIV-infected AGMs (P = 0.06, trending toward significant). The proportions and absolute numbers of total B cells in SIV-infected AGMs were restored to preinfection levels by 13 weeks postinfection and remained constant through week 45 postinfection. Although there appeared to be a more dramatic transient reduction in total B cell number and proportion at
SIV infection, although the naive B cell proportion did significantly decrease from the peak between weeks 2 and 13 postinfection (median decline from 2 weeks postinfection, 14.3%; range, 1.1 to 26.8%; \( P = 0.03 \)). Furthermore, AGMs exhibited a decrease in the absolute number of circulating marginal-zone B cells (median decline from baseline, 11.3%; range, 0 to 27.9 IgD\(^+\) CD27\(^-\) B cells/\( \mu l; P = 0.09 \), trending toward significant), which was followed by an increase in proportions of this population between weeks 2 and 45 postinfection (median increase from 2 weeks postinfection, 7.3%; range, 4.4 to 13.8%; \( P = 0.03 \)) (Fig. 3B and C). Interestingly, the AGMs also had a transient reduction in the proportion of circulating memory B cells at 2 weeks postinfection (median decline from baseline, 7.2%; range, 2.4 to 18.7%; \( P = 0.03 \)) that was restored by week 13 postinfection (Fig. 3B). There was an associated drop in the absolute number of memory B cells at 2 weeks postinfection (median decline from baseline, 41%; range, 0.6 to 133 IgD\(^-\) CD27\(^-\) B cells/\( \mu l; P = 0.03 \)) that rebounded by 13 weeks postinfection (median increase from 2 weeks postinfection, 33.7%; range, 0.8 to 121 IgD\(^-\) CD27\(^-\) B cells/\( \mu l; P = 0.03 \) and then declined again by week 45 postinfection (median decline from 13 weeks postinfection, 18.9%; range, 5.0 to 55.1 IgD\(^-\) CD27\(^-\) B cells/\( \mu l; P = 0.03 \)) (Fig. 3C). In all four RhMs, we observed an increase in the proportion of marginal-zone B cells between weeks 2 and 13 postinfection (median increase from 2 weeks postinfection, 20.9%; range, 5.5 to 24.5%; \( P = 0.12 \)) that was also reflected by an increase in absolute number (median increase from 2 weeks postinfection, 69.8%; range, 0.9 to 92.6 IgD\(^-\) CD27\(^-\) B cells/\( \mu l; P = 0.12 \)) (Fig. 3D and E). Furthermore, naive B cell proportions declined between weeks 13 and 45 postinfection in all four RhMs (median decline from 13 weeks postinfection, 20.3%; range, 6.7 to 27.5%; \( P = 0.12 \)), yet absolute numbers remained similar. Memory B cell proportions and numbers transiently declined and then accumulated in the blood of three of four of the SIV-infected RhMs during acute and chronic infection, but statistical significance for these changes was not achieved due to low numbers of animals (Fig. 3D and E).

Loss of B cell CD21 expression, also known as complement receptor 2, has been described as a reliable marker for B cell dysfunction in HIV-infected individuals (43–45). Accordingly, studies have utilized a combination of CD21 and CD27 surface expression to categorize B cells of pathogenically infected SIV/HIV hosts into naive (CD21\(^+\), CD27\(^-\)), resting memory (CD21\(^-\), CD27\(^+\)), activated memory (CD21\(^-\), CD27\(^+\)) and tissue-like memory (CD21\(^-\), CD27\(^+\)) B cells (40, 44, 46, 47). Therefore, we sought to characterize these B cell subsets in the peripheral blood of SIV-infected AGMs to determine if natural hosts lack the SIV-induced B cell phenotypic alterations that are associated with disease progression in pathogenic SIV/HIV infections. A gating legend and representative dot plots of each B cell subset at preinfection and 45 weeks postinfection is shown for AGMs and RhMs (Fig. 4A). No significant fluctuations in the proportions and absolute numbers of naive B cells were observed during SIV infection in AGMs. However, we did observe an acute decline in the proportion (median decline from baseline, 4.24%; range, 0.6 to 14.2%; \( P = 0.03 \)) and absolute number (median decline from baseline, 15.8%; range, 0.4 to 20 CD21\(^+\) CD27\(^+\) B cells/\( \mu l; P = 0.03 \) of resting memory B cells from preinfection levels by 2 weeks postinfection (Fig. 4B). There was a corresponding increase in the proportion (median increase from baseline, 14.4%; range, 4.1 to 17.7%; \( P = 0.03 \)) of tissue-like memory B cells by 2 weeks postinfection that returned...
to baseline by 13 weeks postinfection (Fig. 4B). However, absolute numbers of tissue-like memory B cells remained relatively constant until they decreased between weeks 13 and 45 postinfection (median decline from week 13 postinfection, 8.3; range, 3 to 13.8 CD21<sup>+</sup> CD27<sup>+</sup> B cells/μL; \( P = 0.03 \)) (Fig. 4C). In RhMs, circulating naive B cell proportions declined between weeks 2 and 13 (median decline from 2 weeks postinfection, 13.6%; range, 6.0 to 19.9%; \( P = 0.12 \)) and between weeks 13 and 45 (median decline

---

**FIG 2** Kinetics of the total B cell population in the peripheral blood of AGMs and RhMs during acute and chronic SIV infection. Shown are proportions (A) and absolute numbers (B) of circulating total B cells, gated as CD20<sup>+</sup> lymphocytes, prior to and during SIV infection of AGMs (\( n = 6 \); closed circles) and RhMs (\( n = 4 \) for all time points, excluding one preinfection time point in which cells were unavailable to perform the analysis; open squares). Each data point represents an individual animal. * \( P = 0.03 \) by the Wilcoxon signed-rank test.

**FIG 3** Naive, marginal-zone, and memory B cell subpopulations in peripheral blood of AGMs and RhMs during acute and chronic SIV infection. A gating legend and representative dot plots of each B cell subset prior to and 45 weeks following SIV infection from each species are shown. (A) Numbers within each quadrant represent the frequency of marker-specific B cells. Proportions and absolute numbers of naive (IgD<sup>−</sup>, CD27<sup>−</sup>), marginal-zone (IgD<sup>−</sup>, CD27<sup>+</sup>), and memory (IgD<sup>+</sup>, CD27<sup>+</sup>) B cells in peripheral blood prior to and during SIV infection of AGMs (B and C; closed symbols) and RhMs (D and E; open symbols) are indicated. * \( P = 0.03 \) by the Wilcoxon signed-rank test.
from 13 weeks postinfection, 9.1%; range, 3.3 to 12.8%; *P* = 0.12). Additionally, we observed small reductions in the proportions of circulating resting memory and tissue-like memory B cells between weeks 13 and 45 postinfection (median decline from 13 weeks postinfection, 0.3 and 9.5%, respectively; range, 0.2 to 1.5% and 5.2 to 17.1%, respectively; **P** = 0.12 for each declining population). In contrast, circulating activated memory B cell proportions increased between weeks 2 and 13 postinfection (median increase from 2 weeks postinfection, 17.7%; range, 0.7 to 24.6%; *P* = 0.12) and between weeks 13 and 45 postinfection (median increase from 13 weeks postinfection, 21.7%; range, 9.4 to 26.8%; **P** = 0.12) (Fig. 4D). Despite these fluctuations in the proportions of naive and memory B cell subsets in RhMs during SIV infection, absolute numbers remained relatively constant throughout infection, except for a slight increase in the absolute number of circulating tissue-like memory B cells between 2 and 13 weeks postinfection (median increase from 2 weeks postinfection, 13.4%; range, 1.3 to 19.7%; *P* = 0.12) (Fig. 4E).

**High proportions of IgD+ CD27- naive B cells in breast milk of chronically SIV-infected AGMs.** Given the differences in the kinetics of circulating naive and memory B cells in AGMs and RhMs during SIV infection, we next investigated these populations in the breast milk of lactating AGMs and RhMs at 20 weeks postinfection. The milk of SIV-infected AGMs (median, 4.2; range, 0.2 to 93 IgD+ CD27- B cells/µl) than in SIV-infected RhMs (median, 0.16; range, 0.03 to 1.3 IgD+ CD27- B cells/µl, **P** = 0.07) (Fig. 5B). However, proportions and numbers of IgD+ CD27- memory B cells were similar between each species (Fig. 5C and D). Comparing the proportions of milk naive and memory B cell subpopulations to other mucosal tissues collected at 44 weeks postinfection in AGMs, we observed a slightly higher proportion of naive B cells in milk than in vaginal (median, 17.9%; range, 7.9 to 24.8%) and colonic tissues (median, 18.4%; range, 10.1 to 23.6%) that trended toward statistically different values (**P** = 0.12 and **P** = 0.06, respectively). Similar proportions of naive B cells were found in the milk and blood of the AGMs (Fig. 6A). Accordingly, there was a reduced proportion of memory B cells in milk (median, 10.4%; range, 6.5 to 25.7%) than in blood (median, 46.0%; range, 30.4 to 57.0%; **P** = 0.03), colonic tissue (median, 22.1%; range, 10.0 to 27.6%; **P** = 0.03), and vaginal tissue (median, 37.3%; range, 11.3 to 50.0%; **P** = 0.06, trending toward significant) (Fig. 6B).

**High-titer, gp120-dominant IgG and IgA binding responses in plasma and milk of chronically SIV-infected AGMs.** We previously described robust autologous virus-specific neutralizing antibody responses in the milk of chronically SIV-infected AGMs (27). To further characterize the antibody responses in these natural SIV hosts, we evaluated the plasma and milk autologous SIV gp120-specific IgG and IgA binding responses at 4 months postinfection and compared them to that of RhMs at the same time point.
following infection and chronically HIV-infected lactating women. Four months postinfection was selected to compare AGM and RhM antibody responses as a balance between adequate time for virus-specific responses to develop and before significant immune dysregulation occurred in the pathogenically infected RhMs. Env gp120 proteins derived from the autologous challenge virus in AGMs (SIVsab92018ivTF gp120) and RhMs (consensus SIVmac251 gp120) were used to assess gp120-specific responses, while a global consensus HIV gp120 protein, group M ConS, was used to assess responses in the HIV-1-infected lactating women. Remarkably, while plasma gp120-specific IgG titers were similar among all species, plasma gp120-specific IgA titers were approximately one to two logs higher in SIV-infected AGMs than in SIV-infected RhMs (P = 0.01) and HIV-infected humans (P = 0.0001), respectively. Moreover, milk gp120-specific IgG and IgA titers in SIV-infected AGMs were approximately one log higher than titers in HIV-infected humans (P = 0.0009 and P = 0.001, respectively) but similar to titers in SIV-infected RhMs (Fig. 7A). When normalized to total plasma IgG and IgA content in each species, plasma gp120-specific IgG and IgA responses were approximately

**FIG 5** Higher proportion of naive B cells in milk of chronically SIV-infected AGMs than in milk of RhMs. Proportions and absolute numbers of naive (IgD⁻, CD27⁻) (A and B) and memory (IgD⁺, CD27⁺) B cells (C and D) of the total breast milk CD20⁺ lymphocyte population of chronically SIV-infected, lactating AGMs (closed circles) and RhMs (closed squares) is shown. Solid lines indicate medians. *, P < 0.05 by the Mann-Whitney test.

**FIG 6** Proportions of naive and memory B cell populations in mucosal compartments of chronically SIV-infected AGMs. Comparison of proportions of naive (A) and memory (B) B cells defined by surface expression of IgD and CD27 in the milk (circles), colon (squares), vagina (triangles), and blood (diamonds) of SIV-infected AGMs. Solid lines indicate medians. Statistical comparisons by the Wilcoxon signed-rank test of milk B cell subsets to other mucosal and systemic compartments are indicated. *, P < 0.05; **, P < 0.005.
one to two logs higher in SIV-infected AGMs than in SIV-infected RhMs (P = 0.001 and P = 0.001, respectively) and HIV-infected women (P = 0.0005 and P = 0.002, respectively, Fig. 7B). Furthermore, normalized milk gp120-specific IgA responses were approximately one to two logs higher in SIV-infected AGMs than in SIV-infected RhMs (P = 0.04) and HIV-infected women (P = 0.0009), while normalized milk gp120-specific IgG responses were comparable between each species (Fig. 7B). We next compared the normalized milk SIV gp120-specific IgG and IgA responses to those of other mucosal compartments in SIV-infected AGMs at 4 months postinfection. While normalized gp120-specific IgA responses in AGMs were highest in plasma, the milk IgA responses were significantly higher than that in rectal secretions (P = 0.03) and trended toward significantly higher values in vaginal and saliva secretions (P = 0.06 and P = 0.09, respectively). In contrast, normalized gp120-specific IgG responses were similar among all systemic and mucosal compartments (Fig. 7C).

Given the robust SIV gp120-specific IgG and IgA binding responses in the milk of SIV-infected AGMs, we next assessed the ability of IgG and non-IgG fractions purified from milk and plasma of SIV-infected AGMs and RhMs at 4 months postinfection to mediate neutralization of the tier 1A TCLA SIVmac251 virus in an Env pseudovirus TZM.bl neutralization assay. No non-specific neutralization was detected in plasma of either species at a dilution of 1:10. We detected more potent neutralization against the neutralization-sensitive tier 1A TCLA SIVmac251 virus in the plasma IgG fraction of SIV-infected RhMs (median 50% inhibitory concentration [IC50], 0.025 μg/ml; range, 0.01 to 0.13 μg/ml) than in SIV-infected AGMs (IC50 median, 1.12 μg/ml; range, 0.48 to 6.09 μg/ml; P = 0.03) at 4 months postinfection, while the tier 1A TCLA SIV neutralizing responses from the milk IgG fraction were comparable between each species (Fig. 7D). Neutralizing responses in the non-IgG fractions from plasma and milk of SIV-infected AGMs remained below the level of detection in our assay (data not shown), suggesting limited contribution of the Env-specific IgA in plasma and milk of AGMs to virus neutralization. As no autologous neutralization was detected in milk at 4 months postinfection in either AGMs or RhMs (data not shown), we reevaluated the neutralization response in the milk and plasma of SIV-infected AGMs and RhMs against the autologous challenge viruses SIVsab90218 T/F and SIVmac251.30, respectively, 1 year after infection. We detected more potent autologous neutralizing responses in the plasma of SIV-infected AGMs (median 50% inhibitory dose [ID50], 1,649; range, 267 to 6,115) than in RhMs (ID50 median, 29.5; range, 10 to 41) (P = 0.02) (Fig. 7E). Moreover, low-level autologous neutralizing responses were detected in the milk of four of six SIV-infected AGMs (ID50 median, 61; range, 25 to 302) but not in the milk of...
SIV-infected RhMs (P = 0.01) (Fig. 7E). As this difference in neutralizing titers against the disparate challenge viruses could be explained by distinct neutralization sensitivities of the viruses, we next characterized the neutralization phenotype of the cloned SIVsab92018ivTF by testing its sensitivity to a panel of sera from chronically SIV-infected natural and nonnatural hosts (Table 1). SIVsab92018ivTF was only occasionally neutralized at low levels by serum of AGMs infected with heterologous viruses SIVagm-Ver90 and SIVsab9351BR. Due to its relative resistance to this panel of neutralizing sera from SIV-infected monkeys, SIVsab92018ivTF appears to be consistent with tier 2 neutralization sensitivity, similar to that of circulating HIV-1 strains (48). Moreover, its level of neutralization sensitivity was equal similar to that of other SIVsab and SIVagm variants (Table 1). In contrast, SIVmac239CS.23, a virus genetically similar to SIVmac251, was only neutralized by plasma of animals infected with an autologous SIVmac251 strain. While the neutralization sensitivity tier designations of SIV variants are still being characterized, the difficult-to-neutralize SIVmac251/SIVmac239 challenge stocks may be more consistent with a tier 3 neutralization phenotype (49, 50), which may explain low/absent autologous neutralization responses in plasma and milk of RhMs.

Previous studies have reported a systemic gp41-biased anti-Env antibody response during acute infection in HIV-infected hosts and in mucosal compartments during acute and chronic infection (51–54). To evaluate the combined IgG and IgA response against the Env gp41 and gp120 protein in the milk and plasma of chronically SIV-infected AGMs and RhMs, we utilized gp140 Env protein derived from the autologous challenge viruses (SIVsab92018ivTF gp140 and SIVmac251 gp140, respectively) to measure gp140-specific responses generated by 4 months postinfection. To compare the response to that of chronically HIV-infected women, we used the global HIV-1 group M consensus gp140 Env protein (ConS gp140) to evaluate gp140-specific IgG and IgA responses. We then performed an intraspecies comparison of normalized plasma and milk gp120- and gp140-specific IgG and IgA responses (Fig. 8). Remarkably, in SIV-infected AGMs, plasma and milk gp120-specific IgA responses were one to two logs higher than gp140-specific IgA responses (P = 0.002 and P = 0.004, respectively), while plasma and milk gp120- and gp140-specific IgG responses were comparable in this natural host species (P = 0.13 and P = 0.93, respectively) (Fig. 8A). In contrast, gp140-specific IgG responses were stronger in plasma (P = 0.03) and milk (P = 0.03) of SIV-infected RhMs than gp120-specific IgG responses, but gp140- and gp120-specific IgA responses were similar in each compartment (Fig. 8B). Similarly, the milk of HIV-infected women had more robust IgA responses against gp140 than gp120 (P = 0.008), but milk IgG and plasma IgG and IgA gp120 and gp140 responses were comparable (Fig. 8C). The gp140-biased antibody response in RhMs and humans is likely accounted for by anti-gp41 antibody responses known to predominate in pathogenic SIV/HIV infection (51, 52, 54). Thus, a gp140-biased mucosal IgA response similar to that reported during chronic pathogenic SIV/HIV infection was not apparent in these nonpathogenically SIV-infected of AGMs.

**DISCUSSION**

The rarity of postnatal virus transmission observed in African-origin natural SIV hosts provides a unique model in which to investigate the mechanisms that have evolved to protect breastfed infants against virus acquisition. We have previously shown that the milk of SIV-infected AGMs harbors RNA virus loads comparable to that of SIV-infected RhMs, nonnatural hosts that readily transmit the virus via breastfeeding, suggesting that the rarity of postnatal SIV transmission in AGMs is not attributable to low-level virus exposure of the infant (27). Therefore, it is plausible that the milk of SIV-infected, lactating AGMs contains immunologic factors which could mediate protection of breastfeeding infants from virus acquisition.

It has also been proposed that natural SIV hosts do not become vertically infected due to low-level surface expression of the coreceptor CCR5 in infant CD4+ T lymphocyte populations (22). A recent report showed that AGMs are readily infected when mucosally inoculated with SIVagm at high viral titers, and that viral susceptibility correlated with higher levels of CCR5-expressing CD4+ T cells in adult and juvenile AGMs, further supporting the notion that the paucity of CCR5- and CD4-expressing T cells in the infant gastrointestinal tract contributes to the rarity of vertical transmission in this species (55). While the limited availability of target cells likely contributes to the rarity of infant infection, SIV infection of the natural host species, sooty mangabeys, has been initiated in the setting of CCR5-null mutations (56). Additionally, certain innate immune factors, including milk cytokines (57), lactoferin (58), and secretory leukocyte peptidase inhibitor (SLPI) (59), have been shown to mitigate viral infectivity and could be playing a role in the relative protection of infants of natural SIV hosts from postnatal infection. However, we previously reported strong autologous challenge virus-specific neutralization responses in the milk and plasma of chronically SIV-infected AGMs but not in the milk of chronically SIV-infected RhMs (27) or HIV-infected women (13), suggesting that autologous virus-specific neutralization responses play a role in the impedance of postnatal virus transmission via breastfeeding in natural SIV host species. Further evaluation of the role of mucosal and systemic B cell responses in the setting of natural and nonnatural SIV host infections could elucidate the protective immune mechanisms that have evolved to thwart postnatal SIV acquisition in natural hosts and inform immunologic strategies to reduce the incidence of HIV transmission via breastfeeding.

Several reports have described loss of peripheral total and memory B cell subsets during acute and chronic pathogenic HIV/SIV infections (39–42, 44, 60, 61). This, in addition to other B cell response perturbations, including hypergammaglobulinemia (37, 38), enhanced polyclonal B cell activation (62), and increased production of autoantibodies (63), has been suggested to play a role in the inability of nonnatural hosts to mount effective antibody responses against HIV/SIV (37, 38). Our data support these reports of a loss in total B cells in SIV-infected RhMs during acute infection, although they were not statistically significant due to small sample size. However, this B cell loss appeared transient in our study, with total B cell proportions and numbers being restored to preinfection levels by the early stages of chronic infection. Interestingly, we also observed a loss in circulating total (CD20+), memory (IgG+ CD27+), resting memory (CD21+ CD27+), and activated memory (CD21− CD27+) B cells and a corresponding expansion in tissue-like memory B cells during acute SIV infection in AGMs, illustrating that natural SIV hosts are susceptible to the early effects of virus-induced B cell perturbations. Notably, these changes in circulating AGM B cell populations were resolved and restored to preinfection levels by week 13 postinfection. More-
<table>
<thead>
<tr>
<th>SIV reference serum</th>
<th>Donor species</th>
<th>Infecting virus</th>
<th>ID$_{50}$ in TZM.bl cells$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SIVab92018ivTF</td>
<td>SIVagm90.35</td>
</tr>
<tr>
<td>SIV 101$^b$</td>
<td>RhM</td>
<td>SIVmac239</td>
<td>&lt;20</td>
</tr>
<tr>
<td>SIV 112$^b$</td>
<td>AGM</td>
<td>SIVagm.sab</td>
<td>&lt;20</td>
</tr>
<tr>
<td>SIV 113$^b$</td>
<td>AGM</td>
<td>SIVagm.sab</td>
<td>&lt;20</td>
</tr>
<tr>
<td>SIV 114$^b$</td>
<td>RhM</td>
<td>SIVagm.sab</td>
<td>&lt;20</td>
</tr>
<tr>
<td>SIV 115$^b$</td>
<td>AGM</td>
<td>SIVagm.sab</td>
<td>&lt;20</td>
</tr>
<tr>
<td>V005 pool A$^b$</td>
<td>AGM</td>
<td>SIVagmver90</td>
<td>&lt;20</td>
</tr>
<tr>
<td>U963 pool A$^b$</td>
<td>AGM</td>
<td>SIVagmver90</td>
<td>&lt;20</td>
</tr>
<tr>
<td>V004 pool A AGM</td>
<td>SIVagmver90</td>
<td>68</td>
<td>29</td>
</tr>
<tr>
<td>V038 pool A AGM</td>
<td>SIVagmver90</td>
<td>528</td>
<td>&lt;20</td>
</tr>
<tr>
<td>242-04 AGM</td>
<td>SIVsab9351BR</td>
<td>438</td>
<td>&lt;20</td>
</tr>
<tr>
<td>361-06 AGM</td>
<td>SIVsab9351BR</td>
<td>843</td>
<td>&lt;20</td>
</tr>
<tr>
<td>362-06 AGM</td>
<td>SIVsab9351BR</td>
<td>&lt;20</td>
<td>29</td>
</tr>
<tr>
<td>363-06 AGM</td>
<td>SIVsab9351BR</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>365-06 AGM</td>
<td>SIVsab9351BR</td>
<td>&lt;20</td>
<td>24</td>
</tr>
<tr>
<td>366-06 AGM</td>
<td>SIVsab9351BR</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>89-10 AGM</td>
<td>SIVab92018ivTF</td>
<td>1,298</td>
<td>&lt;20</td>
</tr>
<tr>
<td>90-10 AGM</td>
<td>SIVab92018ivTF</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>91-10 AGM</td>
<td>SIVab92018ivTF</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>92-10 AGM</td>
<td>SIVab92018ivTF</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>93-10 AGM</td>
<td>SIVab92018ivTF</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>94-10 AGM</td>
<td>SIVab92018ivTF</td>
<td>273</td>
<td>&lt;20</td>
</tr>
<tr>
<td>sCD4$^c$</td>
<td></td>
<td>3.5</td>
<td>6.8</td>
</tr>
</tbody>
</table>

$^a$ Values are the sample dilutions at which relative luminescence units were reduced 50% compared to virus control wells (no test sample). ND, not determined. Values in boldface are considered positive for neutralization.

$^b$ Samples were prescreened for neutralization activity against SIV variants.

$^c$sCD4 values are expressed as IC$_{50}$ (μg/ml).
over, the SIV-infected AGMs did not develop hypergammaglobulinemia during chronic SIV infection, which is observed during chronic SIV/HIV infection of RhMs and humans. It has been suggested that hyperactivated naive B cells are a major source of abnormal plasma IgG levels during chronic HIV infections (37). However, naive B cells declined during chronic infection in all RhMs in this study. Still, the rise in the proportion of activated memory and marginal-zone B cells in all RhMs, but not AGMs, during chronic SIV infection could contribute to the elevated systemic IgG levels observed during pathogenic infection. Activated memory B cells are induced in response to ongoing viral replication and eventually undergo differentiation into Ig-secreting plasmablasts (45), and marginal-zone B cells play a role in priming T cell-dependent IgG production in response to infection (64). Therefore, it is conceivable that these B cell subsets are partially responsible for the hypergammaglobulinemia observed during chronic SIV infection in RhMs. The lack of hypergammaglobulinemia and the restoration of total and memory B cell subsets, in addition to the well-described resolution of immune activation in AGMs during the early stages of SIV infection (65–67), support the notion that natural hosts lack the B cell dysfunction that likely impairs antibody responses during pathogenic SIV infection.

We also identified several differences in the B cell populations and immunoglobulin content in milk of SIV-infected AGMs and RhMs. We have previously shown that the total milk IgA content is similar to the plasma IgA content in SIV-infected AGMs, in contrast to SIV-infected RhMs, in which total IgA content is significantly lower in milk than in plasma (27). Total milk IgG levels are similar between the two species, but total milk IgA content is about a half log higher in SIV-infected AGMs than in SIV-infected RhMs (27). In this study, we identified a predominance of naive B cells in the milk of chronically SIV-infected AGMs compared to that of SIV-infected RhMs. Moreover, the predominance of naive B cells may be unique to the breast milk compartment of SIV-infected AGMs, as the colon, vaginal, and circulating population displayed a higher proportion of memory B cells than the breast milk. However, there may be limited contribution of milk B cells to local antibody production, as a large portion of the total antibody content in milk is transudated plasma antibody or is locally secreted by plasma cells in the stroma of the mammary gland. Thus, the finding of a high proportion of naive B cells in breast milk of SIV-infected AGMs compared to that of RhMs may only be a reflection of the differences in the circulating B cell populations.

Our finding of the high-titer, robust gp120-specific IgA binding response in the milk of chronically SIV-infected AGMs was remarkable but may be expected, given the high milk IgA content in this species. This robust gp120-specific IgA response is in contrast to that of milk of SIV-infected RhMs (14) and HIV-infected humans (13), which exhibited low-magnitude IgA binding responses against gp120. The higher magnitude gp120-specific IgA binding response in the milk of SIV-infected AGMs than in other mucosal compartments indicates a role for these responses in mediating protection of breastfeeding infants against virus acquisition in this species. However, this IgA response appears to be nonneutralizing, as we were not able to detect neutralization against a tier 1A, easy-to-neutralize SIV in the milk non-IgG fraction of SIV-infected AGMs. However, the autologous challenge virus-specific neutralization responses measured in the milk of SIV-infected AGMs is remarkable, as this response was undetectable or of low

FIG 8 gp120-dominant IgG and IgA response in plasma and milk of SIV-infected AGMs. Intraspecies comparison of normalized plasma and milk gp120-specific (closed symbols) and gp140-specific (open symbols) IgG and IgA responses in SIV/HIV-infected, lactating AGMs (A), RhMs (B), and humans (C). Autologous Env gp120/140 proteins were used for assessment of AGM (SIVsab92018ivTF gp120 or gp140) and RhM (SIVmac251 gp120 or gp140) gp120/140-specific responses, whereas consensus gp120/140 Env protein (ConS gp120 or gp140) was used to assess human gp140-specific responses. Solid lines indicate medians. *, P < 0.05; **, P < 0.005 by the Mann-Whitney test.
magnitude in the milk and plasma of SIV-infected RhMs and has not been detected in milk of HIV-infected women (13). Detection of this response in AGMs suggests that lactating, SIV-infected AGMs develop neutralizing mucosal antibody responses against the autologous challenge virus at an earlier stage of infection than do RhMs. Of consideration, however, is that the inoculating viruses used in this study to infect RhMs was the genetically heterogeneous SIVmac251 stock, whereas the AGMs were infected with a cloned transmitted/founder variant of the virus stock SIVab92018 (30). The neutralization phenotype of the AGM challenge virus appears to be consistent with a tier 2 designation; thus, it may be more neutralization sensitive than the highly neutralization-resistant SIVmac251 challenge variants in RhMs. However, detection of autologous/tier 2 neutralization responses in breast milk of chronically SIV-infected AGMs, now in two groups of AGMs infected with distinct SIVab9p variants (27), is remarkable, as this response is not readily detectable in breast milk of HIV-infected women (13). Finally, strong autologous antibody responses were generated in milk of AGMs despite lower antigen/virus loads than RhMs in this study. Thus, the ability of AGMs to mount a robust binding and neutralizing mucosal antibody response against their autologous viruses may be a result of preserved B cell function associated with nonpathogenic SIV infection.

Previous studies have shown that the initial antibody response to HIV-1 is against gp41 and that this response is polyreactive, nonneutralizing, and ineffective at controlling plasma viremia (51, 52), suggesting that gp41-reactive antibodies are preexisting and produced by memory B cells that were previously activated by non-HIV-1 antigens. The gp140-biased IgG and IgA responses in the milk and plasma of chronically SIV-infected RhMs and HIV-infected humans are consistent with these reports of strong gp41-specific responses in nonnatural SIV/HIV hosts, yet they are in contrast to the relatively higher magnitude gp120-binding antibody responses found in SIV-infected AGMs. As the majority of neutralizing antibody responses characterized in HIV-infected individuals are focused against gp120 epitopes, it is plausible that SIV-infected AGMs produce more functional antibodies directed against gp120 than nonnatural HIV/SIV hosts. The strong gp120-specific responses observed in SIV-infected AGMs supports the notion that these nonpathogenically infected hosts make more effective functional antibody responses against SIV during acute and chronic infection than pathogenically infected hosts. Further investigation into the functional role of the high-magnitude milk IgG and IgA gp120-specific binding and neutralizing response in chronically SIV-infected AGMs could help inform maternal vaccine strategies that elicit effective milk antibody responses that will prevent mother-to-child transmission of HIV through breastfeeding.

ACKNOWLEDGMENTS

We thank Beatrice Hahn for generously providing the SIVab92018ivTF infectious molecular clone; Joern Schmitz for helpful discussions; Ivona Ferrantelli, Luke Buckley, and Bradley Lockwood for helpful discussions; Ivona Ferrantelli, Luke Buckley, and Bradley Lockwood for helpful discussions; Ivona Ferrantelli, Luke Buckley, and Bradley Lockwood for helpful discussions; Ivona Ferrantelli, Luke Buckley, and Bradley Lockwood for helpful discussions; Ivona Ferrantelli, Luke Buckley, and Bradley Lockwood for helpful discussions. Support for this work was provided by an R21 grant to S. R. Permar from the National Institutes of Health (NIH; grant R21AI100760), National Institute of Allergy and Infectious Diseases-NIH contract HHSN27201100016C (to D. C. Montefiori), and by the Center For HIV/AIDS Vaccine Immunology (CHAVI; grant U19 AI067854).

REFERENCES

15. Ferrantelli F, Buckley KA, Rasmussen OA, Chalmers A, Wang T, Li PL,


