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The function and affinity maturation of HIV-1 gp120-specific monoclonal antibodies derived from colostral B cells

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Abstract

Despite the risk of transmitting HIV-1, mothers in resource-poor areas are encouraged to breastfeed their infants due to beneficial immunologic and nutritional factors in milk. Interestingly, in the absence of antiretroviral prophylaxis, the overwhelming majority of HIV-1-exposed, breastfeeding infants are naturally protected from infection. To understand the role of HIV-1 Envelope (Env)-specific antibodies in breast milk in natural protection against infant virus transmission, we produced 19 HIV-1 Env-specific monoclonal antibodies (mAbs) isolated from colostrum B cells of HIV-1-infected mothers and investigated their specificity, evolution and anti-HIV-1 functions. Despite the previously reported genetic compartmentalization and gp120-specific bias of colostrum HIV Env-specific B cells, the colostrum Env-specific mAbs described here demonstrated a broad range of gp120 epitope specificities and functions, including inhibition of epithelial cell binding and dendritic cell mediated virus transfer, neutralization, and antibody-dependent cellular cytotoxicity. Interestingly, we also identified divergent patterns of colostrum Env-specific B cell lineage evolution with respect to cross-reactivity to gastrointestinal commensal bacteria, indicating that commensal bacterial antigens play a role in shaping the local breast milk IgG repertoire. Maternal vaccine strategies to specifically target this breast milk B cell population may be necessary to achieve safe breastfeeding for all HIV-1-exposed infants.

Keywords

HIV; monoclonal antibodies; breast milk

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Disclosure

We have no conflicts of interest to disclose.

Introduction

Mother-to-child transmission (MTCT) accounts for approximately 260,000 new HIV-1 infections annually[1], with majority occurring in sub-Saharan Africa. One third to one half of infant infections occur postpartum[2, 3, 4]. With up to 1 liter of virus-containing breast milk ingested daily for up to two years of life by breastfeeding infants born to HIV-1-infected mothers, it is remarkable that the HIV-1 transmission rate via breastfeeding is less than 10%, even in the absence of maternal antiretroviral treatment[5]. This relatively low risk of infection in the face of chronic mucosal HIV-1 exposure of the infant warrants the study of naturally protective immune factors in breast milk.

Breast milk is known to provide protection against a multitude of neonatal infections, and also provides homeostasis between the infant and its gastrointestinal microbiota[6, 7, 8]. Breast milk IgA and IgG, as well as a number of innate antimicrobial factors, likely all contribute to infant antimicrobial protection against neonatal pathogens[9]. HIV-1 exposure in the infant mucosa occurs in the presence of maternal breast milk HIV-1 envelope (Env)-specific antibodies, which have been shown to have neutralizing and antibody dependent cellular cytotoxicity (ADCC) activity[10, 11]. It is possible that these antibodies play a role in protection against infant HIV-1 acquisition via effector functions in the breast milk compartment or at the infant mucosal barrier.

We previously reported that HIV-1 Env-specific antibodies isolated from colostrum of HIV-1-infected, lactating women are exclusively IgG1 isotype, have distinct variable immunoglobulin gene usage from the HIV-1 Env-specific B cells in peripheral blood, and are predominantly specific for the gp120 portion of the HIV-1 Env[12]. As the anti-HIV-1 functions of these compartmentalized, potentially-protective mucosal gp120-specific antibodies have not been well-defined, we sought to provide insight into the evolution and antiviral functions of the HIV-1 Env-specific antibody repertoire of breast milk B cells. Defining the antiviral functions and protective role of milk antibodies directed against HIV-1 would guide the development of immunologic interventions to make breastfeeding safe for all infants in areas of high HIV-1 prevalence.

Results

Selection of colostrum Env-specific IgG1 mAbs

In this study, we aimed to characterize the epitope specificity, function and evolution of colostrum-derived Env-specific IgG1 antibodies. Using our initial panel of 39 HIV-1 Env-specific IgG mAbs previously isolated from colostrum B cells of 17 HIV-1 infected Malawian women[12], we selected a panel of Env-specific colostrum mAbs for functional characterization using the Env-binding properties of the mAbs determined after small scale mAb production by transient transfection. Four criteria were applied to select the panel of mAbs for large scale production and in-depth study: (1) robust binding to the clade C 1086gp140 (EC_{50}<0.05μg/ml) (Table 1); (2) cross-clade Env gp120-binding [bound ≥3 of 4 Env proteins: Consensus (ConS), Clade A (A244), B (MN) and C (1086) gp120 Envs[13]]; (3) part of an isolated clonal B cell lineage[4], included to study lineage evolution and affinity maturation; and (4) gp41-specific mAbs with strong binding to C.1086gp140 (EC_{50}<0.03),

included for functional comparison to the predominant gp120-specific colostrum mAbs. We also selected four blood Env-specific mAbs isolated from two of the subjects from which the majority of the colostrum mAbs were isolated (CH9105 and CH0404) for functional comparisons.

This selected panel of 19 colostrum Env-specific mAbs was isolated from a total of six HIV-1-infected, lactating Malawian women from the CHAVI009 cohort[12] (Table 2), one of whom transmitted the virus postnatally to her infant (CH0404). The breast milk viral load from the non-transmitting colostrum B cell donors was below the limit of quantification, <240 copies/ml[14], and the median peripheral CD4+ T cell count was 324, whereas the single transmitting colostrum B cell donor (CH0404) had high viral load (29,425 copies/ml) and low peripheral CD4+ T cell count (80 cells/ml). Of the selected 15 gp120-specific and 4 gp41-specific colostrum mAbs a high percentage (52.6%) used the immunoglobulin heavy chain variable (V_H) gene 1–69, which is common among HIV-1 Env-specific mAbs[15, 16] and is used at significantly higher rates in colostrum Env-specific mAbs compared to those in blood[12] (Table 1). Since broadly HIV-1-neutralizing antibodies (bnAbs) frequently have a high V_H mutation rate and long complementarity determining region 3 (CDR3) regions[17], we examined these characteristics in our panel of Env-specific colostrum mAbs. The median V_H and light chain variable gene (V_K/V_L) amnio acid mutation rate of the colostrum Env-specific mAb panel was 9% (range: 3–13%) and 5% (range: 1–8%), respectively. The median V_H CDR3 length of the colostrum Env-specific mAb panel was 15 amino acids (range: 12–29 amino acids), with three colostrum gp120-specific mAbs having a CDR3 length >20 amino acids (DH276=22; DH284=23; and DH388=29 AAs). The median V_K/V_L CDR3 length of the colostrum Env-specific mAbs was 9 amino acids (range: 8–11 amino acids). Although the selected colostrum mAbs bound robustly and broadly to HIV-1 Env antigens, they did not demonstrate unusually high mutation frequency and only a minority of colostrum Env-specific mAbs had long CDR3 lengths, and thus did not commonly display characteristics of bnAbs[17].

Diverse epitope specificities of HIV-1 gp120-specific colostrum mAbs

Next, we sought to determine the epitope specificities of the gp120-specific colostrum mAbs. Using a combination of binding and blocking competition ELISA assays, we defined the specificities of 12/15 of our colostrum gp120-specific mAbs (Figure 1 a). Twenty-seven percent (4/15) were specific for constant regions 1 and 2 (C1/C2)[18], defined by blocking the Env binding of mAb A32[19]. However, these C1/C2-specific mAbs did not block galactosyl ceramide (Galcer) liposome binding as previously described for some A32-like C1/C2-specific mAbs[20] (Supplementary File S1). Twenty percent (3/15) of the colostrum gp120-specific mAbs were specific to the 3rd variable loop (V3), binding to both linear peptides and scaffolded V3 proteins[21] (Supplementary File T1). Another twenty percent (3/15) of the colostrum gp120-specific mAbs blocked the binding of soluble CD4 (sCD4) to Env, and are therefore presumably specific for the CD4 binding site. Interestingly, DH388 mAb binding was dependent on an N-linked Asparagine at amino acid position 334 in the C3 region of the gp120 Env (Figure 1 b), a glycosylation site that is often targeted by antibodies in infected subjects who later develop neutralization breadth[22]. DH388 glycan-binding mAb also had the longest V_H CDR3 length (29 amino acids) of the panel of gp120-specific colostrum

mAbs, yet had a rather typical V_H mutation rate (8%). Another antibody, DH285, bound specifically to the V1/V2 loop of the HIV-1 Env, a specificity of antibody responses that predicted reduced risk of infection in the RV144 Thai vaccine trial[23]. Finally, twenty percent (3/15) of colostrum mAbs had gp120-specificity unable to be defined by ELISA.

Colostrum gp120-specific mAb heterologous and autologous HIV-1 neutralization potency

The ability of locally-produced colostrum IgG1 mAbs to neutralize heterologous tier 1 and tier 2 HIV-1 and SHIV variants was investigated in the TZM-bl assay. Whole breast milk neutralization potency against the tier 1 clade C variant MW965 was determined for each subject[14] (Table 2), but this response did not appear to correlate with the isolation of neutralizing colostrum mAbs. Fifty-three percent (8/15) of the colostrum gp120-specific mAbs neutralized the tier 1 clade C virus, MW965 (Supplementary File S2B) at a concentration below 0.01μg/ml, well below the range of concentrations of Env-specific IgG (~1–10 μg/ml) found in milk of HIV-infected women[14]. Of those, seven neutralized other tier 1 clade B and C Simian/Human Immunodeficiency Viruses (SHIVs). Of note, DH378, a CD4 blocking mAb, demonstrated weak neutralization, at a concentration of 41μg/ml, against one tier 2 SHIV variant (SHIV1157ipd3N4) (Supplementary File S2A). Interestingly, despite its glycan-dependence and long CDR3 length, mAb DH388 did not neutralize any of the tested HIV-1 variants. None of the four selected gp41-specific colostrum mAbs neutralized tier 1 or tier 2 variants. In addition to heterologous virus neutralization, we also assessed whether the neutralizing colostrum mAbs isolated from subject CH9105 had autologous neutralization potency against two HIV-1 variants isolated from plasma of that subject at 4 weeks post delivery. Importantly, 4 of 5 of the neutralizing colostrum antibodies isolated from CH9105 (DH374, DH376, DH377, and DH378) did exhibit low-level neutralization of these two autologous viruses (Table 3 and Supplementary File S2C and S2D).

Env-specific colostrum mAbs can inhibit binding of HIV-1 virions to gastrointestinal (GI) epithelial cells

Preventing HIV-1 infectious virions from binding to and crossing the GI tract epithelial cell layer in infants is a potential target for blocking establishment of HIV-1 infection via breastfeeding. Therefore, we sought to determine whether the colostrum Env-specific mAbs can impede this initial step of mucosal infection. Colostrum Env-specific mAbs were tested for their ability to inhibit binding of a Malawian tier 1 clade C HIV-1 variant, C.MW965, to colonic epithelial cells[24]. Eleven of nineteen (57.8%) Env-specific mAbs inhibited viral attachment to colonic epithelial cells (Figure 3 a). Interestingly, all eight mAbs that neutralized MW965 also inhibited epithelial cell binding, at a concentration of 30μg/ml, with a mean percent inhibition (MPI) ranging from 68.7–98.3%. In contrast, DH390, a non-neutralizing gp120-specific mAb that mediated this activity, only had marginal inhibition (12.8% MPI). In addition, two gp41-specific mAbs, DH288 and DH389, from the postnatally transmitting subject CH0404 and nontransmitting subject CH9606, weakly inhibited the binding of HIV-1 MW965 to epithelial cells (23.9% and 10.79% MPI, respectively). These data may indicate that weak inhibition of epithelial cell binding by local, non-neutralizing mAbs was not enough to confer protection from breast milk transmission in the setting of high milk virus load in subject CH0404.

Neutralizing gp120-specific colostrum mAbs inhibit HIV-1 transfer from dendritic cells (DCs) to CD4+ target cells

Another important step for establishment of mucosal infection in the infant GI tract is the transfer of virions from DCs to CD4+ T lymphocytes. We therefore sought to determine whether colostrum Env-specific mAbs can inhibit the transfer of HIV-1 virions from DCs to CD4+ target cells. Six of eight (75%) mAbs that neutralized HIV-1 MW965 also inhibited DC transfer to CD4+/CCR5+ target cells (TZM-bl) at a concentration of 30μg/ml, with a MPI ranging from 91.4–100% (Figure 3 b). While the majority of the neutralizing antibodies efficiently inhibited DC viral transfer, none of the non-neutralizing gp120 or gp41-specific mAbs inhibited DC-C.MW965 virus transfer above background levels. Thus, the ability of colostrum Env-specific mAbs to inhibit infectious virus transfer by DCs may be restricted to neutralizing mAbs.

C1/C2-specific colostrum mAbs mediate antibody-dependent cellular cytotoxicity (ADCC)

Once HIV-1 virions or infected cells cross the mucosal epithelium, there are few mechanisms by which the adaptive immune system may protect from further dissemination of HIV-1. ADCC may be an effective way to locally contain HIV-1 infection once HIV-1 crosses the mucosal barrier[25] and in fact, has been associated with protection against postnatal HIV-1 transmission[11]. Vaccine-induced antibodies specific for the C1/C2 region of the gp120 Env have been previously described to mediate ADCC[26]. Thus, we tested our colostrum mAbs for ADCC activity against NK cells coated with 1086Cgp120 Env and found that 53% mediated ADCC. In concordance with previous findings, all four of our C1/C2-specific colostrum mAbs mediated ADCC, three at a high titer: DH280, DH382 and DH383 (16–27% GzB activity, Figure 3 c). Two of three V3-specific colostrum mAbs also mediated ADCC. Additionally, two conformationally gp120-specific mAbs mediated ADCC at a high level, one neutralizing mAb (DH276) and one non-neutralizing mAb (DH375). The ADCC mediating mAbs demonstrated peak activity at either 10 or 40μg/ml. However, endpoint concentration for the mAbs with high activity was below 0.1 μg/ml (Supplementary File T2). Interestingly, 71% of the non-neutralizing gp120-specific colostrum mAbs mediated ADCC, thus ADCC may be an important role of non-neutralizing Env-specific colostrum mAbs.

A subset of colostrum Env gp120-specific mAbs cross-react with intestinal microbiota antigens

While it has been established that mammary gland IgA-producing B cells originate in the gut-associated lymphoid tissue (GALT)[27, 28], the origin of IgG-producing B cells in milk is still uncertain. Interestingly, it was previously reported that gp41-specific mAbs derived from terminal ileum B cells of acute and chronically HIV-1 infected individuals are frequently cross-reactive to intestinal microbiota whole-cell lysate (WCL)[29] isolated from human stool samples of HIV-1 uninfected individuals. Therefore, we aimed to determine if colostrum Env-specific mAbs are similarly cross-reactive to commensal gastrointestinal microbiota. We tested our panel of 19 Env-specific colostrum mAbs and two previously described colostrum Env-specific mAbs[10] for reactivity against aerobic and anaerobic intestinal microbiota WCL by Western blot analysis (Supplementary File S3). Surprisingly,

none of the gp41-specific colostrum mAbs cross-reacted with the bacterial WCL antigens (Table 4). However, 76% (13/17) of gp120-specific colostrum mAbs cross-reacted with either aerobic or anaerobic intestinal microbiota WCL antigens in Western blot. Since a subset of gp120-specific mAbs induced by Env vaccination cross-react with intestinal microbiota WCLs[30], we sought to determine whether this cross-reactivity was specific to colostrum B cell-derived gp120-specific mAbs. Thus, we selected 18 well-characterized, gp120-specific antibodies isolated from blood of vaccinated or chronically HIV-1-infected individuals with a range of gp120 epitope specificities similar to that of the colostrum mAbs from our panel (Table 4), as well as one blood gp120-specific mAb from the lactating HIV-1-infected subject CH9105, to assess cross reactivity to bacterial WCL. Nine of the nineteen blood gp120-specific mAbs (47%), were reactive to either aerobic or anaerobic commensal bacteria WCL antigens in Western blot analysis, which was not different than the proportion of colostrum gp120-specific mAbs (13 of 17, 76%) that cross-reacted with stool bacteria WCL (p=0.096, Fisher's exact test).

Antibody reactivity to the aerobic and anaerobic commensal bacteria in native non-reduced conformation was then confirmed by surface plasmon resonance (SPR). Of the 13 colostrum gp120-specific mAbs that bound to bacterial WCL in Western blot, 10 were also reactive to bacterial WCL by SPR and two others that were negative by Western blot were reactive by SPR. In addition, four of the blood gp120-specific antibodies that bound to WCL in Western blot were confirmed to bind bacterial antigens by SPR and six additional blood gp120s were also reactive by SPR. The difference in mAbs found to be intestinal microbiota reactive in SDS-PAGE Western blot and SPR may be explained by the reduced versus native conditions of the WCL proteins in each assay, respectively. Overall, colostrum– derived gp120-specific mAbs (15 of 17) did not have significantly higher rate of intestinal microbiota reactivity than blood derived gp120-specific mAbs (15 of 18; p=0.662, Fisher's exact test). Additionally, colostrum antibody reactivity to intestinal microbiota does not appear to depend on gp120-epitope specificity or particular mAb genetic characteristics, as mAbs with varying gp120 specificities (V3, CD4 blocking, C1/C2, N334 glycan dependency and V1/V2) and with long and short CDR3 lengths demonstrated reactivity with bacterial antigens (Table 4). Yet, we explored whether any of the measured antiviral functions were associated with intestinal microbiota cross-reactivity and found that there were significantly less cross-reactive gp120 colostrum mAbs with the ability to inhibit dendritic cell mediated virus transfer compared to non-cross reactive mAbs (p=0.02, Fisher's exact test). For a full summary of antibody characteristics and function, refer to Supplementary File T3.

To determine if intestinal microbiota WCL cross-reactivity could be a product of polyreactive/autoreactive properties of the mAbs, we tested both the colostrum and blood gp120-specific antibodies for reactivity against autoantigens in Luminex AtheNA ANA II and HEp-2 immunofluorescence ANA assays. Only two of 15 colostrum-derived and one of 15 blood-derived intestinal microbiota cross-reactive, gp120-specific mAbs showed reactivity to non-HIV-1 antigens by these assays (Table 4). Thus, there is pervasive cross-reactivity of blood and breast milk gp120-specific mAbs with commensal bacteria antigens that does not appear to primarily be due to antibody polyreactivity[31].

Cross-reactivity of a colostrum isolated gp120-specific mAb with E. coli Chaperonin 60

One gp120 V3-specific colostrum mAb that strongly cross-reacted with commensal bacteria WCL (DH374) by Western blot and SPR, specifically bound to a single 60kDa bacterial antigen band on a reduced and non-reduced SDS-PAGE Western blot (Figure 4 a). This 60 kDa band was isolated by gel extraction and the antigen was identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as Chaperonin 60 from multiple bacterial species (*E. coli, B. thetaiotaomicron, E. eligens*). The binding of DH374 to *E. coli* Chaperonin 60 was confirmed by Western blot and ELISA (Figure 4 b and c) but did not bind by SPR or native gel (Supplementary File S4), potentially due to the native conformation of the antigen in solution when measured by SPR versus the non-native/ reduced conformation of the protein in the SDS-PAGE Western blot and ELISA. As there is limited amino acid homology between the linear Chaperonin 60[32] and the linear V3 sequence (Supplementary File S5), our findings suggest that the colostrum gp120 V3-specific mAb DH374 may cross-react with a conformational epitope on the monomer of the heptameric protein.

Affinity maturation of commensal bacteria cross-reactive colostrum Env-specific mAbs

To define the role that commensal bacterial antigens may play in the development of HIV-1 gp120-specific colostrum mAbs, we inferred the heavy- and light-chain unmutated common ancestors (UCAs)[12] of two gp120 and commensal bacteria-reactive colostrum mAbs, DH284 and DH285, and recombinantly-produced their UCAs and intermediates (Figure 5 a and b). In both clonal lineages, there was a progressive increase in the affinity for the gp120 (DH284) or V1V2 (DH285) Env antigen with antibody maturation (Figure 5 c and d). Yet, differences emerged in the binding affinity for bacterial WCL within each lineage. For DH284, the binding affinity to commensal bacteria WCL antigens increased over the maturation of this mAb (75 peak RU to 350 peak RU) (Figure 5 e) during affinity maturation for gp120. In contrast, the binding strength of the V1V2-specific mAb DH285 to commensal bacteria WCL decreased with maturation (binding affinity peak from 100 peak RU to 25 peak RU) (Figure 5 f), suggesting that this mAb evolved away from its specificity for bacterial antigens as it increased in affinity for HIV-1 Env. Notably, the DH285 clonal lineage developed tier 1 neutralization potency (C.MW965) during affinity maturation Env-specificity (Figure 5 b). These two examples of colostrum gp120-specific mAb evolution indicate that cross-reactivity for commensal bacteria can both be gained (Figure 5G) and lost (Figure 5H) during HIV-1 gp120-specific affinity maturation, demonstrating that GI bacterial antigens may contribute to shaping the milk B cell repertoire.

Discussion

Despite widespread ARV access, breast milk transmission of HIV-1 persists throughout areas of high HIV-1 prevalence. Immunologic strategies that are less dependent on daily adherence to antiretroviral drugs are needed to reduce postnatal HIV-1 transmission while maintaining the benefits of breastfeeding. Natural, innate antiviral factors in breast milk, such as Tenascin-C[33], may contribute to the inherently low rate of HIV-1 transmission via breastfeeding, but with the potential for immunologic interventions to enhance potentially protective maternal antibody responses in breast milk, the characteristics of the natural milk

antibody repertoire needs further exploration. IgG represents less than 10% of immunoglobulin secreted in breast milk, while IgA represents the majority of the remaining of the total milk immunoglobulin[34]. Yet, we and others have reported that the concentration of HIV-1 Env-specific IgG in breast milk, which makes up <10% of the total milk IgG [14, 36], exceeds that of the HIV-1 Env-specific IgA levels in milk by 1–2 logs [2, 14, 35] It is important to define the qualities and functions of the locally-produced milk HIV-1 Env-specific IgG relative to the concentration in milk to develop strategies for enhancing these potentially protective antibodies that can achieve levels that may be functional *in vivo*. Our study also sought to provide insight into the ontogeny of mAbs isolated from colostrum B cells that may contribute to blocking mucosal infection of the breastfeeding infant.

We previously reported that the Env-specific B cells in breast milk produced mAbs that had restricted isotype and gene usage compared to those in blood[12]. Moreover, compared to the systemic Env-specific B cell repertoire, the breast milk Env-specific B cell repertoire was biased towards gp120-specificity. This finding was surprising because of the observed gp41-specific mucosal mAb predominance in acute infection[37] and in the GALT of chronically infected individuals[29]. While most of the milk antibodies characterized in this work were isolated from HIV-1-infected lactating women with a predominance of gp120-specific B cells isolated from milk, the single transmitting mother, CH0404, displayed a predominantly gp41-specific colostrum Env-specific B cell repertoire (Table 2). However, this subject also had considerable risk factors for postnatal transmission, including a high milk viral load and low peripheral CD4+ T cell count[38], raising the question of whether the gp41-specificity predominance of the colostrum B cell repertoire in this subject is related to the high viral load and maternal immunosuppression, yet unrelated to the transmission outcome. Additional studies are necessary to investigate whether a gp41-specific B cell repertoire in breast milk provides less protection of the infant against HIV-1 infection compared to a gp120-biased B cell repertoire.

Despite colostrum Env-specific B cells being compartmentalized from those in blood on the basis of gp120 versus gp41 specificity in this population[12], our panel of colostrum gp120-specific antibodies demonstrate specificity for a wide range of epitopes on the gp120 spike[39]. Twenty percent of the gp120-specific colostrum mAbs blocked the CD4 binding site on the Env, which could be an important function of mucosal antibodies in blocking HIV-1 target cell infection at mucosal surfaces[40]. The isolated colostrum mAbs were also specific for the V3 loop, which could work in concert with those directed against the CD4 binding site[41]. As has been previously reported for weakly-neutralizing anti-V3 mAbs[42], our colostrum mAbs also showed gene preference for V_H5–51 usage. We also showed that colostrum-derived gp120-specific mAbs can target the V1/V2 loop, a response that has been implicated as protective when elicited systemically by HIV-1 vaccination[13]. Interestingly, we additionally identified a colostrum mAb, DH388, specific for a glycan binding site at position 334. This glycosylation site in the Env constant region 3 (C3) is part of a larger area known as the ‘high-mannose patch’, thought to be a promising epitope of antiviral activity for bnAbs[43]. It has been postulated that a high mannose glycan located at the asparagine at amino acid 332 is involved in viral escape from bnAbs and escaped viruses switch the glycosylation to position 334 at around two years following infection[22]. Yet, surprisingly, this mAb did not show neutralizing activity against any of the HIV-1 variants tested. Finally,

despite the high rate of V_H 1–69 usage among colostrum Env-specific B cells[12], we found no correlation between usage of this heavy chain gene and distinct gp120 epitope specificity or function.

This study also characterized the neutralizing potency of colostrum mAbs and their ability to inhibit virion transmission across a mucosal barrier. We found that a high rate of colostrum gp120-specific IgG mAbs could neutralize tier 1 HIV-1 viruses. However, only one colostrum antibody, DH378, weakly neutralized a tier 2 virus variant (SHIV1157ipd3N4). Though, importantly, four tier 1 virus-neutralizing mAbs isolated from colostrum of the non-transmitting subject CH9105 weakly neutralized autologous virus variants. This function, demonstrated in one individual, may exemplify a route to block vertical virus transmission, as the infant is solely exposed to maternal virus variants that coevolved with the autologous breast milk antibody responses. However, the function of our colostrum mAbs was not limited to neutralization, as we found that the neutralizing colostrum Env-specific mAbs also had other functions that may mediate protection against mucosal virus acquisition, including the capacity to inhibit HIV-1 virus transfer from DCs to CD4+ target cells[44,45] and inhibit viral particles binding to an epithelial cell layer[46]. Milk antibody blocking of virion interaction with mucosal cells prior to infection of CD4+ target cells may be key to protection of infants against HIV-1 infection oral cavity or GI tract.

The question of whether bnAbs can be induced at mucosal sites remains unclear; however, we isolated two colostrum-derived gp120 mAbs that may be precursors to HIV-1 bnAbs. DH388 was sensitive to changes in the outer domain glycan at amino acid position 334 in the C3 region of Env, the same high-mannose glycan patch targeted by bnAbs, PGT128, 130 and 131[47]. DH388 additionally had a long CDR3 (29 amino acids) that is characteristic of bnAbs, yet it did not demonstrate any neutralizing function. Another potential bnAb precursor, the CD4bs-directed colostrum mAb DH378, weakly neutralized autologous viruses and a tier 2 heterologous SHIV virus, as well as inhibiting epithelial and DC virus transfer. BnAbs have been shown to be effective at blocking oral virus transmission in neonatal rhesus macaques[48], but have yet to be elicited in mucosal or systemic compartments through vaccination. Thus, it will be important to determine if these nonbroadly neutralizing colostrum IgG mAbs offer any protection against infant oral acquisition.

It is well described that IgA is trafficked from the GALT to the mammary gland through the gut-mammary axis[16,17] and is theorized that IgG-producing B cells in milk follow this same trafficking pattern[49]. It was recently reported that a subset of gp41-specific mAbs isolated from blood and intestine B cells have reactivity to intestinal microbiota WCLs[29,50] and may develop as a result of preinfection B cell repertoire. Williams et al.[30] reported that Env-vaccine induced gp120 mAbs also cross react with intestinal microbiota and we found a large proportion of the gp120-specific colostrum mAbs isolated from infected individuals had cross reactivity to intestinal microbiota antigens. Of note, the panel of gp120-specific mAbs isolated from blood of chronically-infected or vaccinated individuals with matched epitope specificity to our colostrum gp120-specific mAb panel had a similar proportion of commensal bacteria cross-reactive mAbs, suggesting that the preexisting B cell repertoire stimulated by commensal bacterial antigens may contribute to shaping both the systemic and

breast milk Env-specific B cell repertoire. Interestingly, we identified a specific bacterial antigen, Chaperonin 60, that cross-reacted with the gp120-specific colostrum mAb, DH374. This cross-reactive gp120-specific mAb bound to the linear and scaffolded V3 loop, yet there was limited homology between the linear V3 and Chaperonin sequence. As this mAb only bound to the non-native, Chaperonin 60 protein, it may target an epitope only displayed on the monomer of this heptameric protein.

In our analysis of two clonal lineages of gp120-specific colostrum mAbs isolated from the same subject, we observed two distinct patterns of evolution for gp120-specific and commensal microbiota reactive mAbs. In one clonal lineage, DH285, the germline naïve B cell receptor, or UCA, showed affinity maturation for the V1/V2 loop and away from commensal bacteria reactivity. In this case, the IgG producing B cell may have been initially stimulated in the GALT, then through somatic hypermutation, developed a strong affinity for the V1/V2 loop of gp120. A second pattern, demonstrated by the lineage of DH284, also involved affinity maturation toward gp120 Env specificity, however this clonal lineage showed increasing cross-reactivity with commensal bacteria. As is theorized with HIV-1 gp41-specific antibodies[29, 51], this pattern may represent molecular mimicry of gp120 and commensal bacterial antigens. Alternatively, this commensal bacteria antigen cross-reactivity may be a common feature of milk B cell-produced antibodies, since the maternal milk antibodies have also been described as regulators of the infant GI microbiome[52].

There are several limitations to our study. First, we only characterized antibodies from a small number of HIV-1-infected, lactating subjects, focusing on subjects in which we were able to isolate a large number of antibodies[12]. Moreover, we characterized the function of only a few subject-matched systemic antibodies in parallel with the colostrum Env-specific antibody panel. Yet, the systemic Env-specific B cell repertoire has been studied extensively in previous reports[29, 39, 53, 54], and therefore we focused our investigations on the colostrum antibodies. Beyond our association of milk and blood HIV-1 Env-specific and microbiota cross-reactive antibodies, it would be relevant to probe the hypothesis that the preexisting commensal microbiota influences the HIV-1 specific IgG-producing B cell repertoire. Moreover, it would be interesting to isolate milk Env-specific B cells at various time points postpartum to track development of clonal lineages over time and further characterize mammary gland B cell trafficking and evolution.

In summary, these data provide evidence that IgG antibodies produced by B cells resident in breast milk mediate important anti-HIV-1 functions that may contribute to protection of the infant against postnatal virus acquisition. This work supports the development of strategies to specifically target the induction of functional, HIV-1 Env-specific IgG producing B cells in breast milk. In fact, we have previously reported that combined systemic and mucosal maternal HIV-1 Env vaccination can elicit functional antibody responses in milk of lactating nonhuman primates[9, 55]. Maternal vaccination during breastfeeding to specifically target the B cell population which traffics to and resides in breast milk may be a feasible and effective strategy to make breastfeeding safe for all infants born to HIV-1-infected women.

Methods

Study Subjects

The subjects whose blood and colostrum samples were selected for analysis of antibody function, were from the previously described CHAVI009 Malawian HIV-1-infected maternal cohort enrolled between 2008 and 2009[12, 14, 56]. The majority of subjects were untreated or antiretrovirals were initiated during the third trimester. Single dose Nevirapine was provided to all mothers and infants at the time of delivery. Maternal blood and milk samples were collected postpartum through the period of breastfeeding. HIV-1 transmission was determined by infant whole blood HIV-1 DNA PCR[24]. The subjects' baseline plasma viral load and peripheral CD4+ T cell count was measured at the third trimester of pregnancy. Milk virus load was quantified by RT-PCR, with a detection limit of 240 RNA copies/ml, yet if virus RNA was detected in the sample but below the minimum for quantification, 120 RNA copies/ml was assigned[14]. Blood and breast milk samples used in this study to isolate B cells were collected within one week postpartum, and therefore are referred to as colostrum.

Isolation of Env-specific B cells from colostrum and blood, antibody variable region gene amplification, sequencing and screening for HIV-1 Env reactivity

As described, breast milk cells (BMCs) and peripheral blood mononuclear cells (PBMCs) isolated from EDTA-anticoagulated blood were thawed, stained, isolated and sorted according to our previously reported data[12]. B cells were sorted using fluorescently-labeled ConS gp140 staining, with the exception of B cells from PTID CH8802 which used ConC gp120 antigen.

B-cell immunoglobulin gene PCR, sequencing and HIV-1 Env reactivity screening were performed as described[12]. Briefly, RT and nested PCR were utilized to amplify B cell expressed variable heavy and light chain genes. PCR products were then purified and sequenced. Using the first round, functional immunoglobulin, PCR products were transiently transfected in 293T cells and supernatants of the transfected cells were screened for reactivity against HIV-1 Env proteins (C.1086gp120[57] or MNgp41, obtained from Immunodiagnostics) by ELISA.

Selection of colostrum Env-specific mAbs for characterization

The panel of colostrum Env-specific mAbs selected was originally from six HIV-1-infected mothers, five postpartum non-transmitting mothers and one transmitting mother whose B cell repertoires were previously analyzed[12]. We selected colostrum IgG mAbs from three mothers (040-4, 910-5 and 920-8) who had a high number of mAbs isolated (between 7 and 14) and from three other mothers that had few antibodies isolated. We selected 19 colostrum mAbs for further characterization using the criteria of strong Env binding strength and breadth, and membership in an identified B cell lineage. Antibody gene sequences were inserted into competent pcDNA 3.1 expression plasmids using standard molecular technology, then these mAbs heavy and light chain plasmids were transfected into 293F and resulting IgG was protein A purified prior to binding and functional assays.

ELISA binding and blocking assays

Recombinant mAbs expressed in small and large-scale transfections were assayed for antibody reactivity to HIV-1 antigens by ELISA as described[50, 58]. Purified antibodies were incubated with HIV-1 Env antigen-coated wells at concentrations ranging from 100 μg/ml to 5.6E-4 μg/ml at 3-fold dilutions with positivity cutoffs for reactivity set at 3-fold above background and an optical density (OD) of 0.180 at 100 μg/ml. Fine specificity of gp120 colostrum mAbs was determined by binding to BconV3, CconV3[21], gp70MNV3[59], C.1086 V1/V2tags[60], CH505 gp120 and CH505 gp120 N334 mutant. MAb blocking of A32 and sCD4 were performed as described[61]. Briefly, 0.2 μg of protein (C.97ZMgp140, A244gp120 or ConSgp140)[57] was added to each well and blocked with assay diluent (PBS containing 4% [wt/vol] whey protein, 15% normal goat serum, 0.5% Tween 20, and 0.05% sodium azide). Each assay step was conducted in assay diluent and incubated for 1 h at room temperature and was followed by washing with PBS-0.1% Tween 20, except the substrate and subsequent reaction stop step. MAbs were titrated at concentrations ranging from 100 μg/ml to 0.001 μg/ml at 3-fold dilutions. In addition, CH31[62] and CH106[63] were included as positive controls. For the CD4 blocking assay, sCD4 was added to each well at a concentration of 0.64 μg/ml. Biotinylated A32 or OKT-4 (eBioscience) was added at the 50% effective concentration determined by a direct binding curve (biotinylated-MAb versus JRFL protein). Inhibition of biotin-MAb binding was detected with streptavidin-alkaline phosphatase at 1:1,000 (Promega V5591), followed by incubation with alkaline phosphatase substrate (2 mM $MgCl_2$, 1 mg/ml *p*-NPP, CBC, pH 9.6). Plates were read with a plate reader at 405 nm. For duplicate wells, background values were subtracted, and the results were averaged. Percent inhibition was calculated as follows: [1 -(mean of triplicate wells/mean of no-inhibition control)] × 100.

HIV-1 pseudovirus neutralization in TZM-bl cells

HIV-1 Env heterologous pseudovirus neutralization assays were performed as described[64]. Briefly, colostrum mAbs were incubated with virus for 45–90 minutes, after which TZM-bl cells were added at a concentration of 1.0×10^5. Luminometry was used to determine if antibodies are able to inhibit viral entry, inhibitor concentration (IC_{50}) calculated as a 50% reduction of relative light units with respect to virus-only wells. All antibodies were tested for neutralization capacity against C.MW965 and 1086C in TZM-bl cells, and if positive were screened against additional viruses. Virus variants BF 1677.613a, BF942.218d and BF1266.431a are postnatal T/F HIV viruses[24], whereas 0724 and 0711 are 910-5 autologous virus variants. To isolate autologous virus from available plasma with detectable viral load, single genome amplification and sequencing was performed as described[65] on plasma aliquots from PTID 910-5 resulting in only two viral variants, likely due to low virus load. Once functional viruses were amplified and sequenced, cloning and viral production was implemented as described[24].

Epithelial Cell Inhibition Assay

To determine the ability of colostrum IgG mAbs to impede infectious virus binding to colonic epithelial cells (HT29), a modified previously reported protocol was used[24]. Percent inhibition was calculated by dividing the RLUs of each well by the median RLUs of

epithelial-bound virus that was not pre-incubated with isolated colostrum mAbs. The anti-influenza mAb CH65 IgG was used as a negative control, whereas the broadly neutralizing anti–HIV-1 CD4 binding site mAb VRC01 was used as a positive control. The cutoff value was determined by the mean inhibition plus 2SD of CH65 IgG relative to no antibody.

Dendritic cell HIV-1 trans-infection assay

To determine whether our mAbs blocked virus transfer to monocyte-derived dendritic cells (DCs), we used a method reported previously[24]. The ability of various Abs to prevent the DC-mediated transfer of HIV MW965 was measured by comparing the TCID50 of the dendritic cell-bound virus and the total virus added to the dendritic cells. The anti-influenza mAb CH65 IgG was used as a negative control, whereas the broadly neutralizing anti–HIV-1 CD4 binding site mAb VRC01 was used as a positive control. The cutoff value was determined by the mean plus 2SD of CH65 relative to no antibody.

Antibody Dependent Cellular-Cytotoxicity

ADCC activity was determined by the GranToxiLux (GTL) assay as described[66]. Briefly, $CEM.NKR_{CCR5}$ target cells (NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, from Dr. Alexandra Trkola) were coated with recombinant HIV-1 1086.C gp120. NK cells isolated by negative selection with magnetic beads (Miltenyi Biotec GmbH, Germany) from cryopreserved PBMC collected from a healthy HIV-1 seronegative donor with the F/F low-affinity Fc-gamma Receptor (FcRγ) IIIa phenotype were used as effector cells. The NK cells were added to the gp120-coated target cells in wells of a 96-well plate at a ratio of 10:1. The colostrum mAbs were tested at a final concentration range of 40 to 0.01 μg/ml. The RSV-specific mAb Palivizumab and the HIV-1 specific anti-C1 mAb A32[67] were included as negative and positive controls, respectively. The assay plates were incubated for 1 h at 37°C in 5% CO_2. A minimum of 2,500 events representing viable target cells was acquired for each well using a LSRII flow cytometer (BD Biosciences, San Jose, CA). Data analysis was performed using FlowJo 9.8.1 software (Tree Star Inc., Ashland, OR). The % Granzyme B (GzB) activity was defined as the percentage of cells positive for proteolytically active GzB out of the total viable target cell population. The final results are expressed after subtracting the background %GzB activity observed in wells containing no mAb samples. ADCC Ab titers were determined by interpolating the concentration of mAb that intersect the positive cut-off (>average+3SD %GzB activity of mAbs tested against uncoated target cells) using Graph Pad Prism 5 software (Graph Pad, La Jallo CA).

Western Blot Analysis of Intestinal microbiota WCLs

Colostrum mAbs were tested for gastrointestinal microbiota antigen reactivity as described[29]. Previously characterized[10], CH07 and CH08 were added to our panel of colostrum mAbs to round out the panel of 17 colostrum mAbs. A panel of gp120-specific mAbs isolated from the blood, of both HIV-1-infected and vaccinated individuals, matched by epitope specificity to the panel of colostrum-isolated gp120-specific mAbs including V2-specific mAbs CH58[60], CH59[60], HG107[60]; V3-specific mAbs F39F[68], 19b[69], CH22[70], CH16 (provided by B. Haynes and the Center for HIV/AIDS Vaccine Immunology); V1/V2 mAb PG9[71], V2/V3 mAb PG16[71]; CD4i mAbs 17b[72], A32[73], CH38[74]; CD4bs mAbs CH31[75], VRC01[76], CH13, CH17 and CH18 (provided by B. Haynes and the Center for

HIV/AIDS Vaccine Immunology); conformationally dependent mAbs CH21[74], and DH379 were included for comparison. Briefly, western blot analysis of intestinal microbiota WCL reactivity, 100 μg of human stool aerobic and anaerobic bacterial whole cell lysates were run on 4%–12% Tris-Bis SDS-PAGE (Life Technologies) for 1.5 h at 150 V in both reduced and nonreduced conditions. NuPAGE sample reducing agent at 1× was used for reducing conditions (Life Technologies). Antigens were transferred to nitrocellulose using Life Technologies iBlot Gel Transfer system. Antibody binding was tested at 20 μg/ml for all antibodies, and the Anti-Human IgG (whole molecule)-Alkaline Phosphatase antibody produced in goat (Sigma) was used at a 1:5,000 dilution. Detection occurred directly on the nitrocellulose using Western Blue (Promega).

Autoantibody Assays

Assays to test autoreactivity of isolated colostrum mAbs were performed as described[31]. Briefly, antibodies were assayed for reactivity to HEp-2 cells at 50μg/ml and 25μg/ml (Inverness Medical Professional Diagnostics) by indirect immunofluorescence staining. Autoreactivity was also determined by antibody multiplex AtheNA Multi-Lyte ANA II test (Wampole Laboratories) with a dose dilution starting at 50μg/ml and determined positive when assay scores were 225 MFI or above.

Surface Plasmon Resonance and Galcer blocking assays

To confirm the reactivity of colostrum mAbs to commensal bacteria WCL and C.1086 gp120 Env, SPR binding assays were performed on a Biacore 4000 (GE Healthcare) maintained at 25°C as described[30]. Briefly, the colostrum mAbs were immobilized on a Series S CM5 sensor chip to 5,000 to 6,000 response units (RU) using standard amine coupling chemistry. Uninfected human stool bacterial WCL, 1086Cgp120 or C.1086V1/V2 protein was injected over the immobilized colostrum mAbs. Injection time was 150s and the dissociation activity was monitored for an additional 100s. The maximal RU of binding at 150s was reported. The dissociation constant (kd) was calculated using a 1:1 Langmuir model from 160s to 250s. For bacterial WCL, avidity scores were calculated using the formula, RU/kd. All data analysis was performed using the BIAevaluation 4.1 analysis software. (GE Healthcare).

The lipids, Lyophilized powder of D-galactosyl-β-1,1' N-octanoyl-D-erythro-sphingosine (Galcer) and chloroform stock of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), were purchased from Avanti Polar Lipids (Alabaster, AL).The Galcer liposomes were prepared at a 1:1 Galcer:POPC molar ratio and used in the Galcer blocking assay as detailed[20].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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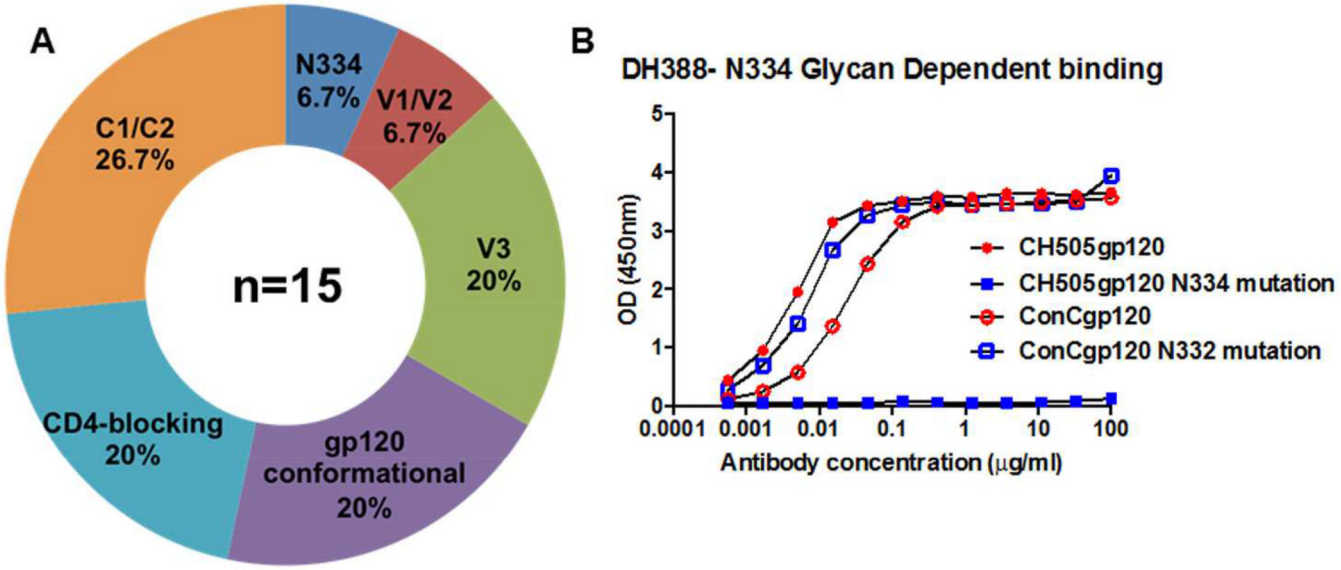


Figure 1. Fine specificity of isolated colostrum anti-gp120 IgG mAbs

(A) V3 and V1/V2 specificity was determined by direct V3 peptide and scaffolded V1/V2 protein binding ELISA. CD4 blocking mAbs were defined by Env sCD4-blocking ELISA. C1/C2 specificity was determined by mAb A32-blocking ELISA. (B) MAb DH388 Env binding was dependent on N334 glycan for binding by ELISA using CH505gp120 and a mutant CH505gp120 that has an alanine substitution at position 334. DH388 binding was not dependent on the glycan located at amino acid position 332.

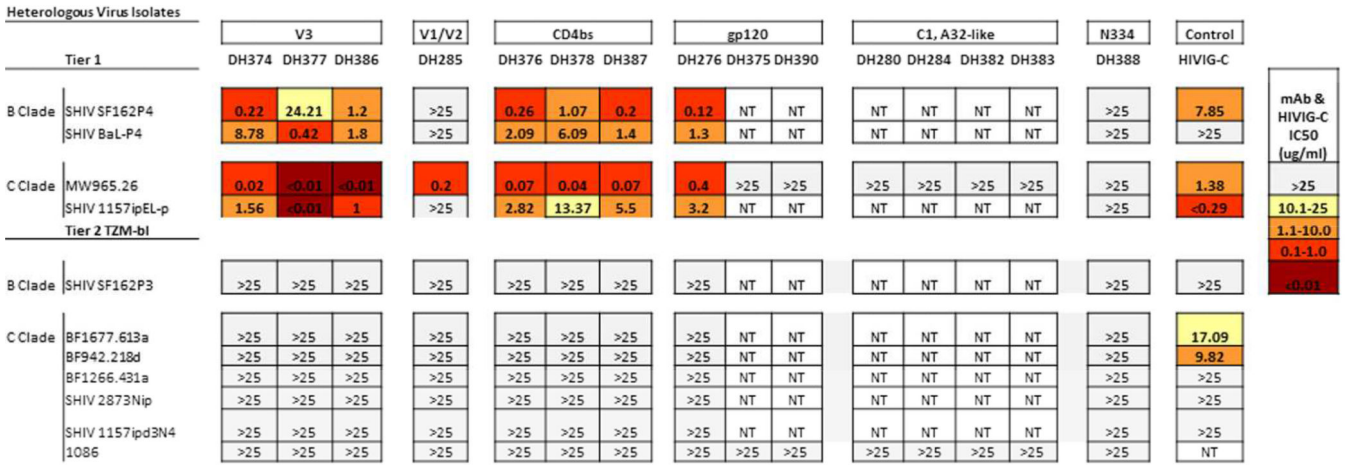


Figure 2. Gp120 Env-specific colostrum IgG mAbs isolated from non-transmitting subjects showed neutralization activity against tier 1 HIV-1/SHIV variants in TZM-bl cells

The inhibitory concentration 50% (IC_{50}) was determined against a panel of tier 1 and 2 HIV-1 and SHIV variants in TZM-bl cells using an initial concentration of 25μg/ml. When assayed at an initial concentration of 50ug/ml, DH378, a CD4 blocking mAb, neutralized the tier 2 SHIV1157ipd3N4 variant (IC50=41μg/ml). All mAbs were screened against tier 1 C.MW965 and tier 2 C.1086. Mabs with neutralizing activity or BnAb properties (DH388) were then screened against a wider panel of tier 1 and tier 2 heterologous viruses.

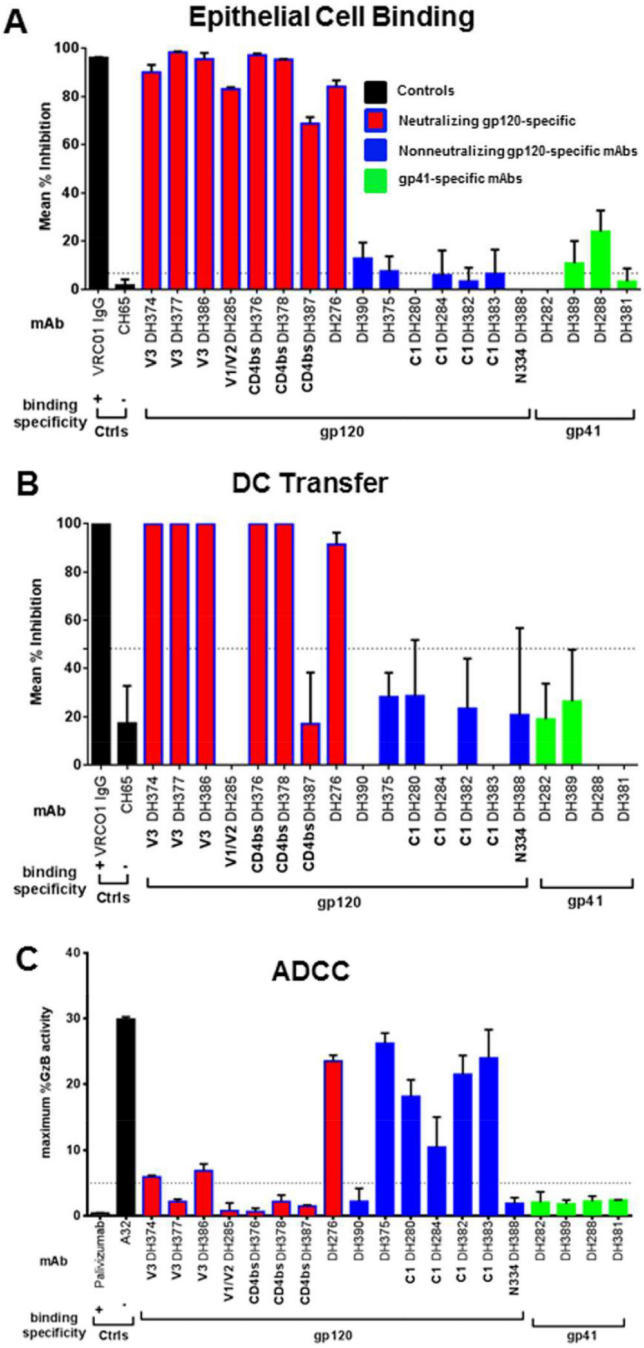


Figure 3. Inhibition of HIV-1 epithelial cell binding and DC-mediated transinfection by Env-specific colostrum mAbs

(A) Eleven of fifteen colostrum Env-specific mAbs inhibit epithelial cell binding of the clade C HIV-1 variant MW965. Epithelial cell binding inhibition was mediated at a high level by the HIV-1 MW965-neutralizing mAbs (red bars), and at a low level by one nonneutralizing gp120 specific mAb (DH390) and gp41 mAb's (DH288 and DH389). (B) Six of fifteen colostrum gp120 specific antibodies inhibit DC transfer of clade C HIV-1 variant MW965 from mature DC's to CD4+/CCR5+ target cells (TZM-bl). The cut off for significant response in both assays was determined by the mean response of the negative control (anti-

influenza mAb CH65) plus two standard deviations (dotted line). (C) Maximum ADCC activity is reported indicated that eight of fifteen colostrum gp120 specific mAbs mediate ADCC of 1086Cgp120 coated NK cells. All of the antibodies reported to mediate ADCC of 1086C coated NK cells were compared to uncoated NK cells as controls and all of the ADCC activity against uncoated NK cells was found to be below the cutoff of positivity. For the three assays above, mAbs were considered positive if their mean was above the cut off.

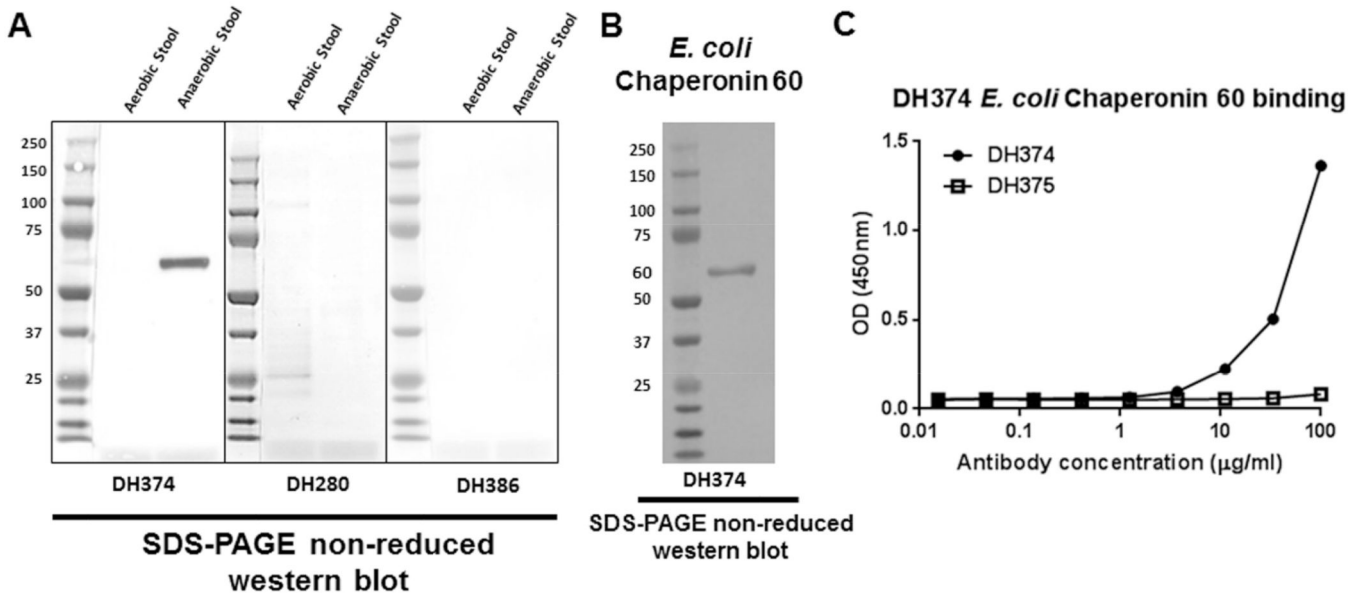
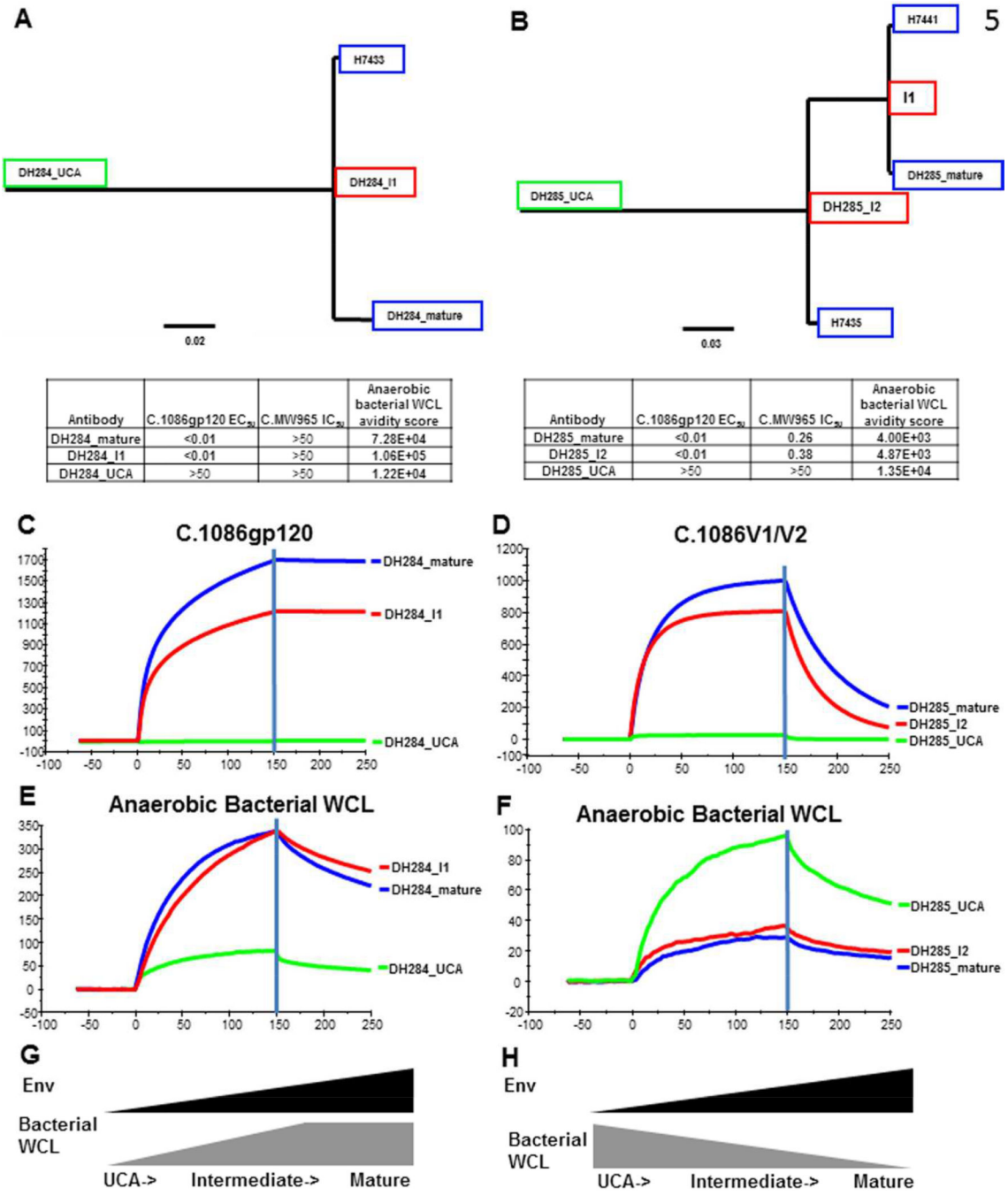


Figure 4. HIV-1 gp120-specific colostrum mAbs are cross reactive with commensal bacteria whole cell lysate (WCL) by western blot, including specific reactivity against *E. coli* Chaperonin 60

(A) gp120-specific mAbs were tested for reactivity to bacterial WCL by SDS-Page reduced and non-reduced western blot. MAb DH374 was strongly reactive (++) against a single protein band in the WCL, whereas DH280 was weakly reactive (+) against a number of protein bands and DH386 was non-reactive (−) to gut microbiota WCL. (B) The specificity of DH374 for *E. coli* Chaperonin 60 was confirmed by both reduced and non-reduced SDS-Page western blot. (C) DH374 binding to Chaperonin 60 was also confirmed by binding ELISA to the non-reduced *E. coli* Chaperonin 60 protein (3-fold dilutions, ranging from 100 µg/ml to 0.01 µg/ml). DH375, a noncommensal bacteria-reactive colostrum mAb, was used as a negative control.



Antibody	C.1086gp120 EC_{50}	C.MW965 IC_{50}	Anaerobic bacterial WCL avidity score
DH284_mature	<0.01	>50	7.28E+04
DH284_I1	<0.01	>50	1.06E+05
DH284_UCA	>50	>50	1.22E+04

Antibody	C.1086gp120 EC_{50}	C.MW965 IC_{50}	Anaerobic bacterial WCL avidity score
DH285_mature	<0.01	0.26	4.00E+03
DH285_I2	<0.01	0.38	4.87E+03
DH285_UCA	>50	>50	1.35E+04

Figure 5. Affinity maturation of colostrum gp120-specific and commensal bacteria cross reactive mAbs

(A–B) Phylogenetic trees are rooted on the inferred unmutated common ancestor (UCA). Color boxes surrounding lineage antibodies (UCA green, Intermediate red, Mature blue) indicate the antibody HIV-1 C.1086 Env binding strength (EC_{50}), MW965 neutralization potency (IC_{50}), and aerobic commensal bacteria WCL reactivity (avidity score); color corresponds to graphs below. (C) MAb DH284 clonal lineage C.1086gp120 antigen reactivity by SPR. (D) MAb DH285 clonal lineage C.1086V1/V2 antigen reactivity by SPR. (E–F) MAb DH284 and mAb DH285 clonal lineages commensal bacteria WCL reactivity by

surface plasmon resonance (SPR), respectively. (G–H) Schematic of the reactivity of clonal lineages DH284 and DH285 to anaerobic commensal bacteria and HIV-1 Env through lineage maturation.

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Table 1

Characteristics of selected, recombinantly-produced colostrum Env-specific mAbs

Subj ID	Postnatal Trans-mission Status (Yes/No)	Sample Type	mAb ID	Env binding EC_{50}[ab] (μg/ml)	Env Specificity	Env specificity EC_{50}[a] (μg/ml) or max % blocking	Iso type	V_H Gene	V_H Mutation %	V_H CDR3 Length (AA#)	V_K or V_L Chain	V_K or V_L Gene	V_K or V_L Mutation %	V_K or V_L CDR3 Length (AA#)
CH 9105	No	Colostrum	DH374	<0.01	V3[c]	<0.01	IgG1	5~51*03	8%	13	V_L	3~1*01	4%	9
		Colostrum	DH375	0.01	gp120	n/a	IgG1	1~46*02	10%	13	V_K	6~21*01	4%	9
		Colostrum	DH376	<0.01	CD4 blocking	91.7%	IgG1	1~69*01,11	11%	18	V_K	3~20*01	7%	9
		Colostrum	DH377	<0.01	V3[c]	0.03	IgG1	3~30*02	8%	18	V_K	4~1*01	3%	8
		Colostrum	DH378	<0.01	CD4 blocking	92.4%	IgG1	1~69*01,11	8%	16	V_K	3~20*01	5%	9
		Colostrum	DH276	<0.01	gp120	n/a	IgG1	1~69*01	9%	22	V_K	3~20*01	4%	10
		Colostrum	DH280	0.02	C1, A32-like	85%	IgG1	1~69*01	10%	15	V_K	1~12*02,01	5%	9
		Colostrum	DH282	0.03	gp41	n/a	IgG1	1~2*02	10%	14	V_K	1~12*01,02	8%	9
		Peripheral Blood	DH379	<0.01	gp120	n/a	IgG1	1~2*02	9%	12	V_K	3~20*01	6%	9
		Peripheral Blood	DH380	0.02	gp41	n/a	IgG1	1~2*02	11%	14	V_K	1~12*01,02	8%	9
CH 0404	Yes	Colostrum	DH288	<0.01	gp41	n/a	IgG1	1~69*01	11%	14	V_K	3~11*01	5%	9
		Colostrum	DH381	<0.01	gp41	n/a	IgG1	4~4*02	3%	18	V_K	3~20*01	4%	11
		Colostrum	DH382	<0.01	C1, A32-like	70.9%	IgG1	4~31*03	8%	15	V_L	2~8*01	3%	10
		Colostrum	DH383	0.01	C1, A32-like	85.5%	IgG1	1~2*02	5%	13	V_L	8~61*01	6%	10

Subj ID	Postnatal Trans-mission Status (Yes/No)	Sample Type	mAb ID	Env binding EC_{50}[ab] (μg/ml)	Env Specificity	Env specificity EC_{50}[a] (μg/ml) or max % blocking	Iso type	V_H Gene	V_H Mutation %	V_H CDR3 Length (AA#)	V_K or V_L Chain	V_K or V_L Gene	V_K or V_L Mutation %	V_K or V_L CDR3 Length (AA#)
		Peripheral Blood	DH384	<0.01	gp41	n/a	IgG1	3~30*14	13%	17	V_K	1D~17*01	8%	9
		Peripheral Blood	DH385	<0.01	gp41	n/a	IgG1	1~69*06,10	7%	15	V_K	1~12*01,02	4%	9
CH 9208	No	Colostrum	DH285	0.01	V1/V2[d]	<0.01	IgG1	1~69*01,05	9%	15	V_K	3~20*01	8%	9
		Colostrum	DH386	<0.01	V3[c]	<0.01	IgG1	5~51*03	7%	18	V_L	3~1*01	5%	8
		Colostrum	DH284	0.01	C1, A32-like	64.5%	IgG1	3~49*03	6%	23	V_K	3~20*01	7%	9
		Colostrum	DH387	<0.01	CD4 blocking	93.4%	IgG1	1~69*01,11	10%	16	V_K	1~39*01	5%	9
CH 8908	No	Colostrum	DH388	<0.01	N334 Glycan Dependent	n/a	IgG1	1~69*11	8%	29	V_K	3~20*01	5%	9
CH 9606	No	Colostrum	DH389	0.01	gp41	n/a	IgG1	1~69*01,11	9%	18	V_K	3~11*01	5%	9
CH 8802	No	Colostrum	DH390	<0.01	gp120	n/a	IgG1	3~30-3*01	3%	13	V_L	3~10*01	1%	11

[a] An estimate of the EC50 was calculated by a titration of antibody binding or blocking in ELISA assays. 100 μg/mL was the highest concentration tested.

[b] C.1086gp140

[c] BConV3

[d] 1086CV1/V2Tags

Table 2

Clinical characteristics of HIV-1-infected lactating Malawian women from whom the panel of colostrum Env-specific monoclonal antibodies (mAbs) were isolated and recombinantly produced

Subject ID	Postnatally Transmitted to Infant	Breast Milk Viral Load (copies/ml)[ab]	Peripheral CD4+ T cell count	Breast Milk neutralization of C. MW965 Tier 1 virus IC_{50}[c]	Colostrum Antibodies			
					Isolated		Selected	
					gp120	gp41	gp120	gp41
CH8802	No	<240	382	131	1	0	1	0
CH8908	No	<240	178	24	1	0	1	0
CH9105	No	<240	266	100	12	2	7	1
CH9208	No	<240	447	21	8	0	4	0
CH9606	No	<240	558	46	0	1	0	1
CH0404[a]	Yes	29,425	80	28	2	5	2	2

[a] Measured at 4–6 weeks postpartum.

[b] Assay detection limit= 240 RNA/ml, <240= virus detected in milk, but at a level lower than the assay detection limit

[c] The values indicated are indicated as inhibitory dilution 50% (ID_{50}), determined in TZM-bl cells using an initial dilution of 1:20

Table 3

CH910-5 V3-specific and CD4-blocking tier 1 heterologous HIV-1 neutralizing colostrum mAbs weakly neutralize autologous plasma virus variants

Autologous Virus Isolates	Colostrum mAb Env-specificity				
	V3-specific		CD4-blocking		gp120-specific
	DH374	DH377	DH376	DH378	DH276
CH910–5.0711	**47.41**[a]	**44.48**	**41.75**	**45.42**	>50
CH910–5.0724	**46.37**	**40.69**	**38.56**	**38.97**	>50

[a]The values indicated are indicated as inhibitory concentration 50% (IC_{50}), determined in TZM-bl cells using an initial concentration of 50μg/ml.

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Table 4

Binding characteristics of gp120-specific purified antibodies isolated from the colostrum B cells of HIV-1 transmitting and non-transmitting mothers

Sample Type	Antibody ID	gp120 fine-specificity[a]	SDS- PAGE Western Blot[b]		Surface Plasmon Resonance of Bacteria WCL[c]		Autoreactivity/ Polyreactivity Assays	
			Aerobic Bacteria WCL[d]	Anaerobic Bacteria WCL[d]	Aerobic Peak Response Unit (RU)	Anaerobic Peak Response Unit (RU)	Athena: (Positivity cut off= MFI >125)	HEp-2 Staining @ 25μg/ml[e]
Colostrum B Cell mAbs	DH374	V3	++	++	**39**	**93.7**	no	no
	DH375	conformational	+	+	**196**	**227**	no	**yes**
	DH376	CD4 blocking	+	+	5.38	**19.7**	no	no
	DH377	V3	+	+	−1.21	5.89	no	no
	DH378	CD4 blocking	+	+	**18.7**	**19.9**	no	no
	DH276	conformational	−	+	−6.39	−11.8	no	no
	DH280	C1, A32-like	+	+	7.61	**16.8**	no	no
	DH382	C1, A32-like	+	−	**224**	**179**	no	no
	DH383	C1, A32-like	+	−	**86.3**	**176**	no	no
	DH285	V1/V2	+	−	7.72	**22.8**	no	no
	DH386	V3	−	−	−0.0518	9.29	ND[e]	ND[e]
	DH284	C1, A32-like	−	−	**294**	**300**	ND[e]	ND[e]
	DH387	CD4 blocking	−	−	−0.629	−9.23	ND[e]	ND[e]
	DH388	N334 glycan	+	−	**20.8**	**32.3**	no	no
	DH390	conformational	+	+	**110**	**152**	no	no
	CH07	C5/gp41 fusion	−	−	**19.9**	7.37	**yes**[f]	**yes**
	CH08	CD4i	−	+	0.89	4.77	no	no
Peripheral Blood B cell mAbs	DH379	conformational	−	+	3.55	5.06	no	no
	CH58	V2	−	−	−1.7	**34.2**	no	ND[e]
	F39F	V3	−	−	−4.28	0.769	no	no
	HG107	V2	+	+	**23.7**	2.22	no	**yes**
	17b	CD4i	+	+	12.7	9.57	no	no

Sample Type	Antibody ID	gp120 fine-specificity[a]	SDS- PAGE Western Blot[b]		Surface Plasmon Resonance of Bacteria WCL[c]		Autoreactivity/ Polyreactivity Assays	
			Aerobic Bacteria WCL[d]	Anaerobic Bacteria WCL[d]	Aerobic Peak Response Unit (RU)	Anaerobic Peak Response Unit (RU)	Athena: (Positivity cut off= MFI >125)	HEp-2 Staining @ 25μg/ml[e]
	CD4i	A32	−	−	**294**	**342**	no	no
	19b	V3	−	−	9.95	**23.2**	ND[e]	no
	V2	CH59	−	+	0.464	0.0873	no	no
	CH31	CD4bs	−	−	6.74	**39.9**	no	no
	V1/V2	PG9	+	+	−4.17	14.4	no	no
	PG16	V2/V3	−	−	**47.5**	**68.8**	no	no
	CH21	conformational	+	+	−1.83	11.9	ND[e]	ND[e]
	CH22	V3	−	−	−27.2	0.225	no	ND[e]
	VRC01	CD4bs	−	−	12.3	**60.9**	ND[e]	no
	C1	CH38	+	+	**25.5**	**48.6**	no	no
	CH13	CD4bs	+	++	**24.7**	**46**	ND[e]	ND[e]
	CH16	V3	−	−	−17.2	4.35	ND[e]	ND[e]
	CH17	CD4bs	−	−	−14.1	6.28	ND[e]	ND[e]
	CH18	CD4bs	+	+	**21.5**	**25.2**	ND[e]	ND[e]
	p-value[g]		0.19	1	0.31	0.33		

[a] Specificity data collected from Table 1 or LANL database (http://www.hiv.lanl.gov/content/immunology/antibody_id.html)

[b] Reactivity in western blot and Hep-2 assays was scored with "no" for no reactivity or "yes" for positive reactivity

[c] Commensal bacteria mAb binding considered postive if >15 RU

[d] Examples of '--, '+', '++' western blot reactivity shown in Figure 4

[e] Assays were not done for these antibodies

[f] Reactivity with centromere protien B, autoantigen SCL70 and double stranded DNA

[g] Fisher's exact test comparing the frequency of commensal bacteria-reactive mAbs among gp120-specific mAbs isolated from peripheral blood and colostrum B cells