Secretory Anti–Human Immunodeficiency Virus (HIV) Antibodies in Colostrum and Breast Milk Are Not a Major Determinant of the Protection of Early Postnatal Transmission of HIV

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The immune response to human immunodeficiency virus (HIV) type 1 was evaluated in breast milk from HIV-infected African mothers who had transmitted and those who had not transmitted HIV to their children through breast-feeding. The levels, specific activities against gp160 and 2 HIV-derived peptides from gp41 and gp120 (V3 loop), and inhibitory activity toward viral transcytosis in vitro of secretory IgA (S-IgA) and IgG purified from breast milk were investigated in 8 transmitting mothers and 18 nontransmitting mothers. S-IgA and IgG antibodies to gp160 and to peptides were found in all breast milk samples. The specific activities of S-IgA and IgG to gp160 and peptides were similar between transmitting and nontransmitting mothers. No difference of the capacity of S-IgA and IgG to block HIV transcytosis in vitro was found between the 2 groups. These results suggest that humoral mucosal immunity to HIV does not appear as a predominant factor for protection against viral transmission through breast milk.

Human immunodeficiency virus (HIV) may be transmitted through breast milk [1–8]. Although the risk of perinatal mother-to-child transmission of HIV is significantly decreased with the use of antiretroviral chemoprophylaxis, postnatal transmission of HIV through breast milk remains a major challenge for public health in developing countries. Transmission of HIV through breast milk is determined by maternal infectivity and unknown factors of infant susceptibility. Factors that determine breast milk infectivity include the size of viral inoculum [3, 9–12], the presence of nonspecific antiviral activity in milk [13], and the specific mucosal immune response to HIV [3, 14–16]. Colostrum, early milk, and mature breast milk of HIV-infected women each contain secretory IgA (S-IgA), IgG, and secretory IgM (S-IgM) antibodies to env-encoded surface glycoproteins and pol- and gag-encoded proteins [3, 14–16], with IgG antibodies being the most abundant isotype [3, 8, 14, 15]. In a model system, S-IgA and IgG purified from colostrum are capable of blocking transcytosis of cell-associated HIV through a tight monolayer of epithelial cells in vitro [8, 16]. In a previous study in Kigali, Rwanda, the presence of HIV-specific S-IgA and S-IgM, but not of HIV-specific IgG in noncolostral breast milk, was found to be associated with a decreased risk of mother-to-child transmission of the virus [3]. In this study, we compared the levels, specific activity, and inhibitory activity toward viral transcytosis of S-IgA and IgG purified from colostrum and early milk of transmitting and nontransmitting HIV-seropositive mothers in Bangui, Central African Republic. None of these parameters could discriminate between the 2 groups of mothers, suggesting that humoral mucosal immunity to HIV does not appear as a predominant factor for protection against viral transmission through breast milk.

Materials and Methods

Study population. The study population included 43 unselected breast-feeding mothers who gave birth at the Hôpital Communauteaire in Bangui, Central African Republic, from March 1995 through December 1997, and who had been diagnosed as HIV-1 seropositive during pregnancy. Informed oral consent for participation in the study was obtained from all women. The women were asymptomatic (stage A of the World Health Organization classification), with CD4 T cell counts ranging from 200 to 300 × 10^3 cells/L (mean ± SE, 232 ± 41). None of the women received antiretroviral drugs before or during pregnancy; no anti-retroviral intervention was available at that time in Bangui. Samples of breast milk were obtained at birth and at 1 month and 6 months after delivery and were frozen at –80°C without further processing. Blood samples were collected on filter paper from all children, at the same time points. White blood cells were lysed,
and DNA was extracted by use of phenol-chloroform. Proviral DNA was detected by means of a nested polymerase chain reaction (PCR) on pol and by means of a semi-nested PCR on tat, as described elsewhere [6]. HIV-1 infection of a child was diagnosed if both PCR reactions were positive. Positive PCR reactions at birth or at 1 month defined perinatal (i.e., in utero or peripartum HIV infection). Early postnatal transmission was defined as negative PCR results at birth and at age 1 month, with positive results at age 6 months. Twenty-two infants were diagnosed as HIV infected. Fourteen (32%) of 43 infants (95% confidence interval [CI], 19%–49%) were infected perinatally, including 2 neonates who had positive PCR results at birth. Eight (19%) of 43 infants (95% CI, 8%–33%) were infected in the postnatal period, with the first positive PCR results obtained at age 6 months. Thus, 52% of the children in the cohort were infected with HIV, both in the perinatal and early postnatal period.

Quantitation of immunoglobulins, lactoferrin, and albumin in milk. Milk was thawed and centrifuged at 10,000 × g for 10 min, to discard both the pellet and the fat layer. Levels of S-IgA, IgG, S-IgM, lactoferrin, and albumin in breast milk were then determined by use of a sandwich ELISA, by reference to a standard pool of 20 samples of normal human colostrum, as described elsewhere [17]. The limit of detection of S-IgA, IgG, and S-IgM was 3 ng/mL.

Epitope mapping of anti-gp160 antibodies from milk. Immunoglobulins were purified from milk by 2 successive affinity chromatography steps, by use of protein G-Sepharose (Pharmacia, Uppsala, Sweden) and Sepharose to which anti-Fce antibodies had been coupled [17]. IgG was eluted from the protein G column by use of 0.2 M glycine-HCl (pH 2.5), followed by immediate neutralization by addition of 1 M Tris-HCl (pH 10.0). The IgG-depleted fraction of the milk was then incubated with anti-α-Sepharose. S-IgA was eluted by use of 0.2 M glycine-HCl (pH 2.5). The fraction depleted in IgA and IgG was kept. The purity of S-IgA and IgG was confirmed by the lack of detectable contaminating IgG and S-IgM or of S-IgA and S-IgM, respectively, by immunocapture ELISA [8, 17]. The IgA fraction contained 99% S-IgA.

The amount of S-IgA and IgG antibodies to env-encoded gp160 was determined by ELISA after diluting the samples in PBS containing 2.5% (wt/wt) powdered skim milk, by use of purified baculovirus-expressed recombinant gp160 derived from the envelope of the LAI strain of HIV-1 as antigen, as described elsewhere [8]. Goat biotinylated antibodies to human immunoglobulin (Sigma Chemical, St. Louis, MO) were used as secondary antibodies [17]. We were not able to purify S-IgM because of insufficient volumes of milk specimens available. We thus used the IgA- and IgG-depleted fraction of milk to detect S-IgM antibodies to HIV by using a μ-chain–specific antibody as the revealing conjugate. The cutoff for positivity in ELISA was defined as the mean optical density obtained by testing triplicate samples of PBS-milk plus 0.05.

The epitopic specificity of milk antibodies to HIV surface glycoproteins was determined by means of an indirect ELISA, by use of the HIV-derived peptide gp41/K or gp120/V3, as described elsewhere [8]. The gp41/K peptide was from the LAI strain of HIV-1 (table 1). The peptide contains the linear sequence ELDKWA, which is part of determinant III of gp41 [18] and is conserved in many isolates of HIV-1, irrespective of the viral clade [19, 20]. The gp120/V3 peptide represents the V3 loop from the MN strain of HIV-1. Although the V3 loop peptide originates from virus of clade B, we found it to be recognized by antibodies isolated from milk of all mothers in this study, whether transmitting or nontransmitting. The ELISA results were determined as described earlier. The specific activity of antibodies was expressed as the ratio of arbitrary units (AU) of reactivity per microgram of purified immunoglobulin of a given isotype.

Table 1. Peptides used in indirect ELISA for determination of epitopic specificity of anti-env antibodies in breast milk.

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<th>Peptide</th>
<th>Domain</th>
<th>Amino acid sequence</th>
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<tr>
<td>gp41/K</td>
<td>DIH</td>
<td>EKNEQELLELDKWASLW (659–675 LAI)</td>
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<tr>
<td>gp120/V3</td>
<td>V3 loop</td>
<td>CTRPYNKKRKHIGPGRFYTTKNIHG</td>
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*According to Earl et al. [18]. Determinant III is recognized by monoclonal antibody 2F5, at level of conserved linear sequence ELDKWA (residues 662–667) [19].

Model for postnatal transmission of HIV through breast milk. We have used the results of the present study in a simplified mathematical model to assess the role of anti-HIV antibodies in breast milk in protection against postnatal transmission of HIV. The overall risk r(t) of transmission from birth to time t after birth may be assumed to correspond to the integration over time of breast milk
infectivity $L(u)$ and putative resistance to infection of the child $-R(u)$:

$$\int_{0}^{t} [L(u) - R(u)] du ,$$

where $u$ denotes time. $L(u)$ corresponds to the sum of a function $V(u)$ representing the size of the viral inoculum (breast milk viral load) and a function $-I(u)$ representing breast milk–specific immunity:

$$\int_{0}^{t} [V(u) - I(u) - R(u)] du .$$

$I(u)$ represents both humoral immunity, $I_u(u)$, and cellular immunity, $I_c(u)$, in milk. Thus, equation (1) may be represented as follows:

$$\int_{0}^{t} [V(u) - I_u(u) - I_c(u) - R(u)] du .$$

Finally,

$$\int_{0}^{t} [V(u) - I(u) - R(u)] du .$$

where

$$\int_{0}^{t} [V(u) - I(u) - R(u)] du .$$

For the purpose of the analysis, we assumed that $V(u)$, $I_u(u)$, and $R(u)$ did not differ between transmitting and nontransmitting mothers. Hence, we limited the analysis to the expression $\int_{0}^{t} I_u(u) du$, which is expressed in nontransmitting (NT) mothers by

$$p^{NT}(t) = \int_{0}^{t} I_u(u) du ,$$

and in transmitting (T) mothers by

$$p^{T}(t) = \int_{0}^{t} I_u(u) du .$$

If $F(u)$ is the flow of milk (in volume/time), $\lbrack Ig\rbrack(u)$ the concentration of immunoglobulin, and $\bar{I}(u)$ a function corresponding to the inhibitory activity of immunoglobulin toward transcytosis (in AU/$\mu$g), anti-HIV humoral immunity in milk $I_u(u)$ may be expressed as follows for each immunoglobulin isotype:

$$I_u(u) = [IgA]_u(u) + [IgG]_u(u) + [IgM]_u(u) ,$$

where $[IgA]_u(u)$, $[IgG]_u(u)$, and $[IgM]_u(u)$ represent both humoral immunity, $I_u(u)$, and putative resistance to infection of the child $I_u(u)$. Hence, we limited the analysis to the expression $\int_{0}^{t} I_u(u) du$, which is expressed in nontransmitting (NT) mothers by

$$\int_{0}^{t} [IgA]_u(u) + [IgG]_u(u) + [IgM]_u(u) du ,$$

where

$$\int_{0}^{t} [IgA]_u(u) + [IgG]_u(u) + [IgM]_u(u) du ,$$

and in transmitting (T) mothers by

$$\int_{0}^{t} [IgA]_u(u) + [IgG]_u(u) + [IgM]_u(u) du .$$

Results

Quantitation of S-IgA, IgG, and S-IgM in the milk of transmitting and nontransmitting mothers. The mean concentration of S-IgA and IgG in the milk of 8 mothers who had transmitted HIV was similar to that in the milk of 18 mothers who had not transmitted the virus, when measured at birth and at 1 month and 6 months after delivery (table 2). The mean level of colostral S-IgM at birth tended to be higher in transmitting mothers, compared with that in nontransmitting mothers, although the difference did not reach significance ($P = .07$). The concentration of S-IgM in milk at 1 month and 6 months after delivery was similar in transmitting and nontransmitting mothers. Mean levels of lactoferrin and albumin were higher in transmitting mothers than in nontransmitting mothers at 1 month ($P < .001$ and $P < .01$, respectively) and showed no difference

| Table 2. Levels of S-IgA, IgG, S-IgM, lactoferrin, and albumin in breast milk samples collected at birth and at 1 month and 6 months after delivery from HIV-infected African mothers who had either transmitted or not transmitted HIV to their child via breast-feeding. |
|-----------------|-----------------|-----------------|
| S-IgA           | Nontransmitting mothers | Transmitting mothers |
| Birth           | 15,770 ± 5540    | 11,950 ± 4290   |
| 1 month         | 493 ± 261        | 302 ± 62        |
| 6 months        | 196 ± 49         | 187 ± 25        |
| IgG             |                 |                 |
| Birth           | 573 ± 277        | 652 ± 210       |
| 1 month         | 246 ± 63         | 172 ± 31        |
| 6 months        | 102 ± 33         | 117 ± 38        |
| S-IgM           |                 |                 |
| Birth           | 1630 ± 600       | 755 ± 157       |
| 1 month         | 23 ± 6           | 35 ± 11         |
| 6 months        | 6 ± 3            | 9 ± 1           |
| Lactoferrin     |                 |                 |
| Birth           | 3820 ± 817       | 2180 ± 210      |
| 1 month         | 1130 ± 142       | 592 ± 201a      |
| 6 months        | 840 ± 209        | 752 ± 223       |
| Albumin         |                 |                 |
| Birth           | 221 ± 147        | 320 ± 72        |
| 1 month         | 75 ± 12          | 36 ± 5b         |
| 6 months        | 84 ± 17          | 46 ± 15         |

NOTE. Data are mean ± SE, $\mu$g/mL. S-IgA, secretory IgA; S-IgM, secretory IgM; HIV, human immunodeficiency virus.

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$^a$ $P < .01$ vs. transmitting mothers.

$^b$ $P < .001$ vs. transmitting mothers.
between the 2 groups of mothers at birth and at 6 months after birth.

**HIV-specific S-IgA, IgG, and S-IgM in the milk of transmitting and nontransmitting mothers.** S-IgA and IgG to gp160 were present in all samples of transmitting and nontransmitting mothers, with a specific anti-gp160 activity of 20–52 AU/μg for S-IgA and 607–1134 AU/μg for IgG. As shown in figure 1, the mean specific activity of IgG antibodies to gp160 in milk was 21-, 30-, and 31-fold higher than that of S-IgA to gp160, at birth and at 1 month and 6 months after delivery, respectively (P < .005). There was no difference in the concentration of specific S-IgA and IgG antibodies to gp160 in the milk of transmitting and nontransmitting mothers. The fraction of colostrum from transmitting and nontransmitting mothers that had been depleted in IgG and S-IgA contained detectable S-IgM to gp160 at low concentrations (71%). No S-IgM to gp160 was detected in the IgA- and IgG-depleted fraction of milk collected at 1 month and 6 months after delivery. Thus, S-IgA and IgG represent the major isotypes of HIV-specific antibodies in colostrum and early breast milk of HIV-seropositive mothers.

The epitopic specificity of antibodies was further assessed by testing the reactivity of S-IgA and IgG purified from milk with env-encoded peptides. S-IgA and IgG of milk obtained at birth and at 1 month and 6 months after delivery contained antibodies to the gp41/K and gp120/V3 peptides. Neither specific antibody activity nor changes in antibody titers over time differed between transmitting and nontransmitting mothers (figure 2).

**Inhibition of transcytosis of HIV by S-IgA and IgG from milk.** The ability of purified S-IgA and IgG from milk to inhibit HIV-1 transcytosis through an epithelial cell monolayer was investigated in 8 transmitting and 10 nontransmitting mothers, who were selected on the basis of high titers of anti-env S-IgA and IgG antibodies in colostrum and early breast milk of HIV-seropositive mothers. The epitopic specificity of antibodies was further assessed by testing the reactivity of S-IgA and IgG purified from milk with env-encoded peptides. S-IgA and IgG of milk obtained at birth and at 1 month and 6 months after delivery contained antibodies to the gp41/K and gp120/V3 peptides. Neither specific antibody activity nor changes in antibody titers over time differed between transmitting and nontransmitting mothers (figure 2).

Inhibition of transcytosis of HIV by S-IgA and IgG from milk. The ability of purified S-IgA and IgG from milk to inhibit HIV-1 transcytosis through an epithelial cell monolayer was investigated in 8 transmitting and 10 nontransmitting mothers, who were selected on the basis of high titers of anti-env S-IgA and IgG antibodies in milk. The addition of S-IgA and IgG fractions to U1 cells (1.0 μg/2 × 10⁶ cells) on the apical pole of the epithelial monolayer resulted in significant inhibition of HIV transcytosis (figure 3). The inhibitory activity of antibodies did not differ between transmitting and nontransmitting mothers. Thus, the mean percentages of inhibition (± SD) of transcytosis by S-IgA and IgG in transmitting and nontransmitting mothers, respectively, were 48.5% ± 14.9% versus 66.2% ± 10.4% (P > .05) and 53.8% ± 7.4% versus 64.5% ± 19.5% (P > .05) at birth; 48.5% ± 14.9% versus 66.2% ± 10.4% (P > .05) and 61.5% ± 16.8% versus 71.2% ± 13.3% (P > .05) at 1 month after delivery; and 48.5% ± 14.9% versus 66.2% ± 10.4% (P > .05) and 71.7% ± 13.7% versus 73% ± 1.0% (P > .05) at 6 months after delivery. The inhibitory activity of S-IgA was similar to that of IgG on a weight basis, although S-IgA exhibited lower anti-gp160-specific activity than did IgG (figure 4).

**Mathematical model for assessing the role of antibodies in breast milk in protection against early postnatal transmission of HIV.** When using the model described in Materials and Methods, the mean p̄₄(τ₁) calculated for 8 transmitting mothers was not different from the mean p̄₄(τ₁) calculated for 10 nontransmitting mothers, of whom we had examined the inhibitory activity of breast milk antibodies toward viral transcytosis. The lack of difference indicates that the factor I₄ is not sufficient to explain the difference between the 2 groups of mothers in transmission of HIV to the child.

**Discussion**

In the present study, we investigated the specific activity against env-encoded surface glycoproteins and the inhibitory activity toward viral transcytosis in vitro of S-IgA and IgG in the colostrum and breast milk from HIV-seropositive mothers who had transmitted and those who had not transmitted the virus to their child. None of the immunochemical and functional characteristics of the antibodies allowed us to distinguish between transmitting and nontransmitting mothers. By use of a simplified mathematical model for postnatal transmission of HIV, HIV-specific humoral immunity in breast milk was not found to differ between women who had transmitted and those who had not transmit HIV via breast-feeding. Our observations emphasize that postnatal transmission of HIV is primarily a complex multifactorial process and suggest that the specific humoral mucosal immune response to HIV in milk does not appear as a predominant factor for protection of viral transmission.

We found no significant difference in the mean concentra-
Figure 2. Specific activity of secretory IgA (S-IgA) and IgG antibodies against env-encoded gp41/K and gp120/V3 peptides in samples of breast milk obtained at birth and at 1 month (M1) and 6 months (M6) after delivery from human immunodeficiency virus (HIV)-infected African mothers who had transmitted (●; blackened bars) and those who had not transmitted (●; hatched bars) HIV. Specific antibody activity is expressed as arbitrary units per microgram of total immunoglobulin of a given isotype (mean ± SE).

tions of S-IgA, S-IgM, and IgG in milk from the group of mothers who had transmitted HIV and those who had not transmitted the virus, when measured at birth and at 1 month and 6 months after delivery. We were not able to measure HIV-1 viral load in breast milk because of insufficient amounts of material, although recent data suggest that viral load in milk may be correlated with the risk of transmission [24]. The groups of transmitting and nontransmitting mothers did not differ, however, in terms of disease stage, mean CD4 cell count, and CD4 : CD8 ratio (data not shown). The mean concentration of lactoferrin, a major locally produced nonspecific antimicrobial factor [25], and that of albumin, which is exclusively of plasma origin, was higher in transmitting mothers than in nontransmitting mothers at 1 month after birth, suggesting enhanced mucosal activation and an alteration in the mucosal barrier integrity at that time. It is possible that the mammary epithelium is intermittently activated in transmitting mothers as a consequence of as-yet-unknown causes, including local replication of HIV. Antibodies of the S-IgA and IgG isotypes directed against gp160 and 2 env-encoded gp160 peptides were found in all samples of breast milk that we tested. In contrast, S-IgM to gp160 was only found in colostrum samples. The calculated specific activity of IgG to gp160 was higher than that of S-IgA. The predominance of HIV-specific IgG over HIV-specific S-IgA was in strong contrast with the predominance of total S-IgA over total IgG in breast milk. These observations are consistent with previous reports on anti-HIV antibodies in breast milk [3, 8, 14–16] and with findings in cervicovaginal secretions of HIV-1–infected women [26]. The predominance of specific anti-HIV antibodies of the IgG isotype in the mucosal compartment, where the production of IgA has been recognized as the essential component of the local immune response, may be considered as a major feature of mucosal impairment in HIV infection [26, 27]. In this study, we only detected HIV-specific S-IgM antibodies in colostrum. When present, S-IgM antibodies exhibited low specific activity to env-encoded proteins, as assessed by ELISA. These observations are in contrast with the high frequency at which S-IgM to HIV was detected by Western blot in previous studies [3]. Such differences may not be explained by the processing of milk samples. Thus, we searched for anti-gp160 S-IgM in the S-IgA– and IgG-depleted fractions of milk samples; in previous studies, IgG-depleted samples were examined [3]. In a previous report, we had noticed that breast milk samples of some HIV-1–seronegative African mothers contained antibodies that bound to gag-encoded HIV proteins on Western blot strips [15]. We further evaluated the specific antibody activity of S-IgA and IgG purified from breast milk against 2 linear epitopes of the HIV-1 surface glycoproteins, gp41/K and gp120/V3, which are considered to be important for HIV neutralization [28]. Specific antibodies directed to these env-encoded viral epitopes were detected in all colostrum and breast milk samples collected at birth and at 1 month and 6 months after delivery, whether mothers belonged to the transmitting or nontransmitting group. We further assessed the inhibitory properties toward viral
Inhibitory activity toward transcytosis of human immunodeficiency virus (HIV) through a tight epithelial barrier in vitro of secretory IgA (S-IgA; 1 μg) and IgG (1 μg) purified from breast milk. Inhibition of transcytosis was investigated, as described in Materials and Methods. Samples of breast milk were obtained at birth and at 1 month (M1) and 6 months (M6) after delivery from 8 HIV-seropositive mothers who transmitted HIV and from 10 mothers who did not transmit HIV to their children. Positive control in blocking assay was IgG (1 μg) purified from pooled serum of HIV-1-seropositive individuals. Negative control was IgG purified from serum of HIV-seronegative blood donors. Data are expressed as percentage of inhibition of transcytosis. The dashed line indicates threshold of significant inhibition of transcytosis in assay.

transcytosis of S-IgA and IgG antibodies, in an in vitro model of transcytosis of cell-associated HIV-1, through a tight monolayer of endometrial epithelial cells [16, 22, 23, 29]. Several lines of evidence support the relevance of this in vitro model for the transmission of HIV from mother to child through breast milk: (1) breast milk contains 10^5–10^6 cells/mL, including target cells for HIV such as macrophages, lymphocytes, and ductal epithelial cells [30]; (2) prevalence of HIV-1 proviral DNA in breast milk of HIV-positive mothers is high, ranging from 44% to 58% [3, 9–11]; (3) oral mucosa, at least in the adult, is not favorable for HIV penetration [31] and, thus, the monostratified mucosal surface of the intestinal tract could be the target tissue for HIV in breast-fed children; and (4) infection of intestinal cells with HIV has been achieved in vitro [32–35], although it has rarely been observed in vivo [36–38]. An inhibitory activity against transcytosis of cell-associated HIV-1 was shown for purified S-IgA and IgG in all breast milk samples from transmitting and nontransmitting mothers. Interestingly, despite a lower specific activity against gp160, purified S-IgA from colostrum and breast milk exhibited inhibitory activity against HIV-1 transcytosis similar to that in purified IgG, as reported elsewhere for the case of antibodies in colostrum [16]. S-IgA is considered to be more efficient than IgG in immune exclusion at mucosal surfaces, because it exhibits a dimeric structure with 4 antibody valencies.

The role of HIV-specific humoral immunity in breast milk was examined in transmitting and nontransmitting mothers by use of a mathematical model that took into account the specific activity and blocking index of S-IgA and IgG, the 2 major isotypes of HIV-specific antibodies in breast milk, according to duration of child exposure through breast-feeding. We assumed that exposure of the child to HIV-infected milk is a continuous process and that the volume of breast-fed milk received is similar among children. The model assumed that natural resistance to infection by the child, size of the viral breast milk inoculum, and HIV-specific cellular immunity were similar among the children. By using this approach, we found no difference between the HIV-specific humoral immunity of transmitting and nontransmitting mothers.

The present observations are not fully consistent with the previously suggested protective role of HIV-specific humoral mucosal immunity in breast milk in vivo [3]. Our findings suggest that postnatal transmission of HIV is probably dependent on several complex associated factors of the mother, the child, and possibly the virus, since it is also believed that sexual trans-

Figure 3. Inhibitory activity toward transcytosis of human immunodeficiency virus (HIV) through a tight epithelial barrier in vitro of secretory IgA (S-IgA; 1 μg) and IgG (1 μg) purified from breast milk. Inhibition of transcytosis was investigated, as described in Materials and Methods. Samples of breast milk were obtained at birth and at 1 month (M1) and 6 months (M6) after delivery from 8 HIV-seropositive mothers who transmitted HIV and from 10 mothers who did not transmit HIV to their children. Positive control in blocking assay was IgG (1 μg) purified from pooled serum of HIV-1-seropositive individuals. Negative control was IgG purified from serum of HIV-seronegative blood donors. Data are expressed as percentage of inhibition of transcytosis (mean ± SE). The dashed line indicates threshold of significant inhibition of transcytosis in assay.

Figure 4. Relationship between inhibitory activity toward viral transcytosis and specific anti-gp160 activity of secretory IgA (S-IgA) (unblackened symbols) and IgG (blackened symbols) purified from breast milk obtained at birth from transmitting (circles) and nontransmitting (squares) mothers. The vertical dashed line indicates a heuristic delineation between S-IgA and IgG.
mission of HIV involves several factors [39]. Thus, the amount of free HIV particles [12, 24], number of HIV-infected cells [3, 9–11], presence of nonspecific antiviral substances such as polyanionic milk proteins [13], local HIV-specific cellular immunity, HIV phenotype, and other uncharacterized factors may play a more important role than the local humoral immune response in determining infant susceptibility to infection.

Acknowledgments

We thank B. Garin and J. Morvan (Institut Pasteur, Bangui, Central African Republic) for providing samples from patients; and J. P. Bouvet (INSERM U430, Paris), F. Ouaz (INSERM U430, Paris), and Y. Hu-Wong (Columbia University, New York) for discussion and critical review of the manuscript.

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