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Natural Antibodies to CCR5 from Breast Milk Block Infection of Macrophages and Dendritic Cells with Primary R5-Tropic HIV-1

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In the present study, we demonstrate that breast milk of 66% and 83% of HIV-seronegative and seropositive women, respectively, contains natural Abs of the secretory IgA and IgG isotypes directed against the CCR5 coreceptor for R5-tropic strains of HIV-1. Abs to CCR5 were affinity purified on a matrix to which a synthetic peptide corresponding to the second extracellular loop of CCR5 had been coupled. The purified Abs bound to the CCR5 peptide in a dose-dependent fashion and to both native CCR5 expressed by Chinese hamster ovary cells transfected with CCR5 gene, macrophages, and immature dendritic cells. Although the avidity differed, the amount of anti-CCR5 Abs did not significantly differ between breast milk of HIV-seropositive and -seronegative women. Purified anti-CCR5 Abs inhibited up to 75% infection of macrophages and dendritic cells with HIV

HIV JR-CSF. Our observations provide evidence for a role of natural Abs to CCR5 in breast milk in controlling transmissibility of HIV through breastfeeding. The Journal of Immunology, 2005, 174: 7202–7209.

The CCR5 chemokine receptor is the major coreceptor that is associated with mucosal transmission of R5-tropic HIV-1 during sexual intercourse and postnatal transmission through breastfeeding (1–3). The importance of CCR5 for HIV-1 transmission is evidenced by the observation that individuals homozygous for a defective CCR5 allele remain uninfected despite repeated exposure to HIV (4). The CCR5 molecule is thus a target for novel therapeutic strategies aimed at blocking HIV-1 entry into cells (5–7). We have previously demonstrated that natural Abs of therapeutic preparations of IgG (i.v. Ig) contain natural Abs directed against CCR5 that inhibit infection of human macrophages and CD4+ T lymphocytes with laboratory and primary R5-tropic isolates of HIV-1 in vitro (8). Breast milk contains high amounts of immunoglobulins, predominantly of the secretory IgA (sIgA) and to a lesser degree of the IgG and sIgM isotypes that are produced in the absence of deliberate immunization and independently of exposure to Ags. Most natural Abs in the breast milk of healthy women are self-reactive Abs (9, 10).

In the present study, we demonstrate that breast milk contains Abs directed against CCR5 molecule. Following affinity purification, these Abs were shown to bind to the native CCR5 molecule and to inhibit infection of macrophages and dendritic cells by R5-tropic HIV-1.

Materials and Methods

Antibodies and reagents

Breast milk from 18 HIV-seronegative and 12 HIV-seropositive women was collected at the lactarium of the Institut de Puériculture (Paris, France) and at the sexually transmitted diseases and AIDS center and pediatric complex of Bangui (Central African Republic), respectively. All breast milks were collected between 1 and 4 months after childbirth. Milk samples were centrifuged at 10,000 rpm to separate the cellular, supernatant, and lipid fractions. Supernatants were stored at −80°C until use. FITC-conjugated mAb to CD4 (Leu3-a) was purchased from BD Biosciences. PE-conjugated mAb to CCR5 (clone 2D7), PE-conjugated mAb anti-CXCR4 (clone 12G5), and a corresponding isotypic control mAb were purchased from BD Pharmingen. FITC-conjugated anti-CD14 (RM052) was obtained from Immunotech Beckman Coulter, and PE-conjugated goat anti-human Ig was obtained from Jackson ImmunoResearch Laboratories. GM-CSF, M-CSF, IL-4, IL-2, and the recombinant human chemokines RANTES and SDF-1 were purchased from R&D Systems Europe. CD14 magnetic beads were obtained from Miltenyi Biotec. The CCR5 peptide csshfpysqfkwkmfqk that corresponds to the second extracellular loop of CCR5 (ILE/E/C-CCR5) was synthesized by the solid-phase F-moc method using an Applied Biosystem model 433A peptide synthesizer that was a kind gift from Dr. A. Beretta (Fondazione Centro S. Raffaele Del Monte Tabor, Milan, Italy). Dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) irrelevant peptide (YWWRGEPNNVGEDCAEFSGNW NDDKCNL) was synthesized by Séquençia SA. Epithelial Chinese hamster ovary (CHO)-CCR5 and CHO-CXCR4 cells were obtained from National Institutes of Health AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, National Institutes of Health).

HIV strains

Primary R5-tropic HIV JR-CSI, HIV JR-CSF, and X4-tropic HIV NDK were a gift from the Pr. F. Barré-Sinoussi (Institut Pasteur, Paris, France). HIV JR-CSF and HIV JR-CSF were produced in primary macrophages; HIV NDK was produced on IL-2-activated PBL.

Detection of anti-CCR5 Abs in breast milk

Anti-CCR5 activity in breast milk was assessed by ELISA. Plastic plates were coated with the CCR5 peptide (10 µg/ml) in PBS, at pH 7.4 overnight at 4°C. The plates were washed with PBS-Tween 0.1% before saturation with PBS-milk 1%. Dilutions of milk supernatants to be tested were then added and incubated overnight at 4°C. After washing, peroxidase-labeled...
goat anti-human F(ab')₂, Abs were added for 1 h at 37°C before addition of substrate. Positive and negative controls consisted of anti-CCR5 Abs purified from i.v. Ig (8) and of anti-CCR5-depleted IgG, respectively.

**Immunoadfinity purification of anti-CCR5 Abs from breast milk**

The I/E/C-CCR5 peptide was coupled to activated Sepharose 4B (Pharmacia Biotech) according to the manufacturer’s instructions. Total IgS of pools of breast milk samples from either healthy or HIV-seropositive women were purified using anti-F(ab')₂ Abs coupled to activated Sepharose 4B. Purified IgS allowed to interact with the peptide matrix overnight at 4°C before extensive washing of the column with PBS until the OD of the effluent reached 0.001. The column was then eluted with 0.2 M glycine-HCl, pH 2.5. The pH of eluted material was rapidly neutralized with 3.0 M Tris and further dialyzed against PBS overnight. The affinity-purified anti-CCR5 immunoglobulins bound to the CCR5 peptide in a dose-dependent fashion as assessed by ELISA and revealed with anti-F(ab')₂ Abs described above.

The content in IgG, IgA, and IgM of affinity-purified anti-CCR5 Abs was measured by ELISA. Plates were coated with goat anti-human α-chain, goat anti-human γ-chain or goat anti-human μ-chain (all at 3 μg) in PBS overnight at 4°C, before washing with PBS/0.1% Tween 20, and saturation with PBS/1% skim milk. Serial dilutions of immunopurified anti-CCR5 immunoglobulins were then added for 1 h at 37°C. After further washing, goat anti-human F(ab')₂, coupled with peroxidase was added for 1 h at 37°C. After extensive washes, substrate was added, and peroxidase activity was revealed. A pool of normal human sera with known levels of IgG, IgA, and IgM was used to build standard curves. IgA and IgG Abs were purified from total anti-CCR5 peptide Abs using anti-γ- and anti-α-chain Abs coupled to activated Sepharose 4B.

**Determination of avidity of breast milk purified Abs to CCR5 peptide**

The relative avidity of anti-CCR5 Abs purified from breast milk of seronegative and seropositive women was assessed using a potassium thiocyanate (KSCN) dissociation assay. Briefly, 10 μg of CCR5 peptide were coated on 96-well plates overnight at 4°C. After saturation with PBS/1% milk for 1 h at 37°C, the plates were further incubated with 25 μg/well of each preparation of anti-CCR5 Abs for 2 h at 37°C. After several washes, the plates were treated with KSCN at increasing molarities from 0.1 M to 2.0 M for 30 min at room temperature. The plates were washed and incubated with peroxidase-labeled goat anti-human F(ab')₂ Abs for 1 h at 37°C. After extensive washes, substrate was added, and peroxidase activity revealed at 492 nm. The relative avidity (avidity index) was expressed as the molar concentration of KSCN inducing a decrease of 50% in the OD of the control value obtained in the absence of KSCN.

**Dendritic cells and macrophages preparation**

PBMC were isolated from freshly drawn blood of healthy donors by Ficoll-Paque gradient centrifugation. The CD14⁺ fraction was purified from PBMC using magnetic beads (Miltenyi Biotec). Purified CD14⁺ cells contained <1% of CD19⁺ cells. Monocyte-derived macrophages (MDM) were obtained following 7 days of culture of monocytes in the presence of 10 ng/ml M-CSF. At the time of collection, most macrophages expressed CD14 (90%), CD11b (90%). Monocyte-derived dendritic cells were obtained after 6 days of culture in the presence of a combination of GM-CSF and IL-4, both at 10 ng/ml. Nonadherent mononuclear cells exhibited an immature dendritic cell phenotype on the 6th day (CD14⁺, HLA-DR⁺, CD1a⁺) expressing up to 80% DC-SIGN molecule and low levels of CD83 compared with fresh isolated monocytes. In our culture conditions, 45% of macrophages and 25% of immature dendritic cells expressed CCR5 molecule.

**Immunostaining and FACs analysis**

The expression of cell surface Ags was assessed by cytofluorometry using a FACScalibur and CellQuest software (BD Biosciences). Briefly, cells (1 × 10⁶ cells per test) were recovered in EDTA buffer for 15 min at 37°C. The cells were then incubated with mAbs against membrane molecules for 30 min at 4°C, washed with 0.05% PBS/NaN₃ and fixed with 1% paraformaldehyde before analysis.

**Binding of purified anti-CCR5 peptide Abs to CCR5-positive cells**

Epithelial CHO-CCR5⁺ and CHO-CXCR4⁺ cells (1 × 10⁵ cells) were incubated with increasing concentrations of purified anti-CCR5 peptide Abs (0.1–500 μg/ml) for 1 h at 4°C. Cells were then washed, incubated with PE-conjugated goat anti-human immunoglobulin Abs for 30 min on ice. The cells were washed, fixed with 1% paraformaldehyde, and analyzed by FACS. In some experiments, the purified anti-CCR5 Abs (100 μg) were preincubated with a saturating amount of CCR5 peptide (20 μg) for 30 min at room temperature before incubation with cells. Positive controls consisted of anti-CCR5 mAb 2D7. Anti-CCR5-depleted Ig was used as negative control. For binding experiments of purified anti-CCR5 Abs to MDM at day 7 of culture and immature monocyte-derived dendritic cells (IM-DC) at day 6 of culture, cells (1 × 10⁶ cells) were recovered by adding cold RPMI 1640 and scraping with a rubber policeman and then incubated with purified anti-CCR5 Abs (50 and 100 μg/ml), corresponding anti-CCR5-depleted antibodies, mAb anti-CCR5 (clone 2D7; 10 μg) for 1 h at 4°C. After washes, cells were incubated with PE-conjugated goat anti-human Ig Abs for 30 min on ice and fixed with 1% paraformaldehyde and analyzed by cytofluorometry using a FACScalibur and CellQuest software.

**Inhibition of HIV-1 infection by Abs to CCR5 peptide**

Macrophages and dendritic cells (5 × 10⁵ cells) in 48-well plates were infected with 5 ng/ml p24 HIVBal, HIVJR-CSF, and HIVNDK, for 3 h at 37°C. The cells were washed and then cultured for 6 days. Supernatants were collected every 2 days, and HIV p24 levels were measured by ELISA. In some experiments, cells were preincubated with purified anti-CCR5 Abs (100, 250, 500, and 1000 μg/ml) with or without corresponding amounts of anti-CCR5-depleted immunoglobulins used as negative control. As positive control of inhibition of HIV infection, purified anti-CCR5 from i.v. Ig, or the chemokines RANTES and SDF-1 (500 ng/ml) were added to cells before infection with HIV.

**Results**

Human breast milk contains natural Abs directed against CCR5

We first investigated the reactivity with the CCR5 peptide of immunoglobulins in each of the 18 breast milk samples from HIV-seronegative women and 12 samples from HIV-seropositive women by ELISA. Fifteen of 18 (83%) and 8 of 12 (66%) samples contained over 1 μg/ml anti-CCR5 Ig (Fig. 1). Based on the total Ig content of breast milk samples from HIV-seropositive and -seropositive women, the mean specific anti-CCR5 activity was calculated as being 0.24 and 0.30 in breast milk samples of HIV-seronegative and HIV-seropositive women, respectively.

Breast milk samples from the 18 healthy and the 12 HIV-seropositive women were then mixed to constitute two breast milk pools. Anti-CCR5 Abs were purified from both pools by affinity chromatography as described in Materials and Methods. The recovered CCR5 peptide-binding fraction represented 0.2 and 0.25% of total Ig in the HIV-seronegative and HIV-seropositive breast milk pools, respectively. Anti-CCR5 activity was of the IgA (39%) and IgG (55%) isotypes in samples from seropositive women. IgA and IgG represented 52 and 48% in seronegative breast milk samples of HIV-seronegative women. Anti-CCR5 IgM was only found in small amounts (6%) in breast milk samples from HIV-seropositive women (Fig. 2).

As shown in Fig. 3A, Abs purified from breast milk of seropositive women exhibited an ~10-fold stronger anti-CCR5 reactivity than those purified from pooled milk of seronegative women. Anti-CCR5 IgG that had been affinity-purified from i.v. Ig, used as a positive control, also bound with lesser intensity to the CCR5 peptide than affinity-purified Abs from breast milk of HIV-seropositive women. No binding to CCR5 peptide was observed when anti-CCR5 completely depleted the fraction of breast milk was tested. Affinity-purified anti-CCR5 Abs from breast milk of healthy and HIV-seropositive women and i.v. Ig reacted specifically with the CCR5 peptide but not with irrelevant peptide corresponding to a sequence of DC-SIGN molecule. Furthermore, addition of CCR5 peptide to mAb 2D7, that recognize the sequence of CCR5-peptide, before incubation with breast milk purified anti-CCR5 Abs induced a total inhibition of binding to CCR5-peptide (Fig. 3B).
To investigate the anti-CCR5 peptide Abs response in depth, IgG and IgA were purified by means of anti-\text{H}9253- and anti-\text{H}9251-chain antibodies. Our results showed that depletion of sIgA from IgA Abs fraction, by means of anti-secretory component Abs insolvabilized on Sepharose beads, induced a complete abolition of binding to CCR5 peptide indicating that 100% of purified IgA directed against CCR5 peptide are sIgA. No significant difference in reactivity against CCR5 peptide was observed between IgG and sIgA purified from the same pool of breast milk for both seropositive and seronegative women. (Fig. 3, C and D).

We further compared the relative avidity of affinity-purified anti-CCR5 Abs using a KSCN dissociation assay. The molarities of KSCN required to dissociate 50% of total anti-CCR5 Abs from the CCR5 peptide were 1.2 M and 0.5 M for Abs purified from HIV-seropositive and HIV-seronegative breast milk pools, respectively (Fig. 4A). No difference in the relative avidity was observed between IgG and sIgA directed to CCR5 peptide purified from the same pool of breast milk of seropositive and seronegative women (Fig. 4B). Thus, the molarities of KSCN required to dissociate 50% of sIgA and IgG anti-CCR5 Abs from the CCR5 peptide were 0.58 and 0.67 M for Abs purified from HIV-seronegative and 0.95 and 1.45 M for Abs purified from HIV-seropositive women, respectively.

Affinity-purified anti-CCR5 Abs from breast milk recognize the native CCR5 molecule

As shown in Fig. 5, affinity-purified anti-CCR5 Abs from HIV-seronegative and -seropositive breast milk pools bound in a dose-dependent fashion to the native CCR5 molecule expressed by CHO cells that had been transfected with the CCR5 gene. A plateau of binding was reached at a concentration of 100 \mu g/ml Abs that stained 40 and 70% of CHO-CCR5 \textsuperscript{+} cells for Abs from seronegative and seropositive pools, respectively (Fig. 5A). The anti-CCR5-depleted fraction of breast milk stained <5% of CCR5 \textsuperscript{+} CHO cells. Anti-CCR5 peptide Abs tested at 100 \mu g/ml recognized 7–9% of CXCR4\textsuperscript{+}CCR5\textsuperscript{−} CHO cells (Fig. 5B). Mouse mAbs to CCR5 (2D7) and CXCR4 (12G5) used at 10 \mu g/ml stained 98 and 78% of CCR5\textsuperscript{+} and CXCR4\textsuperscript{+} cells, respectively (Fig. 5C). In addition, the incubation of affinity-purified anti-CCR5 Abs with saturating amounts of CCR5 peptide before using the Abs in a binding assay, resulted in staining of <6% of CCR5\textsuperscript{−} CHO cells (Fig. 5D). In our culture conditions, 45% of macrophages and 25% of dendritic cells expressed CCR5 coreceptor. Used at 50 and 100 \mu g/ml, the affinity-purified anti-CCR5 Abs from seronegative women stained 9 and 25% of MDM and 7 and 18% of iMDDC, respectively. Similarly, affinity-purified anti-CCR5 Abs from seropositive patients used at 50 and 100 \mu g/ml,
stained 14 and 22% of MDM and 13 and 15% of iMDDC, respectively (Fig. 5E). No binding was observed when anti-CCR5-depleted Abs were used.

**Natural anti-CCR5 Abs from breast milk inhibit CCR5-dependent infection of macrophages and dendritic cells**

Affinity-purified anti-CCR5 Abs from the breast milk pool of HIV-seropositive and -seronegative women dose-dependently inhibited infection of both MDM and iMDDC with HIV BaL and HIVJR-CSF. Thus, at a concentration of 1000 μg/ml, the anti-CCR5 Abs inhibited up to 75% infection with HIV BaL and HIVJR-CSF of macrophages (Fig. 6A) and dendritic cells (Fig. 6B). At a concentration of 250 μg/ml anti-CCR5 peptide Abs from breast milk of HIV-seropositive women were more efficient in inhibition of HIV infection than Abs from breast milk of HIV-seronegative women. RANTES, used as a positive control, inhibited at 85 and 90% infection of macrophages and dendritic cells, respectively. No significant inhibition was observed when using the anti-CCR5-depleted fraction of breast milk.

**Discussion**

Here we report that natural Abs directed against CCR5, the coreceptor for R5-tropic HIV-1, are present in human breast milk and are capable of inhibiting infection of human macrophages and dendritic cells with primary R5-tropic HIV in vitro.

The relative content in anti-CCR5 Abs was found to differ between samples of breast milk from individual women. Anti-CCR5 activity was found by means of ELISA in 66% and 83% of breast milk samples of HIV-seropositive and -seronegative women, respectively. We purified the Abs to CCR5 from pooled breast milk of both HIV-seropositive and -seronegative women by means of affinity chromatography on a synthetic peptide corresponding to a 20-amino acid region (ILE/C-CCR5) derived from the second extracellular loop of CCR5. This loop together with the N-terminal

**FIGURE 3.** Binding of purified anti-CCR5 Abs to CCR5 peptide. A, Anti-CCR5 Abs from breast milk of HIV-seropositive and -seronegative women, affinity-purified anti-CCR5 IgG Abs from i.v. Ig and anti-CCR5-depleted fractions of breast milk (seropositive, seronegative, and i.v. Ig effluents) were assessed at different concentrations for binding to CCR5 peptide. B, HIV-seropositive, -seronegative, and i.v. Ig-purified anti-CCR5 Abs to CCR5 peptide were incubated with CCR5 peptide, irrelevant peptide (DC-SIGN peptide), and with CCR5 peptide already preincubated for 1 h with anti-CCR5 peptide 2D7 mAb. Anti-CCR5 IgG and sIgA Abs from breast milk of HIV-seropositive (C) and -seronegative (D) women were assessed at different concentrations for binding to CCR5 peptide.

**FIGURE 4.** Avidity index of natural anti-CCR5 antibodies. HIV-seropositive and -seronegative women. Total (A) and purified IgG and sIgA (B) anti-CCR5 Abs were incubated with CCR5 peptide for 2 h at 37°C before treating the wells with increasing amounts of KSCN (0.1–2 M) for 30 min at room temperature. Wells were washed and incubated with goat anti-human F(ab’)2 coupled to peroxidase for 1 h at 37°C. The molarity of KSCN required for dissociating 50% of bound Abs was then determined.
sequence (aa 1–20) and the first extracellular loop (aa 89–102) is involved in the binding of gp120 and of natural CCR5 ligands to the CCR5 molecule (6, 11–13). We focused on the second extracellular loop of CCR5 that was shown to be immunogenic and induce a B and T cell response. Affinity-purified Abs from breast milk bound specifically to CCR5 peptide as demonstrated by the lack of binding to an irrelevant peptide and by the abrogation of the binding in the presence of 2D7 mAb directed against the CCR5 peptide.

Affinity-purified Abs from breast milk bound to the immobilized CCR5 peptide in a dose-dependent manner, with total Abs from seropositive women exhibiting a 10-fold higher reactivity than those from HIV-seronegative women. However, no difference was observed when comparing the binding of IgG and IgA purified from the same pool of breast milk. Anti-CCR5 activity was found in IgA, IgG, and to a small degree in IgM. Depletion of sIgA by means of anti-secretory component Abs induced a complete ablation of binding of purified IgA to CCR5 peptide indicating that 100% of IgA directed against CCR5 peptide are sIgA.

By determining a dissociation index of immune complexes with KSCN, anti-CCR5 Abs purified from breast milk of HIV-seropositive women seemed to have a 2.4-fold higher avidity for the CCR5 peptide.

**FIGURE 5.** Cytofluorometric analysis of the binding of anti-CCR5 Abs to CCR5+ CHO cells. Increasing amounts of anti-CCR5 Abs from seropositive and seronegative breast milk (0.1–500 μg) were incubated with CHO-CCR5+ positive cells (A) or with CHO-CXCR4+ positive cells (B). Positive controls included anti-CCR5 mAb 2D7 clone and anti-CXCR4 12G5 clone at 10 μg (C). Anti-CCR5 Abs from breast milk of seropositive and seronegative women (100 μg) were incubated with CCR5 peptide (20 μg) before being used in a binding assay to CHO-CCR5 positive cells (D). Binding of affinity-purified anti-CCR5 Abs from seropositive and seronegative breast milks (50 and 100 μg) to macrophages (day 7) and immature dendritic cells (day 6) (E). The percentage of positive cells is indicated in each gate. A representative experiment of four independent experiments is depicted in the figure.
peptide than Abs from HIV-seronegative women. However, no difference was observed when we compared the relative avidity index for the CCR5 peptide of IgG and sIgA purified from the same pool of breast milk. The increased avidity against CCR5 of Abs from breast milk of infected women may reflect polyclonal B cell stimulation in the mammary mucosae and the expansion of more avid natural Ig-producing B cell clones.

Antibodies purified from breast milk of HIV-seropositive women exhibited a 2-fold higher ability to bind to the native CCR5 molecule expressed by CHO cells than Abs purified from HIV-seronegative breast milk. The binding of affinity-purified anti-CCR5 Abs to CCR5−/CHO cells was abrogated by presaturation of the Abs with CCR5 peptide. Compared with CHO CCR5+ cells, the binding of affinity-purified anti-CCR5 peptide Abs to primary cultured macrophages and immature dendritic cells was less efficient. Using macrophages and immature dendritic cells, a slight difference in binding to CCR5 was observed between anti-CCR5 peptide Abs purified from breast milk of HIV-seropositive and -seronegative women. Although the avidity differed, the amount of anti-CCR5 Abs did not significantly differ between breast milk of HIV-seropositive and -seronegative women.

HIV infection is predominantly transmitted through mucosae (14). The CCR5 chemokine receptor functions as the major coreceptor for the R5-tropic strains that are primarily transmitted through the mucosal route (15). The role of CCR5 in HIV-1 transmission has been evidenced by observations that individuals homozygous for a defective CCR5 allele (CCR5Δ32) remained uninfected despite repeated exposure to HIV (4), and that protection against HIV infection was associated with the presence of natural anti-CCR5 Abs in serum in some exposed uninfected individuals in sero-discordant couples (16). Anti-CCR5 Abs were shown to down-modulate surface expression of CCR5 and to neutralize the infectivity of HIV R5-tropic strains, providing a basis for the acquisition of resistance to infection (16). After crossing the epithelial barrier, HIV spreads rapidly, through contact between dendritic cells and CD4 T lymphocytes (17). Hence, the blockade of CCR5 with mucosal anti-CCR5 Abs could result in inhibition of spreading of R5-tropic viruses.

Transmission of HIV-1 to the infant through breastfeeding is a major cause of new pediatric HIV-1 infections worldwide. Although extended breastfeeding accounts for approximately one- to two-thirds of infant HIV infections, most breastfed infants remain
uninfected, despite prolonged and repeated exposure to HIV-1. The risk of transmission of HIV-1 through breastfeeding has been reported to be between 5 and 23% (18), depending on a number of factors, including viral load in breast milk and the intensity and quality of the specific mucosal anti-HIV immune response (19). Both free and cell-associated HIV-1 are detected in early and mature breast milk of HIV-infected women (3). Breast milk of HIV-seropositive women also contains high levels of sIgA and IgG to env-encoded HIV surface glycoproteins (20). The Ab response to HIV in breast milk appears to be compartmentalized, when analyzed compared with that in serum (21). Anti-HIV Abs in breast milk were not found to be a relevant factor in protection against transmission of HIV through breastfeeding (22). We thus examined the effect of anti-CCR5 Abs purified from breast milk on infection of macrophages and dendritic cells that express CCR5 (23) and are considered as primary targets for HIV following trans-epithelial passage of the virus (24). Here we demonstrate that natural anti-CCR5 Abs from breast milk of HIV-seropositive and -seronegative women, inhibit infection of macrophages and dendritic cells with the R5-tropic strains HIV BaL and HIV JR-CSF in an HLA-A2-seronegative women, inhibit infection of macrophages and dendritic cells with the R5-tropic strains HIV BaL and HIV JR-CSF in a dose-dependent manner. At similar concentrations, the anti-CCR5-depleted fraction of breast milk inhibited <10% of infection of the cells. Such “residual” inhibitory effect could be due to the presence of anti-CCR5 Abs in the effluents of affinity columns that recognize extramembrane CCR5 domains outside the second extracellular loop. Concentration of anti-CCR5 peptide Abs needed to induce a significant inhibition of HIV infection was always higher than that inducing a maximum binding on CHO-CCR5 cells, macrophages, and immature dendritic cells. This discrepancy may be the result of either the amount of the virus used or of the higher affinity of HIV (gp160) to CCR5 receptor than that of purified natural anti-CCR5 peptide antibodies. We found a difference in the inhibitory capacity toward infection between Abs from breast milk of HIV-seronegative and -seropositive women. Thus, at the same amount, Abs purified from seropositive women exhibited a higher capability of inhibiting HIV infection.

Our observations suggest that anti-CCR5 Abs in breast milk may control postnatal transmissibility of HIV through breastfeeding. The relationship between the presence/amounts and avidity of anti-CCR5 Abs and postnatal transmission of HIV-1 remains to be investigated in clinical cohorts. Abs to both viral-neutralizing glycoprotein epitopes (25, 26) and to chemokine coreceptors have been suggested to play a role in protection against HIV infection (6, 27). The presence of anti-CCR5 Abs has previously been documented in sera of HIV-seropositive (28), HIV-exposed uninfected (16), healthy (8), and homozygous CCR5 Δ32 individuals (29), who had been repeatedly exposed to CCR5expressing cells through sexual activity. Furthermore, Abs to CCR5 molecule induced by vaccination in macaques were shown to play an important role in inhibition of infection with SIV (30). Anti-CCR5 Abs and CCR5-binding chemokines such as RANTES that are secreted in breast milk with a considerable variability among women (31, 32) may compete for the binding to CCR5 and inhibit infection of peripheral blood mononuclear cells with R5 but not with X4-tropic primary isolates of HIV-1. Interestingly, Abs to the second extracellular loop of CCR5 block CCR5-mediated HIV transmission without affecting CCR5 signaling by chemokines (33), which could represent an advantage of passive therapy with such Abs warranting further research.

Disclosures

The authors have no financial conflict of interest.

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