

Human Milk Contains Elements That Block Binding of Noroviruses to Human Histo–Blood Group Antigens in Saliva

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Noroviruses (NVs) recognize human histo–blood group antigens (HBGAs) as receptors. We characterized the interaction of human milk samples with recombinant virus-like particles representing VA387, Norwalk, VA207, and MOH. Milk samples from 60 healthy women were tested for human HBGAs and for their ability to block the binding of NVs. Fifty-four women were secretors (Se⁺), and 6 were nonsecretors (Se⁻). No women had detectable A or B antigens in their milk samples. All 54 Se⁺ milk samples, but 0 of 6 Se⁻ milk samples, blocked VA387 and Norwalk virus (Se⁺ binders) from binding to saliva samples. All 6 Lewis-positive Se⁻ milk samples blocked binding to VA207, and variable blocking activities were exhibited by the Se⁺ milk samples. No milk samples blocked the binding of MOH to A and B antigens. Secretor and Lewis, but not A or B antigens, were present in human milk and were responsible for blocking NV binding to receptors and therefore are likely to be decoy receptors that protect breast-fed infants from NV infection.

Noroviruses (NVs)—previously known as Norwalk-like viruses or small round-structured viruses—belong to 1 of 4 genera of *Caliciviridae*. NVs have been found to be the most important cause of nonbacterial acute gastroenteritis in all ages in both developing and developed countries. Surveillance using molecular diagnostic methods that were developed during the past decade has also shown that NVs are, next to rotavirus (RV), the most important cause of pediatric acute gastroenteritis [1, 3]. Studies in many countries have shown that children acquire antibody against NVs at an early age and that the prevalence of antibody continues to increase throughout the school-age years to adulthood

[2–6]. NVs have also been detected commonly in children with acute gastroenteritis among hospitalized patients (range, 4%–53%; mean, 15%) [7–10], in the emergency room (mean, 31%) [11], and in outpatient clinics (range, 1.3%–16%) [12–15], which indicates that NVs may cause severe diarrhea in children who require a physician visit or hospitalization.

NVs remain difficult to study because there is a lack of cell-culture and animal models. Studies of host range and viral receptors for NVs have enabled new approaches to define the pathogenesis of NVs. Using recombinant (r) virus-like particles (VLPs) of NVs expressed in baculovirus as probes, we have found that NVs recognize human histo–blood group antigens (HBGAs) as receptors [16, 17]. Different NVs recognize different receptors, and 4 major NV-receptor binding patterns have been described [16]. The prototype Norwalk virus represents 1 pattern that specifically binds to human intestinal tissue sections and the saliva of secretors but not of nonsecretors [17]. The other 3 binding patterns are binders of type A, B, and O secretors (VA387); binders of A and B secretors (MOH); and binders of Lewis (Le)–positive secretors and nonse-

Received 28 January 2004; accepted 30 April 2004; electronically published 11 October 2004.

Financial support: National Institute of Child Health and Human Development (grant HD13021); National Institute of Allergy and Infectious Diseases (grant AI 37093).

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The Journal of Infectious Diseases 2004;190:1850–9

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0022-1899/2004/19010-0019\$15.00

cretors (VA207). The terminal 3 sugars containing the antigenic epitopes of human HBGAs are responsible for binding specificity. Direct evidence of human HBGA involved in NV infection has been shown in a human volunteer challenge study that showed that nonsecretors who do not express the H receptor antigens were naturally resistant to NV challenge [18]. Evidence of human HBGAs related with Norwalk virus also has been obtained by other studies [19–21], but direct evidence for the other 3 binding patterns remains lacking.

Human milk is rich in oligosaccharides that are found in both free and conjugated forms, such as glycoproteins and glycolipids. The synthesis pathways of the major human HBGAs are shown in figure 1. These oligosaccharides and glycocon-

jugates can contain the same epitopes of human HBGAs as those found on mucosal epithelial cells and in other body fluids, such as saliva and intestinal contents. Our previous studies have shown that the binding of Norwalk virus to the intestinal epithelial cells of secretors could be blocked by milk from a secretor [17], which suggests that human milk may play a role in blocking the interaction of NVs with human HBGA receptors in the infant gastrointestinal tract.

In the present study, we further characterized human milk samples from 2 populations for their expression of HBGAs and the interaction of these antigens with different strains of NVs, using rVLPs as probes. Our data indicate that similar, but not all, human HBGAs found in the mucosal surface and other

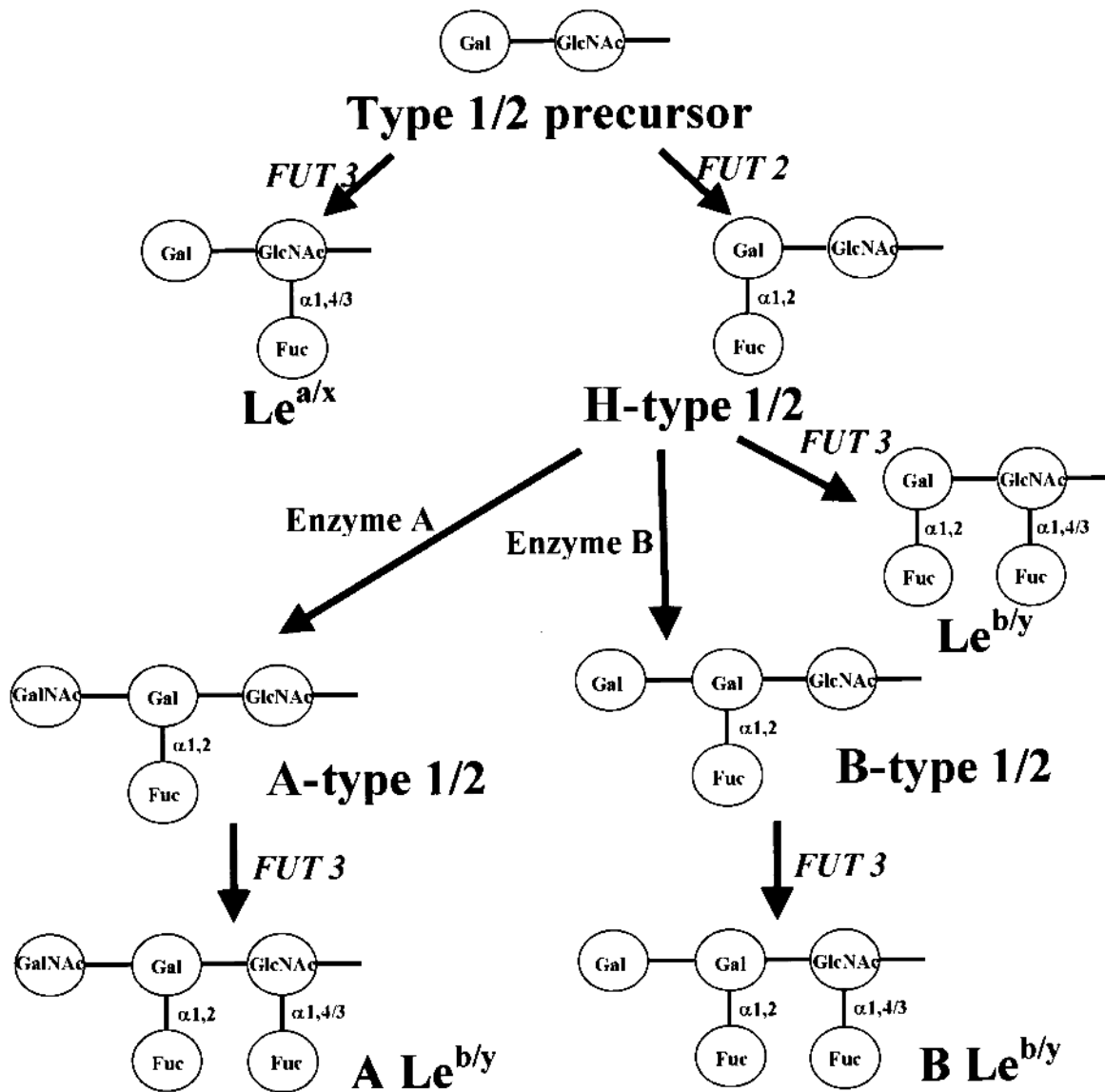


Figure 1. Diagram of the biosynthesis pathways of the human ABH and Lewis histo-blood group antigens based on the type 1 and 2 precursors. Enzyme A, N-acetylgalactosaminetransferase; enzyme B, α -galactosyltransferase; Fuc, L-fucose; *FUT2*, $\alpha(1, 2)$ fucosyltransferase; *FUT3*, $\alpha(1, 3/4)$ fucosyltransferase; Gal, D-galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine.

body fluids also are expressed in human milk. These soluble HBGAs blocked NV binding to specific receptors. The potential role of these receptors in the protection of breast-fed infants from NV infection is discussed.

PATIENTS, MATERIALS, AND METHODS

Study population. A total of 60 women, 30 residing in Mexico City and 30 residing in Norfolk, Virginia, were enrolled in a study of immune modulation of RV vaccination in milk in 1999. One-half of the women received 1 dose of tetravalent RV vaccine at 30 days after delivery, and one-half of them received placebo. Milk samples were collected weekly for the first month after delivery and monthly thereafter during the lactation period. Serum samples also were collected from each individual before and 2 weeks after vaccination. The milk and serum samples were stored at -80°C . A single milk sample from each woman collected 4–7 days after delivery was tested for binding to NV, for blocking NV binding to HBGAs, and to determine the secretor and Le blood types of the women. Milk samples collected from 6 secretors at different time points during lactation also were tested for the expression of the Le^a and Le^b antigens. Because we found that the A and B antigens were undetectable in milk samples, the ABO blood types of the 60 women were determined by an immune hemagglutination test that used serum samples.

Determination of women's histo-blood types on the basis of milk samples. Milk samples were diluted at 1:1000 in PBS and used to coat microtiter plates (Dynex Immulon; Dynatech) overnight at 4°C . After blocking with 5% Blotto (dried milk), monoclonal antibodies (MAbs) specific to Le, secretor, type A, and type B antigens were added. After incubation for 1 h at 37°C , horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG or IgM antibodies were added. After each step, the plates were washed 5 times with PBS. The enzyme signals were detected by use of the TMB (3,3',5,5'-tetramethylbenzidine) kit (Kirkegard & Perry Laboratories) and then read at a wavelength of 450 nm by use of an EIA spectra reader (Tecan).

The following MAbs specific to human HBGA types were used for phenotyping: BG-4 anti-H type 1, BG-5 anti-Le^a, BG-6 anti-Le^b, BG-7 anti-Le^x, BG-8 anti-Le^y (Signet Laboratories), BCR9031 anti-H type 2, BCR 9010 anti-A, and BCRM 11007 anti-B (Accurate Chemical and Scientific).

Determination of ABO blood types. The ABO blood types of the 60 women were determined by immunohemagglutination (Immucor). In brief, microplates were blocked with 1% bovine serum albumin for 30 min at 37°C and rinsed with PBS 6 times. Each serum sample was centrifuged at 5900 g for 10 min to remove the debris, and 40 μL of reference red blood cells were added to each well, along with 20 μL of each sample, and the plates were incubated for 1–3 h at room temperature before the results were read. Hemagglutination was scored ac-

ording to the standard described in the kit. When necessary, the plates were tapped to resuspend the reference cells before recording the results.

Blocking of NV binding to saliva samples by human milk. Conditions of NV/saliva EIAs were used [16], with the addition of an incubation step with human milk. Milk samples from the 60 women described above were added to the plate at a dilution of 1:200. rNVs (rNorwalk, rVA387, rVA207, and rMOH) [22–24] were added at 0.3–0.6 $\mu\text{g}/\text{mL}$, and the captured rNV capsid proteins were detected by secondary antibody HRP conjugates, as described above. Blocking of binding by a human milk sample was determined by a comparison of optical density values between wells with or without incubation with the human milk sample.

Isolation of oligosaccharides. Milk samples (1.0 mL) were thawed immediately before use and centrifuged at 4000 g for 45 min at 4°C . The viscous, upper cream layer, consisting primarily of fats and other lipids, was removed by filtering through a glass wool plug in a Pasteur pipette. Then, 2 mL of ethanol was added to the filtrate (to give 66.7% ethanol), and the mixture was kept overnight at 4°C . The precipitate, consisting predominantly of proteins and lactose, was removed by centrifugation at 4000 g for 15 min at 4°C . The clear supernatant, consisting primarily of oligosaccharides and residual lactose (the total oligosaccharide fraction), was transferred to a screw-cap tube, dried by passage of nitrogen, lyophilized, and weighed [32].

Binding of NV capsid antigen to human milk samples. Baculovirus-expressed capsid proteins of rNorwalk, rVA387, rMOH, and rVA207—representing 4 distinct binding patterns to human saliva samples—were studied. The same protocol of EIA that had been used to measure NV VLP binding to HBGAs in saliva samples was followed, with minor modification. In brief, milk samples were boiled and centrifuged, and the supernatant that formed below the lipid phase was stored frozen until use. This phase is defined as the “aqueous fraction” of milk samples in the study. Similar procedures also were used to prepare the aqueous fraction from unboiled milk samples for antibody testing. Therefore, the only difference between the 2 types of aqueous fraction was boiled versus not boiled.

For testing rNV binding to milk, microtiter plates (Dynex Immulon; Dynatech) were coated with milk samples at a dilution of 1:1500 in PBS (pH 7.4). After blocking the plates with 5% dried milk (Blotto), rNV capsid proteins at 0.4–2.0 $\mu\text{g}/\text{mL}$ in PBS were added. The bound rNV capsid proteins were detected by use of a pooled guinea pig anti-NV antiserum, followed by the addition of HRP-conjugated goat anti-guinea pig IgG (ICN). During each step, the plates were incubated for 1 h at 37°C and subsequently washed 5 times with PBS. The color reaction was developed and recorded as above.

Virus overlay protein binding assays (VOPBAs). VOPBAs were performed to determine the molecules in human milk

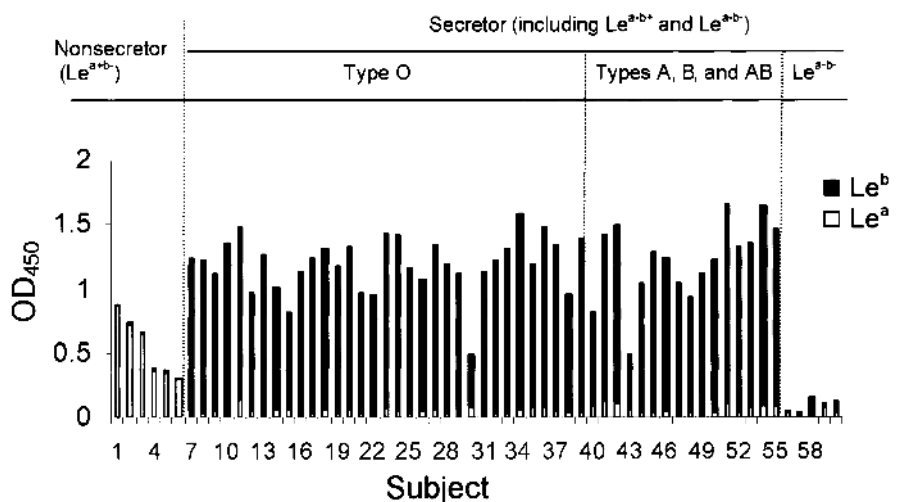


Figure 2. Detection of Le^a and Le^b antigens in milk from the 60 women living in Norfolk or Mexico City. Milk samples collected during the first week after delivery were studied. The secretor and Lewis types of the 60 women were inferred from the relative levels of the Le^a and Le^b antigens in the individuals' milk. Subjects 1–6 were designated as nonsecretors, and the remaining 54 women were designated as secretors (33 type O and 21 types A, B, and AB). Five Lewis-negative women (Le^{a-b-}) are shown at the far right of the figure. These 5 women were designated as secretors on the basis of the detection of the H type 1 and/or Le^y antigens in their milk.

and saliva samples that bind NV VLPs [25]. In brief, the aqueous fractions of human milk and saliva samples were resolved by SDS electrophoresis in 10% polyacrylamide gel and transferred to a nitrocellulose membrane by electroelution. The membrane was incubated in rNV VLPs and then in a guinea pig anti-NV VLP antibody. The bound capsid proteins were detected by hyperimmune guinea pig anti-NV antiserum, followed by the addition of HRP-conjugated goat anti-guinea pig IgG (ICN). The HRP activity was detected by use of the ECL Western Blotting Detection kit (Amersham Bioscience).

Detection of secretory IgA against NVs in milk samples.

EIAs for the detection of serum antibodies against NVs were modified for detecting antibodies in milk samples, according to standard protocols, except that unboiled milk was substituted for serum [2, 26]. Recombinant capsid antigens derived from strains Norwalk, VA387, MOH, and VA207 were studied.

Statistical analysis. The χ^2 or Fisher's exact test was used to distinguish antibody titers of groups of individuals whose fluids bound or did not bind to individual rNVs.

RESULTS

Distribution of Le, secretor, and ABO blood types of the 60 women. The levels of Le^a and Le^b antigens in milk samples from the 60 women are shown in figure 2, and the assignments of blood types of the 60 women are shown in table 1. Six (10%) women had high levels (OD > 0.2) of Le^a and low or undetectable Le^b in their milk; these 6 women were designated as nonsecretors. Forty-nine (82%) women had high levels (OD > 0.4) of Le^b and low or undetectable Le^a in their milk; these 49 women were designated as secretors. Five (8%) women had

low levels of both Le^a and Le^b, but 4 of these were H type 1 antigen positive in their milk samples, and the remaining 1 was strongly positive for Le^y antigen in her milk; thus, these 5 women were designated as Le-negative secretors. The distributions of secretor and Le types were similar between women living in Norfolk and those living in Mexico City, except that a lower rate of nonsecretors (7%) was found in those living in Mexico City than in those living in Norfolk (13%). In all subsequent analyses, the 60 women are considered to be 1 group.

When the 60 milk samples were tested for type A and B antigens, none of them revealed a positive reaction, which suggests that the A and B antigens are not expressed in human milk. This result was consistent with the lack of blocking of MOH (A and B binder) binding to A and B antigens (see below). However, when the serum samples from the 60 women were tested for ABO types by use of the immune hemagglutination assay, a normal distribution of ABO types among the 60 women was found. Sixteen women were type A, 4 were type B, 1 was type AB, and the remaining 39 were type O (table 1). This result

Table 1. Distribution of histo-blood types among the 60 lactating women from Norfolk, Virginia, and Mexico City, Mexico.

Study location	Lewis type, ^a no.			ABO type, ^b no.			
	a ⁺ b ⁻	a ⁻ b ⁺	a ⁻ b ⁻	A	B	AB	O
Mexico City (n = 30)	2	26	2	5	0	1	24
Norfolk (n = 30)	4	23	3	11	4	0	15
Total	6	49	5	16	4	1	39

^a Determined by monoclonal antibody-based ELISA of milk samples.

^b Determined by a hemagglutination test that used serum samples, because the type A and B antigens were undetectable in milk.

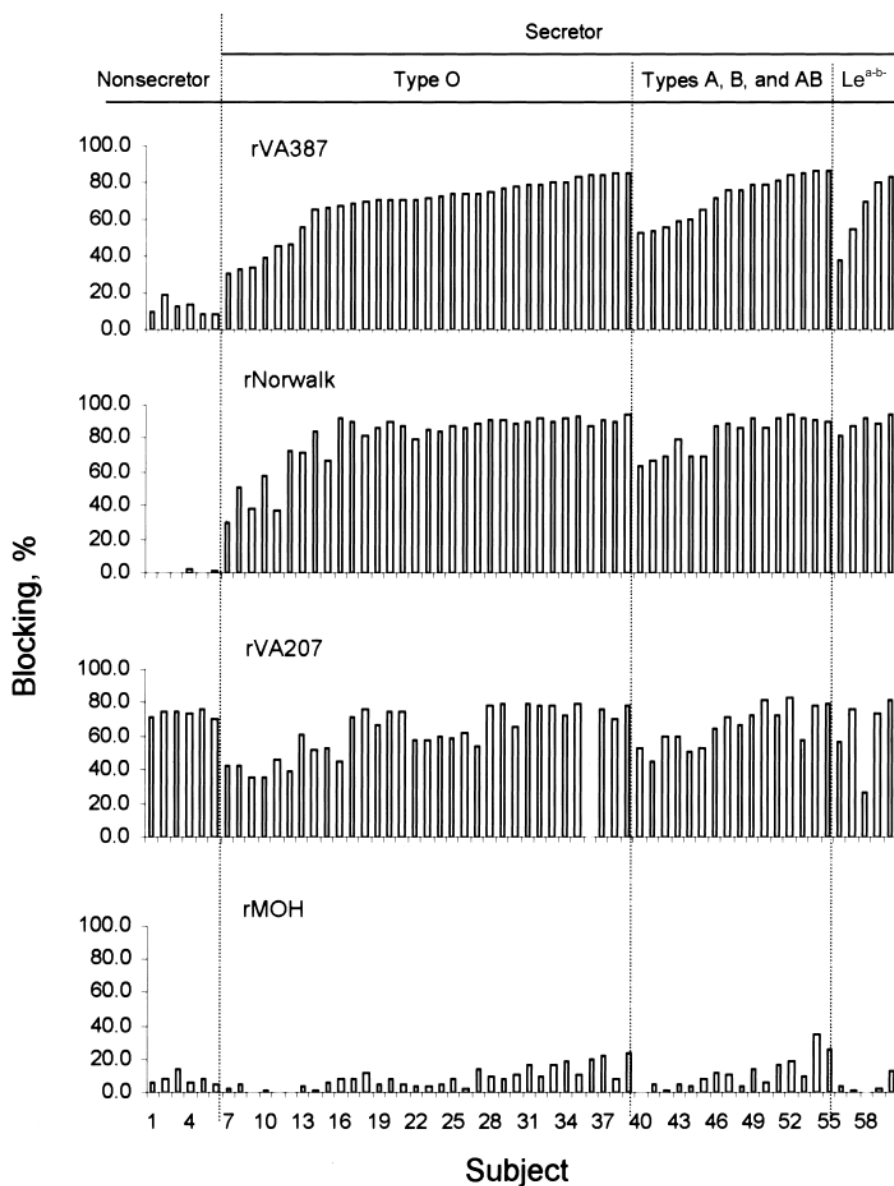


Figure 3. Blocking of Norwalk virus (NV) binding to saliva by 60 human milk samples. The blocking activities of the 60 women' milk samples of binding of the 4 strains of NVs (VA387, Norwalk, VA207, and MOH) to specific receptors were measured by the saliva/receptor binding assays in the presence and absence of milk samples. Milk samples collected during the first week after delivery were studied. The levels of blocking were calculated on the basis of differences in optical density values in the presence or absence of milk samples. The order of subjects is the same as that in figure 1, and the histo-blood types of the subjects are shown at the top of the panel. Subjects 1–6 were nonsecretors, and the remaining 54 subjects were secretors. A correlation of the Lewis and secretor types of the 60 women and the blocking activities of their milk samples were observed for VA387, Norwalk, and VA207. No blocking activities were observed for MOH. The data of blocking VA207 binding to saliva by subject 36 was lacking because of depletion of the saliva sample. r, recombinant.

suggests that the A and B antigens are not expressed in the mammary glands of women with A, B, or AB blood types.

Blocking of rNV binding to saliva by human milk. Milk samples from all 54 secretors, but not from the 6 nonsecretors, blocked the binding of VA387 and Norwalk virus to a secretor (type O) saliva sample (figure 3). Milk samples from all 6 nonsecretors and 54 secretors blocked VA207 (Le epitope

binder) binding to a nonsecretor saliva sample (figure 3). The blocking was stronger for nonsecretors than for secretors (figure 3), which is consistent with the level of Le epitopes being higher in nonsecretors than in secretors [16]. None of the 60 milk samples—including those from the 21 women whose blood types were A, B, or AB—had significant blocking activities to MOH binding to a saliva sample from a woman with type A

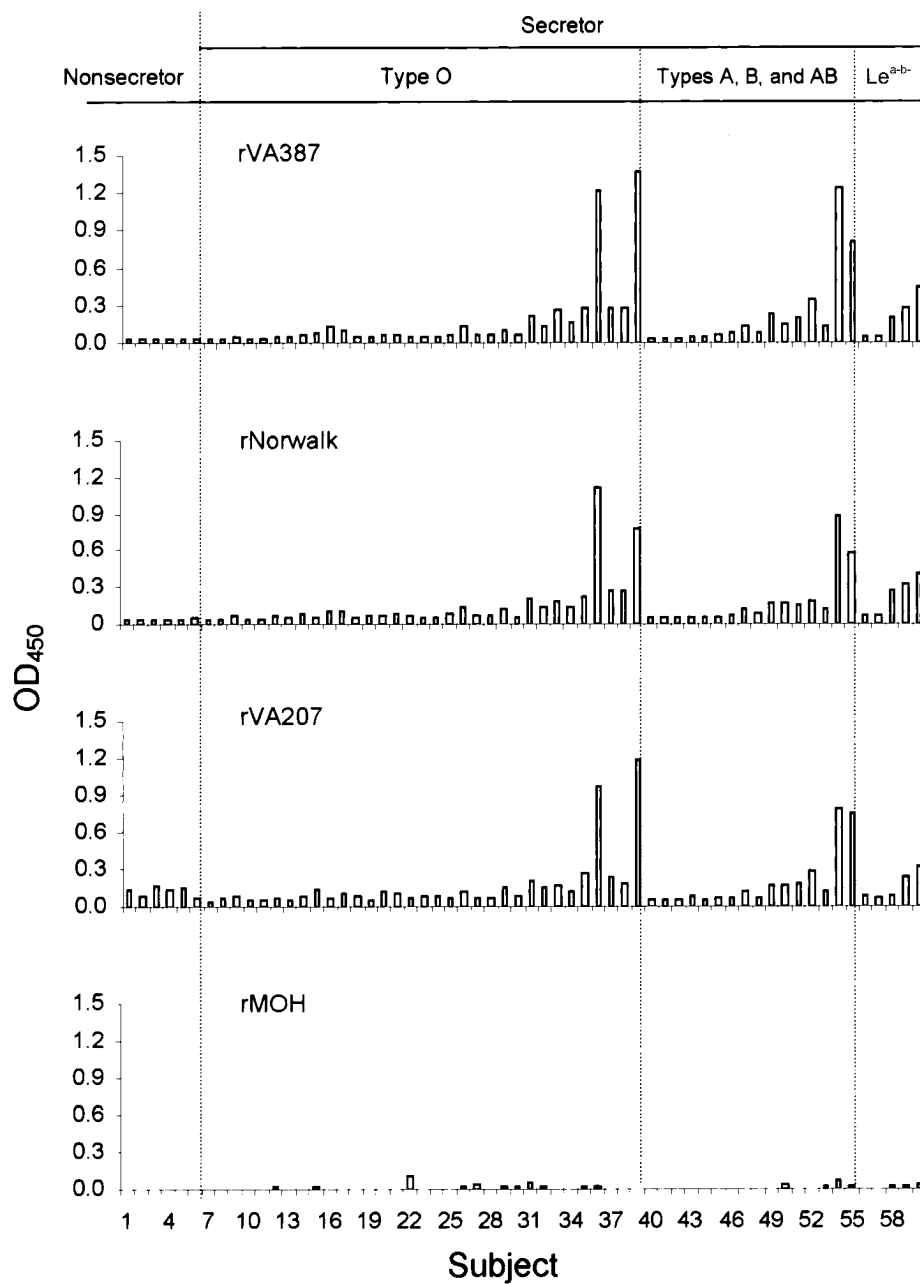


Figure 4. Binding of the 4 recombinant (r) Norwalk virus (NV) virus-like particles (VLPs; VA387, Norwalk, VA207, and MOH) to human milk samples from 60 women. A protocol similar to that of saliva-binding assays was used, except that human milk samples were used in the first step to coat the plates. Milk samples were collected at the first week after delivery. The order of subjects is the same as that in figure 1, and the histo-blood types of subjects are shown at the top of the panel.

blood (figure 3). The observed low levels (~20%) of blocking were interpreted as background, because they did not reveal any pattern related with the ABO blood types of the women.

Binding of rNV to human milk samples. The results of milk binding to NVs were generally in concordance with the blocking results. For example, milk samples from most (>80%) of the secretors, but not most of the nonsecretors, bound to

VA387 and Norwalk virus, milk samples from 5 of 6 nonsecretors reacted with VA207, and 0 of 60 milk samples bound to MOH (figure 4). Other results were not consistent with the blocking results: milk samples from some secretors failed to bind to VA387 and Norwalk virus, whereas milk samples from other secretors had unexplained high binding to these 2 viruses. The high binders to VA387 and Norwalk were also high binders

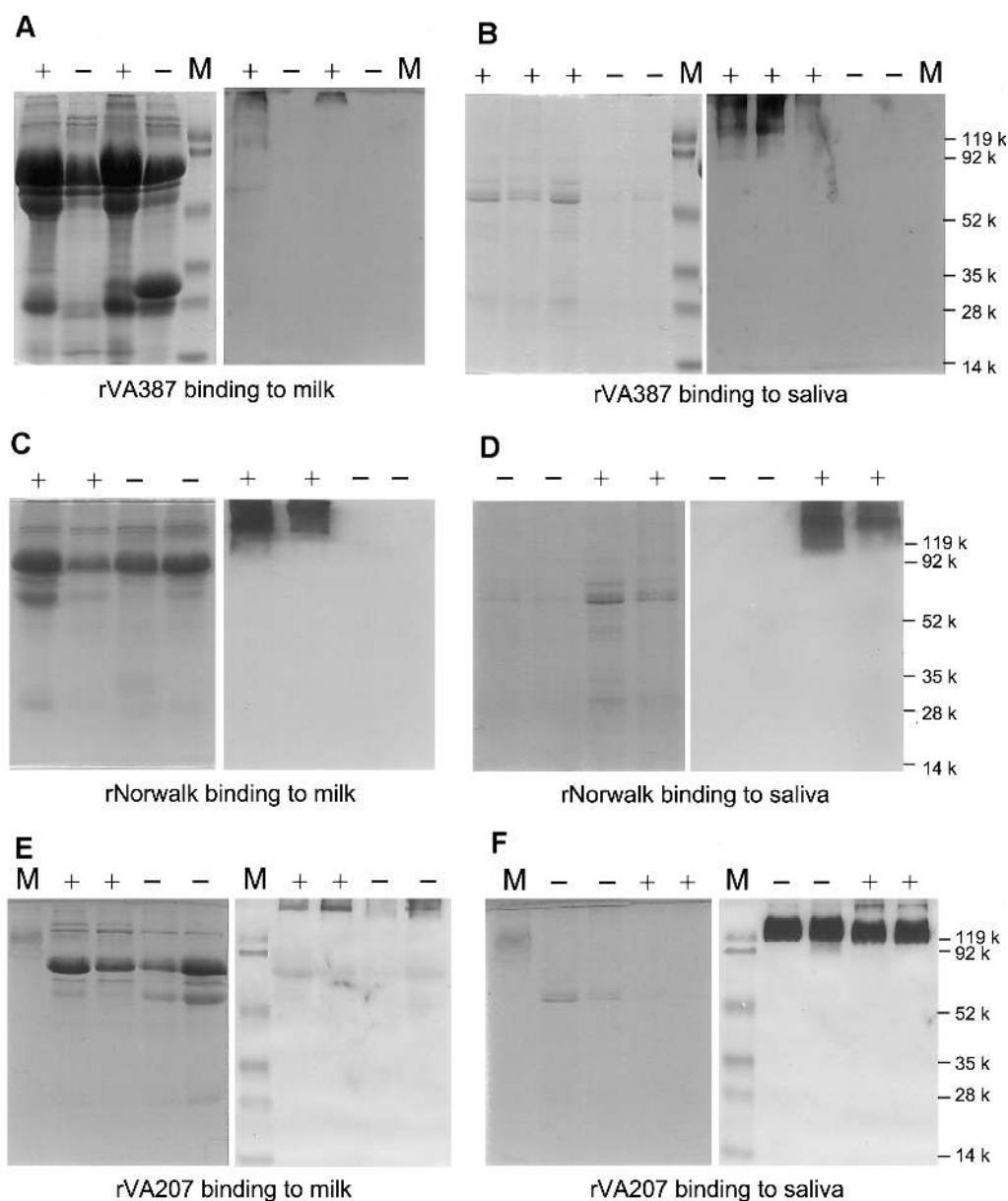


Figure 5. Detection of ligands in human milk and saliva, for recombinant (r) Norwalk virus (NV) capsids, by virus-overlay protein binding assays (VOPBA). Milk and saliva samples from Lewis-positive secretors (+) and nonsecretors (–) were selected for the assays. Recombinant virus-like particles from 3 binding patterns (VA387, Norwalk, and VA207) were used as the probes. Duplicate milk and saliva samples were analyzed by PAGE in the presence of SDS. The resulting gels were stained with Brilliant blue G-250 (Fisher Scientific) (*left*) or tested by VOPBA (*right*). M, molecular-weight markers (the size of the markers is indicated at right).

to VA207. We believe that these results might have been caused by interference from unrelated molecules in the milk. However, different treatments—including freezing/thawing, chloroform extraction, sonication, and high-speed centrifugation—did not result in a significant change in the binding patterns, and the highest binding activities were obtained in milk samples with the least treatment (data not shown). Thus, we cannot exclude the possibility that, in addition to the HBGAs, other factors in human milk also contribute to NV receptor interaction.

Blocking of NV binding to receptors by proteins in human milk with high molecular weight. To identify the ligands in human milk responsible for blocking NV binding to receptors, we performed blocking experiments, using total oligosaccharides purified from human milk. Using total oligosaccharides from milk samples pooled from 40 women, we did not observe blocking of any of the 4 NV strains to their receptors by use of a wide range of concentrations of total oligosaccharides (data not shown). On the basis of previous biochemical results, such

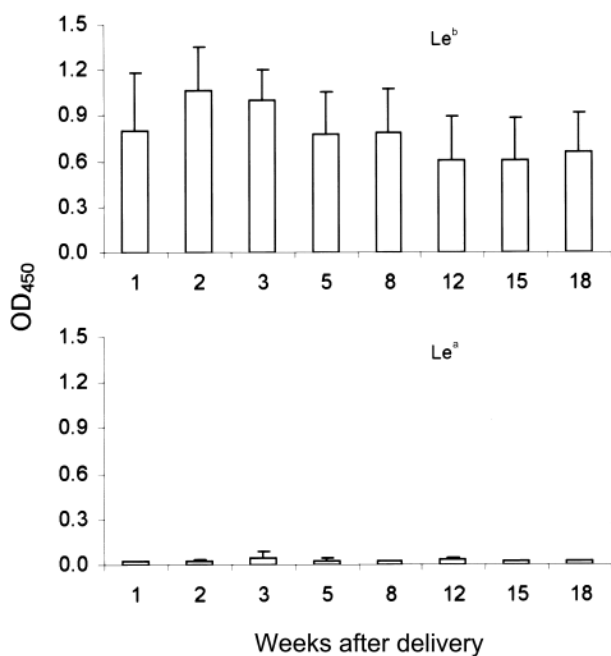


Figure 6. Dynamic detection of Lewis^a and Lewis^b antigens in milk samples from 6 secretors at different time points during the lactation period. Milk samples collected at 1–18 weeks after delivery were studied. Columns show the average optical density with an SD at each time point.

oligosaccharide preparations are known to contain a wide range of oligosaccharide sizes, including fucosyl oligosaccharides, whose Le epitopes are similar to those of the histo–blood group epitopes. Our data suggest that the free oligosaccharides from human milk are not involved in the NV receptor interaction, even though they contain the same epitopes.

We then characterized the biologically active ligand found in human milk, using VOPBAs. As shown in figure 5, the VA387 and Norwalk VLPs bound to proteins with a high molecular weight (>150 kDa) in secretors but not in nonsecretors (figure 5A and 5C). Because mucins are the major glycoproteins with high molecular weight in human milk [27, 28], we hypothesize that they could be the potential ligands in human milk that block NV binding to host receptors. Similar-molecular-weight bands also were observed in saliva samples from secretors (figure 5B and 5D). As had been predicted from the receptor binding specificity, strain VA207 bound to large proteins in milk samples from both Le-positive secretors and nonsecretors (figure 5E). In addition, VA207 also reacted with a band at ~80 kDa. When saliva samples were used for VOPBA, a strong signal was observed at ~130 kDa, in addition to a high-molecular-weight protein of both secretors and nonsecretors (figure 5F). These data suggest that the Le epitopes specific for VA207 exist in multiple glycoproteins of human milk and saliva.

Dynamics of Le-antigen expression in human milk. The expression of Le^a and Le^b antigens in human milk over the course of the first 18 weeks of lactation was studied in 6 se-

cretors. A fairly constant expression of both Le^a and Le^b antigens was observed in all 6 women from the first to the last week (week 18), although the expression of Le^a antigen remained at a constant low level (figure 6). A tendency toward the maximal expression of both Le^a and Le^b was observed at weeks 2 and 3 in the 6 women.

Detection of milk secretory IgA against different rNVs. Milk secretory IgAs against rNorwalk, rVA387, rVA207, and rMOH were tested to determine their exposure to NVs. Significant numbers of secretors, but 0 of 6 nonsecretors, had antibodies (titers >1:160) against Norwalk and VA387 (19 [35%] [$P = .079$] and 23 [43%] [$P < .05$] of the 54 secretors, respectively) (table 2). One nonsecretor (17%) and 18 secretors (33%) had antibodies (titer >1:160) against VA207. Milk antibodies against MOH were detected in 13 secretors (24%) but not in the 6 nonsecretors. Three (17%) of 18 type A and/or B secretors and 10 (28%) of 36 type O secretors had antibodies against MOH (table 2). All 13 women who had antibodies against MOH also had antibodies against VA387, which suggests that shared antigenic epitopes may exist between MOH and VA387.

DISCUSSION

Milk is a natural gift of women to their infants not only for nutrition but also for other functions, such as the protection of infants from infectious diseases during their early development. In addition to acquired immune protection, milk may contain natural products that provide innate protection [29]. Many pathogens rely on specific receptors on host cell surfaces for the initiation of infection. One type of natural product in milk is the decoy receptors that may interfere or compete with pathogens for specific receptors. The findings that Norwalk virus binds to human HBGAs [17] and that nonsecretors were naturally resistant to Norwalk virus infection [18] indicate that NVs recognize human HBGAs as receptors for infection. Our finding of the specific blocking of Norwalk virus binding to receptors by a secretor's milk [17] provided the first example

Table 2. Prevalence of milk secretory (s) IgA against Norwalk, VA387, VA207, and MOH among the 60 lactating women.

Virus type	No. (%) of women with sIgA titers >1:160	
	Nonsecretor (n = 6)	Secretor (n = 54)
Norwalk	0	19 (35)
VA387	0	23 (43) ^a
VA207	1 (17)	18 (33)
MOH	0	13 (24) ^b

^a Pearson $\chi^2 = 4.144$; $P < .05$.

^b Among the 13 secretors, 10 were type O, and 3 were types A, B, or AB.

of such decoy receptors in NV/receptor interaction. Direct evidence of milk protecting infants from NV infection also has been shown by one of our recent studies, in which breast-fed infants were less likely to have NV diarrhea if the concentrations of secretor antigens (Le^b antigens) in their mothers' milk were high [30].

In the present study, we further showed that the expression of HBGAs in human milk correlated with the host blood types, and this correlation was associated directly with the blocking of NV binding to HBGAs. In previous studies, we have shown that different NVs recognize different receptors and that the expression of different genes is likely to be associated with host susceptibility to specific strains [16]. In the present study, we have shown that human milk offers varying protection against different NV strains, depending on the maternal histo-blood group type. Protection of the breast-fed infant against different NVs is thus dependent on the combination of NV strain, the infant histo-blood group genotype, and the maternal histo-blood group genotype.

Moreover, we found that not all human HBGAs are expressed in human milk. The Le (Le^a and Le^x) and secretor (Le^b, Le^v, H-type 1, and H-type 2) antigens, which contain the fucosyl side chains that are added by the fucosyl-transferase gene products (FUT2 and FUT3, respectively), are expressed in human milk. This result is consistent with the reports of high levels of fucosyl oligosaccharides in human milk [31, 32]. Thus, we predict that human milk may provide protection to breast-fed infants from secretor- and/or Le-binding strains of NVs, which cover 3 (VA387, Norwalk, and VA207) of the 4 binding patterns described in our previous study [16].

However, A and B antigens are not expressed in human milk. This raises a question about the evolution of human histo-blood group genes; the A and B antigens are involved in some NV infections (i.e., MOH-like strains), and natural protection against these strains is necessary. A and B antigens are strong immunogens that can cause antibody-related hemolysis in blood transfusion; one hypothesis is that A and B antigen may have been turned off in the mammary gland because of a selection of such potential risk to the immature infant.

Our previous studies have shown that synthetic free oligosaccharides containing H-type epitopes block NV binding to human HBGAs [17]. In the present study, using total oligosaccharides purified from human milk, we did not observe any blocking of NV binding to saliva samples. One possible explanation is that the total fucosyl epitopes in the preparation of the total milk oligosaccharides might be low, compared with those of the synthetic oligosaccharide used in the previous study [16]. In addition, both the HBGAs and the VLPs involved in the saliva-binding assays were large molecules with multiple interaction sites. Thus, the lack of blocking by the total milk oligosaccharides could be because of a low affinity and/or a

low avidity of the free oligosaccharides used in the assay. This information is important for designing new antivirals based on the NV/receptor interaction.

The present study also characterized the relative role of acquired immunity in human milk in the protection of infants from NV infection. The prevalence of such antibodies in maternal milk is relatively low, compared with the prevalence of HBGAs expressed in human milk. Thus, secretory IgA may not provide the same level of protection as that of the oligosaccharide receptors. The finding of the constant expression of Le^b and H type 1 [33] in human milk throughout the first 18 weeks of lactation indicates that human milk continues to provide protection to infants against infection, although direct evidence of human milk in the protection of infants from NV infection remains to be obtained. The antibody prevalence in milk also showed a correlation between HBGA expression in the women and the presence of antibody against specific strains of NV. This result is consistent with our previously observed correlation between the prevalence of antibodies in saliva and host specificity to different strains of NV, which further indicates that individuals with certain blood types are susceptible to certain strains of NV.

These novel data link HBGA expression in human milk to the pattern of protection of infants from NV infection, but many questions remain. For example, the hypothesis that milk HBGAs protect infants from different NVs representing different binding patterns remains to be confirmed. The results of the direct binding assays did not completely agree with those of the blocking assays. This could be due to competition by other milk factors (lipid, proteins, and polysaccharides) with the HBGAs during the coating step of the EIA. In conclusion, human milk may play an important role in the immune protection of breast-fed infants against NVs. Further studies for a better understanding of this role and for the development of a strategy to promote breast-feeding are necessary.

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