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Detection of IgE Anti-Parvovirus Antibodies in Human Breast Milk

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Abstract. Breast milk is a complex fluid, rich in nutrients and non-nutritional bioactive components, including antimicrobial factors, immunoglobulins, cytokines, and anti-inflammatory substances. Although IgE is implicated in viral immunity, its role in breast milk in parvovirus B19 immunity has not been studied. Total immunoglobulin levels of IgE, IgG, and IgE anti-parvovirus B19 antibodies were determined by ELISA and Western blot analysis in breast milk and in sera from a mother and her nursing infant (female, 10 mo). For specific IgE protein determination, breast milk was fractionated by chromatography on G-100 Sephadex; 3 peaks were collected and separated by SDS PAGE. The levels of total IgE in breast milk and its fractions were low (<2.4 ng/ml), and those of maternal and infant serum were negligible (18 and 4.3 IU/ml, respectively). Nevertheless, the breast milk and maternal and infant sera contained IgE anti-parvovirus B19 antibodies, even though the infant was never infected with parvovirus B19. Total serum levels of maternal IgG were within the normal range and those of infant IgG were low (473 mg/dl); total IgG in breast milk was not determined. Maternal serum contained some detectable IgG anti-parvovirus antibodies that were not present in infant serum or breast milk. Total maternal and infant serum levels of IgM and IgA were within the normal ranges. The presence of IgE anti-parvovirus B19 antibodies in breast milk suggests that IgE anti-viral antibodies are transmitted in breast milk and may provide protective responses in nursing children.

Keywords: breast milk, immunoglobulin E (IgE), IgE anti-parvovirus B19 antibodies

Introduction

Breast milk is a complex fluid, rich in nutrients and non-nutritional bioactive components, including antimicrobial factors, immunoglobulins, cytokines, and anti-inflammatory substances [1]. Allergens, also present in breast milk, may be either sensitizing or protective, depending on various factors [2]. Human breast milk also contains components that protect the infant against infections, including factors that provide specific immunity, eg, antibodies and lymphocytes [3]. Breast milk has been reported to contain secretory IgA (sIgA), IgM, and IgG [4,5], IgE, and IgD, which are also found in colostrum [6]. Whether IgE antibodies in breast milk have specificities for certain viruses is unknown. Although IgE has been implicated in viral immunity, its role in breast milk in regard to parvovirus B19 immunity has not been studied.

Previous studies in our laboratory were the first to demonstrate the presence of IgE anti-parvovirus B19 in serum of a parvovirus B19-infected child, and its persistence in serum 7 mo post-infection [7], as well as in IgA deficiency [8]. Studies in our laboratory also showed the presence, persistence, and function of IgE anti-HIV in serum of a subset of HIV-1 seropositive, nonprogressor pediatric

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patients [9-11], and showed that specific IgE anti-HIV-1 antibodies may protect against HIV-1 disease progression by suppressing virus production [10,11]. Recent studies in our laboratory investigated the presence and persistence of IgE anti-*Borrelia burgdorferi* antibodies in pediatric patients with Lyme disease [12].

Studies of Cantisani et al [13] demonstrated that IgE specific for human milk whey proteins is present in serum of atopic infants. Other studies have reported that IgE specific for cow's milk protein (CMP) is present in sera of breast-fed atopic children [14]. It has been suggested that sensitization to CMP can occur through ingestion by the mother of cow's milk and passage of CMP into breast milk [14]. Protective effects of breast feeding against gastrointestinal and respiratory tract infections have been shown [6,15,16]. However, the role of breastfeeding in the prevention of asthma or allergic diseases, and possibly of virus infections, is more controversial and not well-documented [6,17].

In the present study we detected IgE anti-parvovirus B19 antibodies in human breast milk. This suggests that anti-parvovirus B19 antibodies are transmitted via breast milk and may provide anti-viral protective responses in nursing children.

Materials and Methods

Subjects. Peripheral blood (5 ml total) was obtained from a pediatric patient (female, age 10 mo) and her mother; they had serum total IgE levels of 4.3 and 18 IU/ml, respectively. The child had no known allergies, while the mother reported current allergies for ragweed, house dust, and dust mites (skin test positive). The mother had documented parvovrius B19 infection as an 8-yr-old child. Her child did not have prior exposure to parvovirus B19, as verified by serology. The child had been only breast-fed, until low allergen solid foods (eg, rice cereal) were introduced at 6 mo. Informed consent was obtained from the child's parents for use of the blood serum and breast milk samples for this study.

Blood was collected into red-top monoject tubes (Sherwood Medical, St. Louis, MO). Samples of breast milk were collected from the child's mother in the morning using a hand pump or an electric breast pump into sterile tubes and were frozen immediately at -20°C.

Total serum immunoglobulins (IgM, IgG, and IgA) were determined by Universal Diagnostic Laboratories (Brooklyn, NY) and Quest Diagnostics (Teterboro, NJ), using nephelometry, which was performed according to standard procedures. Serum total IgE levels were detected by the UniCAP Total IgE Fluoroenzyme immunoassay kit (Pharmacia & Upjohn Diagnostics, Kalamazoo, MI), which was performed by the Clinical Immunology Laboratory at SUNY Downstate (Brooklyn, NY). IgE data were expressed as IU/ml.

Breast milk total IgE levels were detected using IgE ELISA test kits (Hope Diagnostics, Belmont, CA) according to the manufacturer's protocol. Specimens were analyzed in duplicate and a standard curve was derived from known concentrations of IgE. Plates were read using an automated microplate reader (Model Elx800; Bio-Tek Instruments, Winooski, VT). Data were expressed as IU/ml.

For separation of breast milk proteins, frozen breast milk (1 ml) was thawed and centrifuged for 2 min to separate cells and fat. The opaque fluid was centrifuged a second time to remove casein and residual lipids. The clear fluid was removed and saved for protein determination, using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). The clear milk supernatant sample (0.8 ml) was applied to a Sephadex G-100 column (24 cm x 15 mm) (Pharmacia, Uppsala, Sweden) and eluted with 0.01M phosphate buffered saline (PBS), pH 6.7. Collection of 2-min samples was started after 8 ml (void volume). The collected samples were monitored at 280 nm to determine the protein peaks. The protein peaks eluted from the column were pooled, concentrated by lyophilization, and reconstituted with water. The fractions were dialysed against 0.1M Tris-HCl buffered EDTA (Sigma), and frozen at -20°C. The reconstituted fractions were separated by 12% SDS-PAGE. Molecular weight markers were pepsin (34Kd), lysozyme (14.3 Kd), albumin (66Kd), and beta-lactoglobulin (18.2 Kd). The gels were stained overnight with Coomassie brilliant blue G (Sigma) and destained with a methanol-acetic acid-water mixture.

To detect parvovirus B19 antibodies, parvovirus B19 Western blot strips (Mikrogen, Martinsried, Germany) were incubated in wells with sera or breast milk diluted 1:100 in diluting buffer, consisting of washing buffer (0.05% polysorbate 20, 0.01M PBS) and non-fat dry milk (5%), in 1 ml final volume, on a shaker for 20 hr at room temperature. The strips were washed 3 times in washing buffer. Goat polyclonal anti-human IgE (ICN Biomedicals, Aurora, OH), diluted 1:50 or 1:100 in wash buffer, was added to each well and incubated on a shaker for 1 hr at room temperature. The strips were washed 3 times in wash buffer. Rabbit anti-goat peroxidase-labeled antibody (ICN), diluted 1:1000 in washing buffer, was added to each well and incubated on a shaker for 1 hr at room temperature. The strips were washed 3 times in washing buffer, and developed in 1 ml of a 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution. The reaction was stopped by replacing TMB solution with distilled water, and the strips were read, dried, and mounted. In addition, IgG anti-parvovirus B19 was determined using Western blot strips (Mikrogen) according to the manufacturer's protocol.

Results

Immunoglobulin levels in serum and breast milk. Levels of total IgE in breast milk and its fractions were low, and those of maternal and infant serum

Table 1. IgE levels in serum and breast milk of a nursing mother and in serum of her child (female, 10 mo).

Subject	IgM mg/dl	IgG mg/dl	IgA mg/dl	IgE IU/ml
Mother's serum	79	1083	95	18
Breast milk	nd	nd	nd	1
Child's serum	70	473	82	4.3

nd = not detected. Reference ranges for child serum (7-12 *mo*): IgM, 32-155 mg/dl; IgG, 269-913 mg/dl; IgA, 8.0-54 mg/dl. Reference ranges for adult serum: IgM, 47-367 mg/dl; IgG, 648-2045 mg/dl; IgA, 55-375 mg/dl. Reference range for child and adult serum: IgE, 20-100 IU/ml.

were negligible. Total serum levels of maternal IgG were within normal range, and those of infant IgG were low (total IgG in breast milk not studied). Total maternal and infant serum levels of IgM and IgA were within the normal ranges (Table 1).

Fractionation of breast milk proteins. Breast milk was separated into three chromatographic fractions. Fraction #1 contained proteins that were >30 kDa and likely contained albumin (Fig. 1, compare

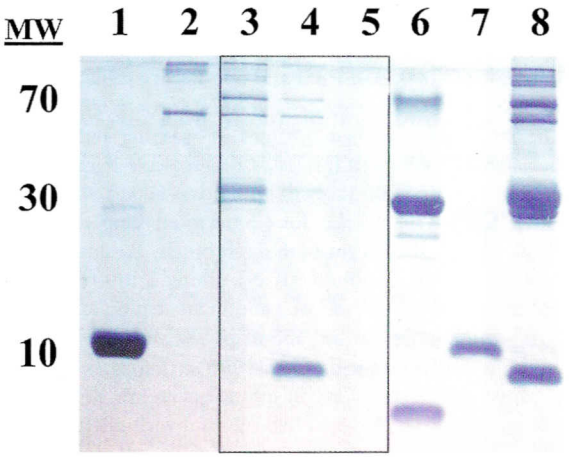

Fig. 1. Analysis of protein fractions of breast milk. Breast milk was separated by Sephadex G-100 chromatography and fractions collected. Three major protein peaks were pooled and analyzed by SDS-PAGE. Lane 1: cytochrome C marker (12 kDa); lane 2: albumin marker (66.5 kDa); lane 3: pool #1; lane 4: pool #2; lane 5: pool #3; lane 6: carbonic anhydrase marker (29 kDa); lane 7: lysozyme marker (14.3 kDa); lane 8: total milk; the box highlights the 3 pooled samples from breast milk.

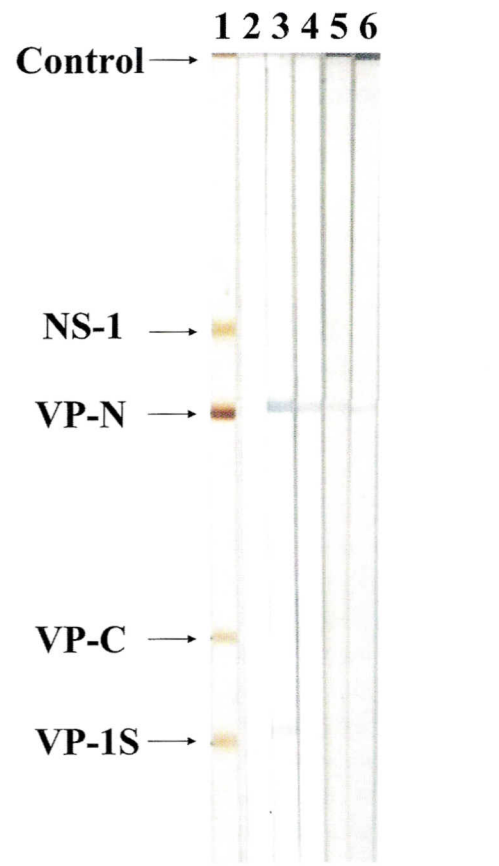

Fig. 2. Western blot analysis of anti-parvovirus B19 antibodies. Serum or breast milk were incubated with Western blot strips containing parvovirus B19 antigens NS-1, VP-N, VP-C, and VP-1S. Lane 1: control strip showing representative bands. Lane 2: serum from a parvovirus B19-negative donor. Lane 3: mother's serum incubated with anti-human IgG (1:1000). Lane 4: mother's serum incubated with anti-human IgE (1:100). Lane 5: mother's breast milk incubated with anti-human IgE (1:20). Lane 6: infant's serum incubated with anti-human IgE (1:100). Control band represents anti-human immunoglobulin.

lanes 2 & 3). Fraction #2 contained residual bands from fraction #1 and a prominent band at ~9 kDa (Fig. 1, lane 4). Fraction #3 contained trace protein bands at ~70 kDa (Fig. 1, lane 5). Unfractionated (total) breast milk contained all of the proteins that were observed in each fraction (Fig. 1, lane 8).

Parvovirus B19 specific antibodies. Maternal sera contained IgG and IgE anti-parvovirus B19 antibodies to VP-N, VP-1S, and trace amounts to VP-C (Fig. 2, lanes 3 and 4, respectively). Both breast milk and infant serum contained IgE anti-parvovirus B19 antibodies to VP-N, even though

the infant was never infected with parvovirus B19 (Fig. 2, lanes 5 and 6, respectively). (The mother had been infected with parvovirus B19 as a child, confirmed by serology.) IgE anti-parvovirus B19 antibodies were not detected in any of the chromatographic fractions of breast milk (data not shown).

Discussion

This is the first report of IgE anti-parvovirus B19 antibodies in human breast milk. IgE has been shown to play a role in viral and bacterial infections. Previous studies in our laboratory demonstrated that IgE is important in parvovirus B19 immunity [7,8], HIV-1 progression [9,10], and spirochete infection [12]. Others have described the presence of virus-specific IgE in several viral infections, such as RSV [18,19], HTLV-1 [20], parainfluenza virus [21], Puumala virus [22], HSV-1, HSV-2, Epstein-Barr virus, and virus-associated encephalopathies [23]. IgE antibodies to bacterial infections, such as Staphylococcus, have been reported in patients with allergies [24], and atopic dermatitis [25]. However, the clinical implications of these IgE anti-viral and anti-bacterial antibodies are yet unknown.

The findings that IgE levels are low in breast milk and breast milk fractions (data not shown), are in agreement with studies of Underdown et al [26], who found little, if any, IgE in breast milk obtained from lactating human mothers who had reported a history of allergy to various common allergens. Duchen and Bjorksten [27] demonstrated that total IgE antibody levels were similar in milk supernatants (colostrum) and blood cells from atopic and non-atopic mothers, albeit the levels were low (median, 0.13 μg/L). However, the authors also reported a strong correlation between total IgE levels in serum and breast milk ($r = 1.0$, $p < 0.001$), suggesting that IgE antibodies might be passively transported from blood into breast milk [27]. Breast milk also contains biologically active molecules that influence the innate immune system, including TGF-beta1, sCD14, IL-10, and IL-12. However these immune factors were not associated with infants' atopic manifestations [28].

In the present study, serum obtained from an atopic mother with low total serum IgE contained IgG and IgE anti-VP-N, anti-VP-1S, and trace amounts of anti-VP-C antibodies. However, the breast milk and the infant's serum both contained IgE anti-VP-N, but not IgE anti-VP-C, anti-NS-1, or anti-VP-1S. These results suggest that IgE anti-parvovirus antibodies in breast milk might be a specific property of breast milk. These antibodies may be immmunoprotective or immunomodulatory for the infant, due to maternal immunity passed through the breast milk. It could be that select maternal IgG or IgE antibodies are passively transferred to the infant via breastmilk and placenta and provide protection against particular viral epitopes. Our assay employed commercially available recombinant versions of parvovirus B19 viral proteins for detection of specific IgE anti-viral antibodies. VP-N represents a common shared component of the 2 alternatively spliced viral shell proteins, VP-1 and VP-2, present in natural parvovirus B19. The VP-N proteins, in addition to the recombinant proximal (VP-1S) and C terminal (VP-C) viral shell proteins and the non-structural (NS-1) proteins of parvovirus, represent linear epitopes on the entire structural protein. IgG antibodies are formed against linear epitopes early in the infection and are later replaced by antibodies that preferentially recognize conformational epitopes after 6-12 mo [29,30]. The specificity of IgE anti-VP-N antibodies, to the exclusion of other epitopes of parvovirus B19, remains to be defined and could be due to preferential recognition of IgE for linear epitopes in a manner distinct from IgG. It is also possible that anti-VP-N antibodies are able to elicit an anti-idiotypic antibody response making this the predominant antibody available for transfer. Similar anti-idiotypic antibody responses to specific antigenic determinants in maternal serum have been described [31].

The lack of observed IgE anti-parvovirus B19 antibodies in fractionated breast milk could be due to IgE remaining on the chromatographic column or possible denaturation of IgE during the fractionation process and thereby being unavailable for assay. In our specific case, the infant was not previously exposed to parvovirus B19 infection. However, Haschke et al [32] showed that healthy

breastfed infants can produce IgG antibodies against cow's milk protein, and in infants at risk for atopic disease specific IgE antibodies were found before cow's milk-based infant formula was introduced to their diets [32]. It is also possible that IgE antibodies specific for microbes or viruses can provide protection from infection. This is supported by studies of Nyindo et al [33] who reported that allergic-type immune responses, particularly IgE, correlate with protective immunity to *Schistosoma mansoni* infection.

The potential biological significance of IgE anti-parvovirus B19 antibodies in breast milk is unclear. Future studies will determine the prevalence and persistence of IgE anti-parvovirus B19 antibodies and their relationship to other immunoglobulins such as IgA. The present finding supports the hypothesis that maternal milk not only contains specific antibodies or idiotypes [34], but also anti-viral immunoregulatory protective factors.

Acknowledgement

Mr. Andrew Nelson, SUNY Downstate Medical Center, Brooklyn, NY, kindly scanned the figures.

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