

Increases of IgA Milk Concentrations Correlate With IgA2 Increment

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IgA, IgA1, and IgA2 concentrations were determined in 81 defatted human milk samples: colostrum (days 1–5, $n = 42$), transitional milk (days 6–14, $n = 18$) and mature milk (days 15–75, $n = 21$) by immunonephelometry. Correlations were found between total IgA levels and the concentrations of both IgA subclasses ($P < 0.0001$). The levels of the three molecules decreased over lactation with significant differences ($P < 0.05$) between colostrum and transitional milk levels and between colostrum and mature milk. Colostral IgA1 and IgA2 mean concentra-

tions dropped respectively from 10.89 ± 2.12 g/L, and 15.41 ± 2.10 g/L to 1.83 ± 0.73 g/L and 3.40 ± 1.25 g/L in transitional milk reaching finally to 0.36 ± 0.07 g/L and 0.27 ± 0.06 g/L in mature milk. IgA2 concentrations were higher than those of IgA1 when the total IgA level was high. The IgA2 levels in colostrum could be an adaptation resistance of IgA to potentially harmful pathogens able to secrete IgA proteases and also a way to regulate colonization of the microflora in the newborn. *J. Clin. Lab. Anal.* 15:55–58, 2001. © 2001 Wiley-Liss, Inc.

Key words: IgA1; IgA2; human milk; lactation

INTRODUCTION

Immunoglobulin A represents the predominant isotype of human milk (1). Dimeric IgA (2), unlike to monomeric serum IgA (3), are synthesized locally in the mammary gland by plasma cells of the lamina propria (4). These cells likely represent the terminal differentiation of B cells activated in the gastrointestinal tract (5). Two IgA subclasses, IgA1 and IgA2, have been described (6) with antigenic, biochemical, and biological distinct properties linked to their structural differences (7). The major difference between IgA1 and IgA2 is a 13-amino-acids deletion of the hinge region in IgA2, which makes them resistant to bacterial IgA1 specific proteases (8). IgA1 and IgA2 display a different distribution in tissues and body fluids (9). IgA1 is the predominant subclass in human serum while IgA2 are more typical of secretory IgA (6). Total IgA level has been measured in human milk in several previous studies (10,11), yet, few reports have described IgA1 and IgA2 concentrations in this fluid (12,13). In these different studies, IgA2 accounts for 22 to 48% of milk IgA. Here we investigated the kinetics of human milk IgA1 and IgA2 levels over lactation using immunonephelometric assays, and compared them to total IgA levels.

MATERIALS AND METHODS

Milk Samples

Human milk samples were collected by 37 volunteer lactating women at the local maternity or at home. A total num-

ber of 81 human milk samples was tested, including 42 colostrum samples obtained between days 1 and 5 postpartum, 18 transitional milk samples collected between days 6 and 14 postpartum, and 21 mature milk samples collected between days 15 and 75 postpartum. All samples were collected approximately midway through a single feeding, aliquoted, frozen immediately at -20°C , and stored until they were assayed. They were thawed at 40°C in a water bath, vigorously homogenized, and precleared by adding (v/v) 1,1,2-trichlorotrifluoroethane (Merck, Darmstadt, Germany). After thorough mixing and centrifugation for 20 min at $4,900g$, assays were carried out using the recovered upper phase of each sample.

Reagents

Antihuman IgA rabbit antiserum and a calibrator for IgA were purchased from Behring (Marburg, Germany). The IgA subclasses standard as well as sheep antihuman IgA1 antiserum and lyophilised microparticle coated with sheep antihuman IgA2 antibodies were obtained from The Binding Site (Birmingham, England). As mentioned by the manufacturer, specificity of anti-IgA1 and IgA2 reagents has been assessed by immunoelectrophoresis.

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Immunonephelometric Assays

Total IgA levels were assayed in immunonephelometry as reported elsewhere (10). For IgA subclass immunoassays, calibration curves were performed with serial dilutions of the calibrator (from 1/20 to 1/320 for IgA1: 8.16–130.55 mg/L and from 1/40 to 1/640 for IgA2: 0.94–15.05 mg/L). All milk and calibrator dilutions were prepared with an automated dilutor (Hamilton, Bonaduz, Switzerland) in 0.01 mol/L phosphate buffer, pH 7.2, containing 0.14 mol/L NaCl. Both IgA1 and IgA2 nephelometric immunoassays were performed in reaction microcuvettes (Nephelia® microcuvette, Sanofi-Diagnostics-Pasteur, Marnes la Coquette, France) by mixing 15 µL of skimmed milk samples diluted according to the total IgA concentration determined previously, or diluted calibrator, with 30 µL of anti-IgA1 or IgA2 reagent and 300 µL of nephelometry buffer (0.05 mol/L borate buffer, pH 8.3, supplemented by 1.5 mmol/L Na₂-EDTA, 0.04 mol/L NaN₃, 2 g/L Triton X100, and 35.26 g/L polyethylene glycol 6000). The scattered light was measured with a nephelometer (Nephelia® N600, Sanofi-Diagnostics-Pasteur) after 30 min incubation at room temperature.

Statistics

All data analyses and statistics were performed using the GraphPad Prism software (GraphPad Software, San Diego CA). Spearman correlations followed by linear regressions were used for the comparison of parameters. The slopes of linear regressions were compared by the Fisher *F*-test. The normality of concentrations distribution was assessed by the Kolmogorov–Smirnov test. When the distribution of data passed the normality test, results were expressed as mean ± SEM. Mean levels in colostrum, and transitional and mature milks were compared using ANOVA followed by the Newman–Keuls multiple comparison post-test. Concentrations of IgA1 and IgA2, in samples of the same period of lactation, were compared by paired Student's *t*-tests. Statistical significance was considered when *P* values were lower than 0.05.

RESULTS

Calibration curves for IgA1 and IgA2 immunonephelometric assays ranged from 0.008 to 0.13 g/L and from 0.0009 to 0.015 g/L respectively. The total IgA concentration was highly corre-

lated ($P < 0.0001$) with the sum of IgA1 and IgA2 as shown in Table 1, yet the latter appeared slightly higher than the total IgA concentration. The concentrations of IgA1 and IgA2 were also strongly correlated to total IgA ($P < 0.0001$). The slopes of linear regressions calculated for IgA1 and IgA2 versus total IgA were significantly different ($P < 0.0001$) with greater concentrations for IgA2 when IgA concentration was high. Similarly, IgA2 and IgA1 concentrations were strongly correlated ($P < 0.0001$) with a slope higher than 1.

The Kolmogorov–Smirnov test, applied to determine the normality of the populations studied when samples were divided into the three stages of lactation, revealed that all populations could be considered normally distributed. Figure 1 shows that IgA, IgA1, and IgA2 levels decrease over lactation. Indeed, IgA mean concentrations were significantly different ($P < 0.01$) between colostrum (19.02 ± 3.11 g/L, $n = 38$) and transitional milk (3.97 ± 1.45 g/L, $n = 12$) and between colostrum and mature milk (1.13 ± 0.19 g/L, $n = 10$). Both IgA1 and IgA2 mean concentrations appeared to be significantly different ($P < 0.05$) between colostrum ($n = 38$ with 10.89 ± 2.12 g/L for IgA1, and 15.41 ± 2.10 g/L for IgA2) and transitional milk ($n = 12$; 1.83 ± 0.73 g/L and 3.40 ± 1.25 g/L) and between colostrum and mature milk, ($n = 10$; 0.36 ± 0.07 g/L and 0.27 ± 0.06 g/L). The comparison of IgA1 and IgA2 mean concentrations for the same stage of lactation revealed significantly higher ($P < 0.05$) IgA2 in colostrum and transitional milk but not in mature milk.

The variation of IgA1 and IgA2 concentrations was followed over 34 days for a mother who displayed very high levels of total IgA in mature milk (Fig. 2). Both subclasses concentration followed the same profile as total IgA. High in colostrum, the concentrations dropped rapidly during the first days, then stabilized until the eighth day before they increased again. IgA2 concentration was higher than that of IgA1 when total IgA was elevated. This was observed not only in colostrum where total IgA concentrations were high but also in mature milk in which total IgA concentration increased.

DISCUSSION

This study reports on the partition of IgA1 and IgA2 among human milk secretory IgA over lactation. Strong correlations

TABLE 1. Correlations and linear regression between parameters

Parameters			Correlation		Linear regression ^d		
<i>Y</i>	<i>X</i>	<i>n</i> ^a	<i>r</i> ^b	<i>P</i> ^c	Intercept	Slope	<i>P</i> ^e
IgA1 + IgA2	IgA	81	0.9684	< 0.0001	-1,4520	1,4990	
IgA1	IgA	81	0.9654	< 0.0001	-0,6264	0,6113	< 0.0001
IgA2	IgA	81	0.9359	< 0.0001	-0,8225	0,8880	
IgA2	IgA1	81	0.9276	< 0.0001	1,2110	1,2590	

^aNumber of paired data.

^bSpearman correlation coefficient between *Y* and *X* parameters.

^cSignificance of *r* against 0.

^dLinear regression $Y = \text{intercept} + \text{slope } X$.

^eSignificance of the different between slopes (Fisher *F*-test).

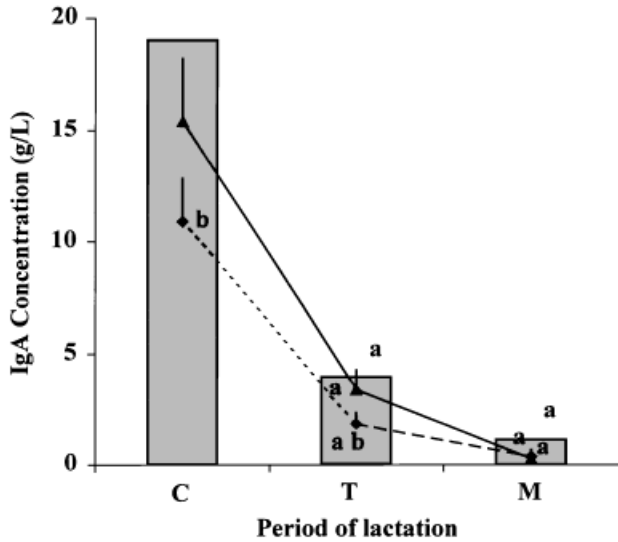


Fig. 1. Changes in IgA, IgA1, and IgA2 concentrations in human milk during lactation. Results are plotted as mean \pm SEM. Shaded bar, total IgA; \blacklozenge , IgA1; \blacktriangle , IgA2. a, concentrations significantly different ($P < 0.05$) vs. colostrum; b, concentrations of IgA1 and IgA2 significantly different ($P < 0.05$) in the same period of lactation.

were noted between the concentrations of the three parameters, and similar kinetics patterns were observed with the highest concentrations in colostrum. The high concentrations

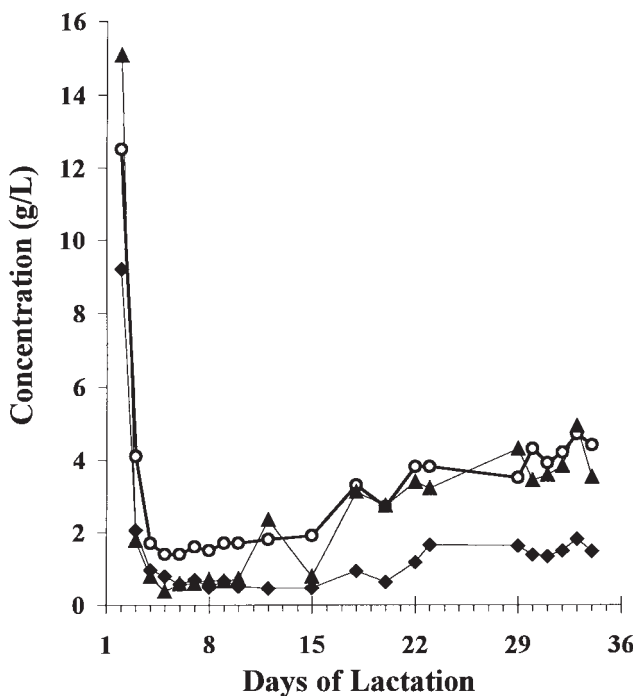


Fig. 2. Kinetics of IgA, IgA1, and IgA2 concentrations in milk samples from one mother with abnormally high levels of total IgA in mature milk. \circ , total IgA; \blacklozenge , IgA1; \blacktriangle , IgA2.

of IgA in colostrum reflect the high density of IgA1 and IgA2 producing cells in the lactating mammary gland (14).

The lower concentration of total IgA compared to the sum of those of IgA1 and IgA2 we obtained is likely to result from the different antisera and calibrators used to measure IgA, IgA1, and IgA2 levels in human milk. Using immunonephelometry, Berth et al. (15), reported the great accuracy of the assay for the determination of IgA1 and IgA2 concentrations in healthy adults' serum with the same anti-IgA subclasses antisera that we used. But they also noticed a discrepancy in the level of total IgA compared to the sum of IgA1 + IgA2.

The high levels of total IgA in colostrum were characterized by a predominance of IgA2. For this reason, we examined sequentially the partition of IgA subclasses in a woman with abnormally high levels of IgA, likely related to breast mastitis (16,17). Interestingly, this increase also involved the predominant secretion of IgA2. The predominance of IgA2 over IgA1 that we observed when total IgA concentrations were elevated can be explained by different hypotheses. First, IgA1 is prone to degradation by bacterial proteases, and this could occur more efficiently through the natural flora of an active mammary gland (18). Another hypothesis could be that IgA1 production is promoted up to a given level of IgA secretion. IgA2 production could then become upregulated and predominant. This is consistent with a report from the literature suggesting independent regulation pathways for IgA1 and IgA2 (19). Preferential homing of cells activated in the gut could also be driven by the luminal and/or cytokinic environment of the mammary gland (20). The feeding mode (breast or formula) also seems important in the colonization of the newborn intestine, with human milk creating an environment favoring the growth of bifidobacteria (21). IgA2 could play a more significant role than IgA1 in promoting a lactic flora. IgA2 have been reported to mediate *E. Coli* agglutination through their abundant mannose side chains accessible to the enterobacteria (22,23). The predominance of IgA2 production by the mammary gland could also be advantageous for mucosal surfaces, as this isotype may avoid inactivation by specific proteases synthesized even by commensal bacteria (24).

CONCLUSION

The measurement of IgA1 and IgA2 by immunonephelometry over lactation reveals that IgA2 is the predominant subclass when total IgA concentrations are elevated, whatever the stage of lactation. This may provide the newborn with a way to regulate its colonic microflora and avoid early infections by IgA1-protease-producing bacteria.

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