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IgE and IgD in Human Colostrum and Plasma

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Summary

Using radioimmunoassay techniques, we measured IgE and IgD levels in paired colostrum and plasma samples obtained within 4 days postpartum. In colostrum, IgE was detected in concentrations of 0.5–6 IU/ml in 16 out of 39 samples (41%) and <0.5 IU/ml in the remainder, whereas IgD was detected in all samples in concentrations of 2–2000 μg/dl. Only a moderate correlation was found between colostral and plasma levels of both IgE ($r = +0.60$) and IgD ($r = +0.74$). The correlation coefficient between IgE and IgD in plasma was 0.23, whereas in colostrum it was only 0.05. The colostrum:plasma ratio of IgE varied strikingly from that of IgD; tbe ratio of IgD was 0.1–22.2 times that of IgE. The findings argue against passive transfer of IgE and IgD from the circulation to milk and suggest possible local mammary production of either or both of these two immunoglobulins.

Speculation

In some patients, local production of IgE and/or IgD probably occurs in the mammary gland. One may hypothesize that previous antigenic exposure at other mucosal surfaces may not only determine the IgA antibody content of milk but also may influence the specificity of antibodies belonging to other immunoglobulin classes that appear in human milk. Whether the IgE and IgD antibodies in breast milk have similar specificities for antigens as the IgA antibodies in milk remains an interesting but unanswered question.

Recent studies of the immunologic composition of human and animal milk have centered on the presence of selected lymphocyte populations in milk (12), migration of lymphoblasts from the gut-associated lymphoid system to the mammary gland (8, 13, 18, 19), and the local production or concentration of immunoglobulins in the mammary gland (11).

Local production of secretory IgA, the principal immunoglobulin of human milk, has been supported by the data of several investigators (5, 11). Studies comparing IgM and IgG antibodies in human milk and serum suggest either a local production or a mechanism of concentrating IgM in the mammary gland. This production or mechanism is not likely for IgG (11). Studies of IgE in milk have been few. Two investigators found either absent or low concentrations of IgE in milk compared with those in serum and concluded that local production or selective secretion by the mammary gland does not take place (16, 17).

Although IgD levels have been examined in milk (7, 15), simultaneous plasma and milk levels have not been compared using a technique as sensitive as radioimmunoassay (RIA). Because of the lack of such information we used RIA to study both IgE and IgD levels in milk and peripheral blood specimens obtained simultaneously from women postpartum. The purpose of our study was to determine whether or not there is evidence to suggest either production or concentration of IgE or IgD in the mammary gland or passive transfer of either of these immunoglobulins from the circulation. By studying both immunoglobulins in paired milk and plasma samples, we obtained data suggesting the local production of one or both in at least some patients.

MATERIALS AND METHODS

Subjects. Women who were hospitalized for delivery were informed of the study. Thirty-nine women, 16–41 years of age, gave informed consent for our obtaining and using breast milk and blood samples. Two volunteers had definite histories of allergy; one had urtcaria from aspirin and from other unknown factors, and the other had asthma and allergic rhinitis. Skin testing for immediate-type hypersensitivity was not a part of the protocol of this study.

Paired samples of breast milk and blood were obtained sometime during the first 4 days after delivery, usually in the morning of postpartum day 1, 2, or 3. Although milk samples were obtained from all 39 women, blood samples were obtained from only 33.

Milk and blood samples. Milk samples, obtained by using a hand pump, were centrifuged at 400 g for 15 min to remove cells and fat.

Blood samples were obtained in a heparinized syringe by venipuncture. Plasma was obtained after centrifuging specimens at 400 X g for 15 min. All specimens were then frozen at −70°C until the assays were performed.

IgE and IgD assays. IgE concentration was measured by a paper disc RIA technique sensitive to 0.5 IU/ml. The procedure, similar to that originally described by Ceska and Lundkvist (6), is described in detail in previous reports (3, 9). The standards from which a standard curve was drawn encompassed a range of 0.1–4200 IU/ml.

IgD concentration was also measured by a paper disc RIA, sensitive to 1 μg/dl. This method has been reported previously (4, 10). The standards used covered a range between 0.25–25,000 μg/dl.

To maximize accuracy, we tested all samples in single assays under identical conditions and duplicated each measurement. The background count was always subtracted and the mean count of the pair of each sample was used for statistical analysis. In no instance did either value deviate from the mean of the pair by more than 8%.

Data analysis. Standard statistical procedures were used for data analysis (2). We transformed the data logarithmically to get the geometric mean and to calculate Pearson's coefficient of correlation. A two-side Student's *t* test was used in comparing two sample mean, using logarithmic values. Analysis of variance was used for comparison of more than two sample means.

RESULTS

IgE. In 13 colostrum samples (33%), IgE concentration was 1–6 IU/ml, with a geometric mean of 2.45 IU/ml. In three samples, IgE concentration was 0.5 IU/ml, and, in the remaining samples, IgE concentration was not detectable (<0.5 IU/ml). Plasma IgE concentrations ranged from 3–1,500 IU/ml, with a geometric mean of 87.7 IU/ml. Data are are presented in Table 1.

A moderate correlation was noted between IgE concentration in colostrum and plasma ($r = +0.60$, $P < 0.001$). When the study group was divided into three subgroups according to colostral IgE concentrations of less than 1, 1–3, and 4–6 IU/ml, the correspond-

Table 1. *IgE and IgD concentrations in colostrum and plasma, and the colostrum:plasma ratios*

Patient no.	IgE IU/ml			IgD μg/dl			
	Colostrum	Plasma	Colostrum / Plasma	Colostrum	Plasma	Colostrum / plasma	IgD colostrum:plasma ratio / IgE colostrum:plasma ratio
1	6.0	1500	0.004	180	5400	0.033	8.3
2[3]	4.6	260	0.018	160	400	0.400	22.2
3	4.0	740	0.005	70	3000	0.023	4.6
4	3.7	620	0.006	34	600	0.057	9.5
5	3.0	640	0.005	80	1400	0.057	11.4
6[3]	3.0	430	0.007	94	1300	0.072	10.3
7	3.0	110	0.027	7	970	0.007	0.3
8	2.6	130	0.020	27	680	0.040	2.0
9	2.0	100	0.020	22	1400	0.016	0.8
10	2.0	10	0.200	10	410	0.024	0.1
11	1.0	480	0.002	60	4700	0.013	6.5
12	1.0	190	0.005	41	4100	0.010	2.0
13	1.0	43	0.023	200	1700	0.118	5.1
14	0.5	90	0.006	8	820	0.010	1.7
15	0.5	3	0.167	3	170	0.018	0.1
16	<0.5	400	NC[2]	2	190	0.011	NC[2]
17	<0.5	240	NC	22	190	0.116	NC
18	<0.5	230	NC	45	620	0.073	NC
19	<0.5	180	NC	220	6400	0.034	NC
20	<0.5	150	NC	8	250	0.032	NC
21	<0.5	120	NC	280	1700	0.165	NC
22	<0.5	92	NC	520	11000	0.047	NC
23	<0.5	80	NC	50	8900	0.006	NC
24	<0.5	74	NC	27	700	0.039	NC
25	<0.5	72	NC	320	2700	0.119	NC
26	<0.5	50	NC	19	450	0.042	NC
27	<0.5	36	NC	58	2100	0.028	NC
28	<0.5	27	NC	9	500	0.018	NC
29	<0.5	13	NC	90	540	0.167	NC
30	<0.5	13	NC	56	1400	0.040	NC
31	<0.5	11	NC	2	10	0.200	NC
32	<0.5	10	NC	82	2100	0.039	NC
33	<0.5	7	NC	14	180	0.078	NC
34	0.5	NA[1]	NA	2000	NA[1]	NA	NC
35	<0.5	NA	NA	190	NA	NA	NC
36	<0.5	NA	NA	70	NA	NA	NC
37	<0.5	NA	NA	62	NA	NA	NC
38	<0.5	NA	NA	56	NA	NA	NC
39	<0.5	NA	NA	14	NA	NA	NC

[1] NA, not available.

[2] NC, not calculated.

[3] Allergy history in 2 subjects: No. 2 (urticaria) and No. 6 (asthma and allergic rhinitis).

ing mean IgE concentrations in plasma were 48.3, 157.8, and 660.8 IU/ml respectively (Fig. 1). Plasma IgE concentrations greater than 200 IU/ml were noted in 10 women, only two of whom had histories of allergy. In our laboratory, the geometric mean concentration for healthy nonpregnant adults is 40 IU/ml. Values greater than 200 IU/ml are greater than 2 standard deviations above the geometric mean and are considered elevated.

IgD. Measurable IgD was present in all 39 colostrum samples. The values had a wide range, 2–2,000 μg/dl, with a geometric mean of 41.3 μg/dl. Seven samples (18%) had less than 10 μg/dl, 26 (67%) had 10–199 μg/dl, and 6 (15%) had 200 μg/dl or more. Plasma IgD concentrations ranged from 10–11,000 μg/dl, with a geometric mean of 953.7 μg/dl (Table 1).

As with IgE, a moderate correlation was also noted between IgD levels in colostrum and plasma ($r = +0.74$, $P < 0.001$). When the study group was divided into three subgroups according to colostral IgD concentrations of less than 10, 10–199, and 200 μg/dl or greater, the corresponding mean plasma IgD values were 229, 1122, and 3548 μg/dl respectively (Fig. 2).

Colostrum: plasma ratios. Of the 26 women with colostral IgE levels less than 1 IU/ml, there were three (11.5%) whose plasma IgE levels were greater than 200 IU/ml, indicating relative or absolute independence of plasma and colostral IgE levels in at least those three. Of the 10 women with colostral IgE levels of 1–3 IU/ml, six had plasma IgE levels of less than 200 IU/ml.

In the 15 with colostral IgE levels in the range of 0.5–6 IU/ml, the colostrum:plasma ratios of IgE varied from 0.002–0.20, with a mean ± S.E. of 0.034 ± 0.016. In the same group the ratios for IgD varied from 0.007–0.4 and had a mean ± S.E. of 0.060 ± 0.026. The variation between the colostrum:plasma ratios for IgE and those for IgD among individual subjects was striking, *i.e.*, the IgD ratio fluctuated widely, being 0.1–22.2 times that of the IgE.

The correlation coefficient between IgE and IgD concentrations in plasma was also strikingly different from that in colostrum (0.23 *versus* 0.05, respectively).

Relationship to postpartum day. Neither colostral IgE nor IgD concentration showed any trend of change during the first 4 days after delivery. The geometric means of colostral IgE on postpartum days 1, 2, and 3 were 0.66, 0.49, and 0.80 IU/ml, and for IgD were 38.3, 41.3, and 21.3 μg/dl, respectively. Analysis of variance revealed no significant difference between days 1, 2, and 3 for either IgE or IgD.

DISCUSSION

Although the three major classes of immunoglobulins—IgG, IgM, and IgA—are present in human milk and colostrum, the preponderant immunoglobulin is secretory IgA (11). Studies examining both total immunoglobulin and specific antibodies have confirmed local production of IgA in the human mammary gland (5, 11). Carlsson *et al.* (5) examined antibodies to *Escherichia coli* antigens in colostrum and milk. The ratio of colostrum antibody to serum antibody was determined for each of the immunoglobulin classes during the wk after delivery. This ratio was always greater than 1 for IgA antibodies, less than 1 for IgG antibodies, and intermediate for IgM antibodies, which suggests some local production of IgM antibodies as well. Although the milk IgA:serum IgA ratio decreased greatly in the first wk postpartum, the decrease in milk IgA concentration was related to an increase in the volume of milk produced. Thus there was a little change in the total IgA secreted into the milk.

Ogra *et al.* (11) compared total immunoglobulin in milk to that in serum. Because the volume of milk changes dramatically during lactation, they compared each immunoglobulin measurement to total protein concentration of the milk and then compared those ratios with serum immunoglobulin:total protein ratios. The data also support local production of IgA in the mammary gland and some local production or concentration of IgM. IgG is present in human colostrum and milk but the quantity in mg per g of total protein changes little during lactation and remains much lower than the serum concentration. During the first few days postpartum, IgA fraction of total milk proteins greatly exceeds that in plasma. As lactation proceeds the IgA fraction in mature milk becomes close to that in plasma. The proportional IgM concentration (mg/g protein) is comparable in colostrum to the serum concentration but decreases to much lower levels in mature milk. Although the techniques used to measure immunoglobulins differed considerably in these studies (5, 11), conclusions were similar, affirming the local production of IgA, some local production of IgM, and little or no local production of IgG.

IgE can be detected in human colostrum and milk. Previous studies have found either small or unmeasurable quantities (16, 17). Our own studies confirm these observations, as IgE was detected in concentrations of 0.5–6 IU/ml in only 41% of the colostrum samples analyzed (16/39) and was undetectable (under 0.5 IU/ml) in the remainder. Case No. 16 is of interest. A high plasma level of IgE (400 IU/dl) was found, yet IgE was not detected in the milk. Such an example militates against a consistent mechanism of passive transfer, as many subjects with low plasma IgE concentrations had easily measurable IgE in milk.

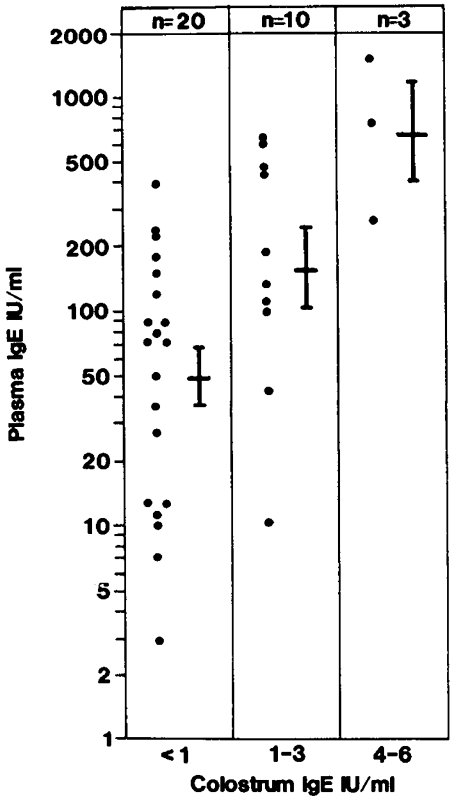

Fig. 1. IgE in colostrum and plasma (distribution and mean ± S.E.).

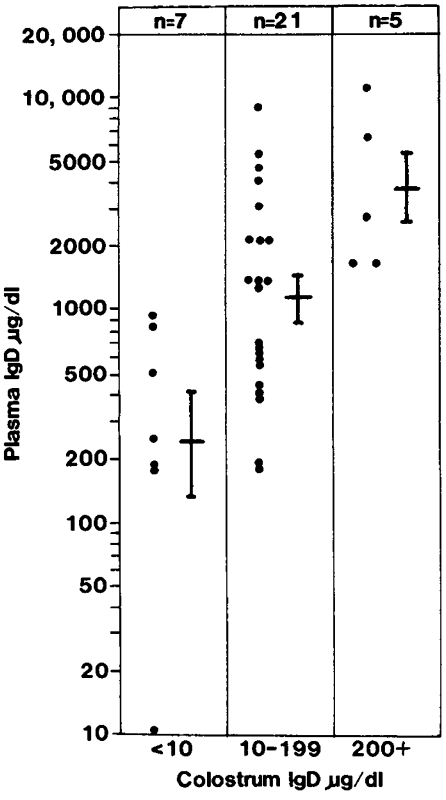

Fig. 2. IgD in colostrum and plasma (distribution and mean ± S.E.).

IgD was detected in all milk samples assayed. Simultaneous measurements of paired plasma specimens revealed a moderate correlation between milk and plasma IgD levels. Although some previous studies demonstrated IgD in milk (7, 15), others were unable to do so (14, 20). No comparisons of simultaneously obtained plasma and milk specimens for IgD content had been made previously by means of radioimmunoassay. The correlation we observed between milk and plasma levels of IgD may indicate that patients with high plasma levels are more likely to passively transfer this immunoglobulin. Alternatively, patients who are genetically programmed to produce high levels of IgD in the plasma may have a similar propensity to produce increased amounts of IgD in the mammary gland. Mammary gland immune system stimulation may at times reflect stimulation of the systemic immune system with increases in antibody at diverse sites, although one would not expect the increases necessarily to be equivalent. The finding of only a moderate degree of correlation between colostral and plasma levels of IgE ($r = 0.6$) and of IgD ($r = 0.7$) raises the question of possible local production of these two immunoglobulins by the mammary gland. In support of this possibility is the finding of a very low correlation coefficient between IgE and IgD concentrations in colostrum ($r = 0.05$) as compared with that in plasma ($r = 0.23$).

The wide variation in milk:plasma ratios of IgE and IgD individual subjects provides additional information. In case No. 2, the milk:plasma ratio of IgD was 22 times greater than the same ratio for IgE. In case No. 15, on the other hand, the IgE ratio was nine times greater than the IgD ratio. If either IgE or IgD was present in milk exclusively as a result of passive transfer from the

plasma, one would anticipate similar milk:plasma ratios. This, however, was not the case. The wide variation in ratios argues strongly for local production of one or both of these classes of immunoglobulin in at least some persons. Local mammary production of IgA varies in individual persons depending on previous antigenic stimulation of the gut mucosal immune system, and it involves homing of lymphocytes to the mammary gland (1, 8). It is not known whether a similar mechanism may also operate for IgE or IgD.

The varying milk:plasma ratios of IgE and IgD observed in the present study are most readily explained by local production of IgD in some subjects, local production of IgE in others, or by local production of both immunoglobulins in differing amounts in some or in all subjects. Differing milk:plasma ratios of IgE and IgD in individual subjects suggest independent mammary secretion of these two immunoglobulins. The only alternative would be extremely differing passive transfer or local concentrating mechanisms. It is certain from the data presented that considerable individual variability exists. Research on mucosal immunoglobulins to date suggests that IgA plasmablasts migrate from the gastrointestinal tract to the mammary gland (13, 19). It seems likely that stimulation of distant mucosal lymph nodes, along with genetic factors regulating the immune responses, will determine for a particular individual the content of IgE and IgD in her milk. IgE antibodies in breast milk could readily contribute to the infants' defense mechanisms by combining with antigens in the gut lumen, causing local release of chemical mediators, leading in turn to increased vascular permeability, and thereby permitting egress of IgG antibodies from the blood (largely derived originally by transplacental passage from the mother) to inactivate the antigen (*e.g.*, bacteria, virus, parasite) and speed its removal. The potential role of IgD antibodies in breast milk is less well understood but no less intriguing. It would be interesting to look at specific antibodies of the IgE and IgD classes in milk. This could not be done in our series because only two subjects gave a history of allergy; one had urticaria from aspirin and from other unknown factors, and the second was not sure of the precipitating agents of her respiratory allergies. Also none of this series underwent allergy skin testing. Studying a group of parturient women with known allergies would be most helpful in looking at specific antibodies in the circulation and milk.

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