

Detection of IgA antibodies to cat, β -lactoglobulin, and ovalbumin allergens in human milk

Rosaura Casas, MSc, Malin F. Böttcher, MSc, Karel Duchén, MD, PhD, and Bengt Björkstén MD, PhD *Linköping, Sweden*

Background: The relationship between the development of allergy during infancy and breast-feeding remains controversial. This controversy may be due to individual variations in the composition of human milk. Antibodies to food antigens to which the mother is commonly exposed are present in the milk, but their relationship to allergy is still unknown. IgA antibodies to inhalant allergens have not been previously detected.

Objective: Our purpose was to analyze secretory IgA antibody levels to cat, β -lactoglobulin, and ovalbumin allergens in colostrum and mature milk in relation to maternal allergy.

Methods: Colostrum and samples of mature milk were obtained after 1 and 3 months of lactation from 53 nursing mothers (17 allergic and 36 nonallergic mothers) and were analyzed for total secretory IgA levels by ELISA and secretory IgA antibodies to cat, β -lactoglobulin, and ovalbumin by an enzyme-amplified ELISA. The specificity of the assays was confirmed by inhibition experiments.

Results: Secretory IgA to cat, β -lactoglobulin, and ovalbumin allergens were detected in colostrum as well as mature milk. The levels of secretory IgA to ovalbumin were lower in colostrum from allergic mothers with $P = .016$, whereas the levels to β -lactoglobulin and cat were similar in the 2 groups. IgA antibodies to ovalbumin were detected in 94% of the colostrum samples from allergic and in all samples from non-allergic mothers, in 82% and 96%, respectively at 1 month, and 53% and 65% at 3 months. Fewer samples had detectable secretory IgA antibodies to β -lactoglobulin than to ovalbumin and cat, and only 33% and 10% of the samples from the allergic and nonallergic mothers, respectively, remained positive at 3 months. All the allergic mothers had detectable IgA to cat in colostrum, whereas 83% and 73% of the samples were positive at 1 and 3 months. The corresponding numbers were 93%, 81%, and 81% in the nonallergic mothers (not significant).

Conclusion: Even a low level of exposure of the mucosa (eg, by

inhalant allergens) can induce antibody secretion into the milk, both in allergic and nonallergic mothers. (*J Allergy Clin Immunol* 2000;105:1236-40.)

Key words: Human milk, breast-feeding, antibodies, IgA, cat, β -lactoglobulin, ovalbumin, ELISA, allergens

Several studies have shown that breast-feeding not only provides the nutritional requirements of the infant but is also important for the transfer of immunologic information from the mother to the offspring.¹ Human milk contains several components that may protect the child against infections. It also contains various factors that may enhance the maturation of the immune system. Furthermore, the maternal immunity is an environmental factor that may influence the allergy risk in children.²⁻⁴ The relationship between the appearance of allergy during infancy and breast-feeding is still unclear. Some studies have suggested that breast-feeding prevents, or at least delays, the development of allergic diseases, whereas other studies have indicated that this is not the case.⁵

Human milk contains secretory IgA (sIgA) antibodies against food that the mother eats,^{6,7} but their relationship to maternal allergy is unknown. The mother is thus an environmental factor in early infancy, and individual variations in the antibody levels in human milk could conceivably affect the breast-fed infant. It has been reported that low levels of breast milk IgA antibodies against allergens represent a risk in the development of allergic diseases in children,^{3,8,9} although the relationship is controversial.⁷

Exposure to various food antigens does not influence to a major extent the levels of specific IgA antibodies in breast milk. For instance, manipulation of the maternal diet did not alter the levels of food-specific IgA in breast milk.⁷ Similar studies of IgA antibodies to inhalant allergens have not been performed.

The current study was undertaken to analyze the presence of IgA antibody levels to cat, β -lactoglobulin (BLG), and ovalbumin (OVA) in colostrum and mature milk in relation to maternal allergy with the hypothesis that low levels of secretory antibodies could be associated with allergy.

MATERIAL AND METHODS

Milk samples

Samples from 53 nursing mothers (17 allergic and 36 nonallergic mothers), participating with their infants in a prospective study, were

From the Division of Pediatrics, Department of Health and Environment, Faculty of Health Sciences, Linköping, Sweden.

Supported by the Swedish Institute, the National Health and Lung Association, the National Association for the Prevention of Asthma and Allergies, the Vårdal Foundation, and the Swedish Medical Research Council (grant No. 7510).

Received for publication Mar 29, 1999; revised Jan 18, 2000; accepted for publication Jan 18, 2000.

Reprint requests: Rosaura Casas, MSc, Department of Health and Environment, Division of Pediatrics, Faculty of Health Sciences, S-581 85 Linköping, Sweden.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/105805

doi:10.1067/mai.2000.105805

Abbreviations used

AU:	Arbitrary units
BLG:	β -Lactoglobulin
HSA:	Human serum albumin
OVA:	Ovalbumin
PHT:	PBS with Tween 0.05% and HSA 0.5%
sIgA:	Secretory IgA

selected on the basis of the availability of both colostrum and mature milk samples. The allergic status of the mothers was established by a clinical history and the presence of circulating IgE antibodies to a panel of inhalant allergens as measured by an Allergy Screen (Magic Lite SQ Allergy System, ALK, Hørsholm, Denmark).

Colostrum was collected within 4 days post partum, and mature milk at 1 and 3 months with use of a manual breast pump, pumping into sterile tubes, and immediately frozen at -20°C until processed. The samples were centrifuged at $13,000g$ for 15 minutes to remove the fatty layer and cellular components. The supernatants were then centrifuged at $29,000g$ for 20 minutes to 5°C and stored at -20°C until analysis.

A questionnaire was used to assess the mothers' dietary intake of milk, milk products, and egg, as well as exposure to cat.

Antibody analyses

sIgA antibody levels. A conventional ELISA was used to measure total sIgA levels in the breast milk. High-binding Costar 3590 plates were coated with $200\ \mu\text{L}$ per well of polyclonal rabbit antihuman secretory component antibody (DakoPatts, Stockholm, Sweden) diluted 1:500 in PBS and incubated for 2 hours in a moist chamber at 37°C and then for at least 3 hours at 4°C . After being washed 4 times with a washing buffer (PBS-Tween 0.05%), the plates were blocked with $200\ \mu\text{L}$ per well of 0.5% human serum albumin (HSA) in PBS and incubated in a moist chamber for 2 hours at 37°C and then overnight at 4°C . The incubations were performed in a moist chamber at 37°C for 1 hour unless otherwise stated. After being washed, $100\ \mu\text{L}$ per well of each standard, milk samples diluted 1:8000 to 1:32,000 in PBS-Tween 0.05% and HSA 0.5% (PHT), or for controls PHT only, were added to the wells in duplicate. Human IgA (Sigma-Aldrich, Stockholm, Sweden) diluted 2-fold (range 16–1000 ng/mL) in PHT was used as standard. The plates were washed and incubated with $100\ \mu\text{L}$ per well of monoclonal antihuman IgA (clone 2D7 Bio Zac AB, Järfälla, Sweden) diluted 1:2000 in PHT and subsequently, after washing, incubated with $100\ \mu\text{L}$ per well of alkaline phosphatase-conjugated rabbit antimouse antibody (Sigma-Aldrich) diluted 1:5000 in PHT. This was followed by a last wash. To develop a reaction, $200\ \mu\text{L}$ per well of *p*-nitrophenyl phosphate (FAST pNPP) (Sigma-Aldrich) was used as substrate. The reaction was stopped after 30 minutes by adding $50\ \mu\text{L}$ per well of 3 mol/L sodium hydroxide. The OD was read at 405 nm. The ODs of the controls were subtracted from the standard and sample values.

Specific IgA antibody levels. An enzyme amplification system was used to increase the sensitivity of the assay for detection of sIgA antibodies to cat, OVA, and BLG.

Costar 5390 microplates (Life Technologies AB) were coated with $100\ \mu\text{L}$ of 10 $\mu\text{g/mL}$ cat allergen extract (ALK, Hørsholm, Denmark), $200\ \mu\text{L}$ of 10 $\mu\text{g/mL}$ BLG (Sigma-Aldrich) or $200\ \mu\text{L}$ of 25 $\mu\text{g/mL}$ OVA purified grade IV (Sigma-Aldrich) in PBS. The plates were incu-

bated in a moist chamber at 37°C for 2 hours and then for at least 3 hours at 4°C . After aspiration, the plates were washed 4 times with a washing buffer (PBS-Tween 0.05%). Further washing steps were included between the incubations until the substrate was added. The free binding sites at the wells were blocked with 2% HSA in PBS, $250\ \mu\text{L}$ for cat allergen and $200\ \mu\text{L}$ for BLG and OVA, at 37°C for 2 hours, and then overnight at 4°C . Human milk samples diluted 1:25 in PBS were added in duplicate. Samples were also applied in duplicate to uncoated rows, blocked with HSA in PBS as individual controls. Duplicate wells with only PHT were used as blanks. The monoclonal antihuman IgA clone 2D7 (Bio Zac AB, Järfälla, Sweden) diluted 1:2000 in PHT was added as detecting antibody, followed by alkaline phosphatase-conjugated rabbit antimouse IgG (Sigma-Aldrich) diluted 1:40,000 for antibodies to cat and 1:20,000 for antibodies to BLG and OVA in AMPAK wash buffer and HSA 0.5%. AMPAK kits (Dakopatts AB, Älvsjö, Sweden) were used for the substrate reaction. After incubation with the conjugate, the plates were washed with AMPAK washing buffer. The plates were then incubated for 20 minutes with reduced nicotinamide adenine dinucleotide phosphate in diethanolamine buffer and for 10 minutes with the amplifier solution. The reaction was stopped by adding $50\ \mu\text{L}$ per well of 0.46 mol/L sulfuric acid. The OD was measured at 492 nm. A reference curve was prepared with use of a sample with high specific IgA levels to the different antigens and low background at the different dilutions. The antibody levels in the samples were calculated from the OD of the reference curve and expressed as arbitrary units (AU), after subtracting the OD of the blanks and the OD values for the individual controls. The specificity of the system was confirmed by pilot experiments, using an antihuman IgG antibody in the detection step. Also, several monoclonal antihuman IgA antibodies were tested in preliminary experiments before one of them was selected for the study.

The specificity of the methods was confirmed with the respective antigens. Breast milk samples with high levels of IgA to cat were incubated for 1 hour at 37°C with serial dilutions (400, 100, 25, 6.2, and 1.3 $\mu\text{g/mL}$ of freeze-dried material) of cat allergen; *Betula verrucosa* (ALK-Abelló, Denmark), *Dermatophagoides pteronyssinus*, and *Blomia tropicalis* allergens (BioCen, Havana, Cuba); and BLG and egg antigens (Sigma-Aldrich). The specificity of the BLG and OVA assays was confirmed by incubation of milk samples overnight at 4°C with 1, 10, and 100 $\mu\text{g/mL}$ of OVA and BLG in PBS. The samples were then analyzed as described above for IgA antibodies to cat, BLG, and OVA.

Statistical analysis

Because the antibody levels were not normally distributed, paired analyses were performed with the Wilcoxon signed-rank test, unpaired analyses with the Mann-Whitney *U* test, and correlations with Spearman's rank order correlation coefficient test. The Bonferroni correction was applied to discard a Type I error. A probability level of $<5\%$ was considered to be statistically significant. The calculations were made with the statistical package StatView 4.5 for Macintosh (Abacus Concepts Berkeley, Calif).

Ethical aspects

The Regional Ethics Committee approved the study for Human Research, Linköping University Hospital, Sweden.

RESULTS

Optimal conditions for the detection of total and specific secretory IgA antibodies to cat, BLG, and OVA by

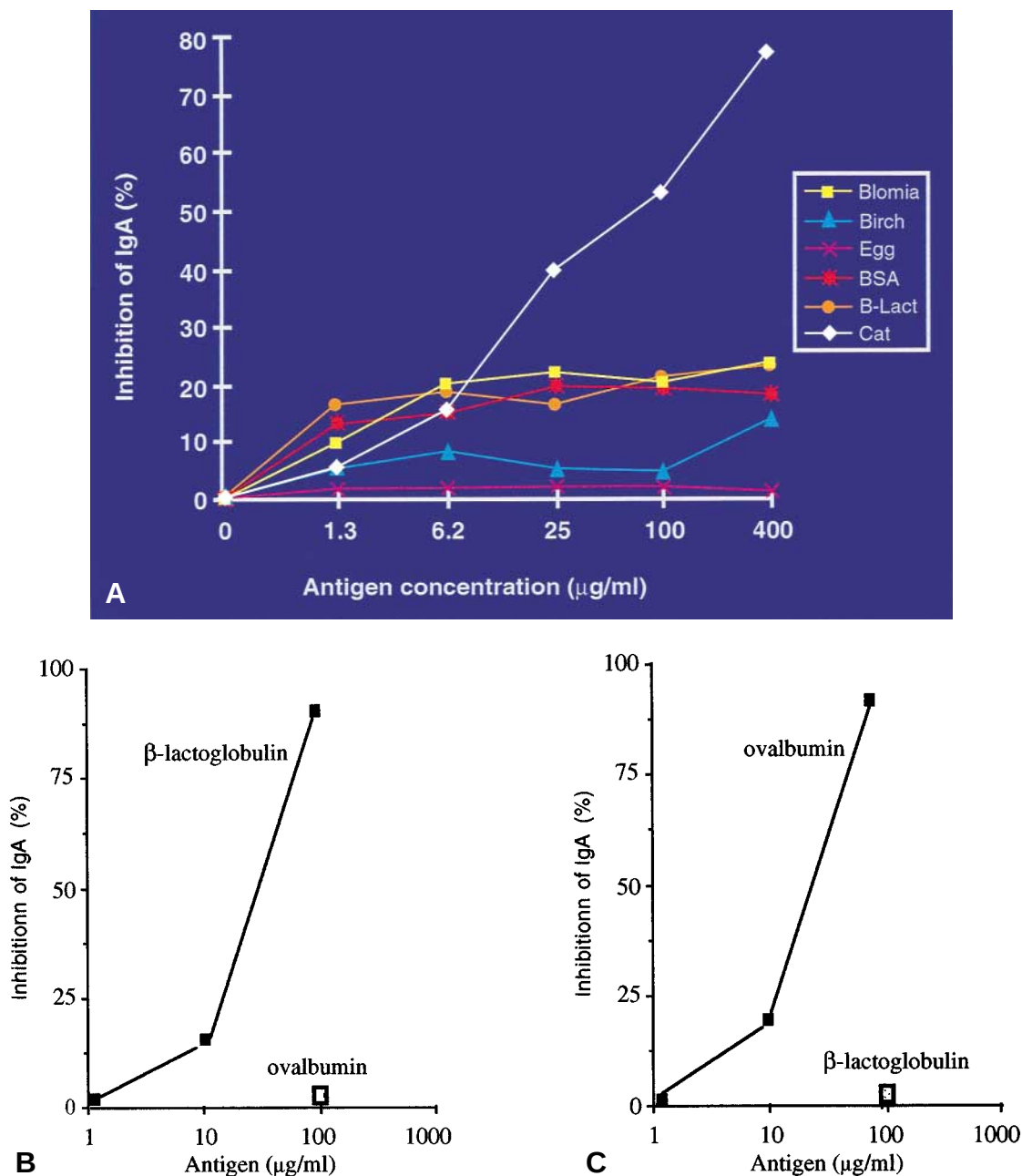


FIG 1. Inhibition of ELISA for detection of human specific IgA antibodies in human milk against (A) cat, (B) BLG, (C) OVA by different antigens.

ELISA were established. The specificity of the assays was confirmed by ELISA inhibition (Fig 1).

sIgA antibodies to cat, BLG, and OVA were detected in milk from both allergic and nonallergic mothers, and the highest levels were recorded in colostrum (Table I). There was a strong correlation between the milk levels of specific IgA throughout the lactation period (eg, between colostrum and 1 month [$r = 0.8, 0.6$, and 0.8 for cat, BLG, and OVA, respectively; $P < .001$ for all] and levels at 1 and 3 months [$r = 0.6, 0.6$, and 0.7 for cat, BLG, and OVA, respectively; $P < .001$ for all]). Between colostrum

and 3-month samples a strong correlation was found for cat and BLG antibodies ($r = 0.6, P < .001$) and a lower correlation for BLG antibodies ($r = 0.5, P < .01$).

All the allergic mothers had detectable IgA to cat in colostrum, whereas 83% and 73% of the samples were positive at 1 and 3 months. The corresponding figures in the nonallergic mothers were 93%, 81%, and 81% (not significant). IgA antibodies to OVA were detected in 94% of the colostrum samples from allergic mothers and in all samples from nonallergic mothers, in 82% and 96% respectively, at 1 month and 53% and 65% at 3 months.

TABLE I. Number of samples (n = positives/total) and levels of sIgA to cat, BLG, and OVA, expressed in AU, in colostrum and mature milk obtained at 1 and 3 months from allergic and nonallergic mothers

	Allergics			Nonallergics		
	No.	Median	Range	No.	Median	Range
IgA to cat						
Colostrum	17/17	77	22-793	33/36	101	5-553
1 mo	14/17	37	5-121	26/32	30	5-134
3 mo	11/15	24	5-64	25/31	23	5-128
IgA to BLG						
Colostrum	9/17	16	8-183	18/36	19	8-9492
1 mo	3/17	8	8-50	4/32	8	8-50
3 mo	5/15	8	8-32	3/31	8	8-53
IgA to OVA						
Colostrum	16/17	23	4-557	36/36	39	8-1498
1 mo	14/17	14	4-88	31/32	27	4-732
3 mo	8/15	8	4-70	20/31	11	4-184

Fewer samples had detectable sIgA antibodies to BLG than to OVA and cat, and only 33% and 10% of the samples from the allergic and nonallergic mothers, respectively, remained positive at 3 months.

The levels of antibodies to OVA were lower in colostrum with $P = .016$ ($P < .017$ with Bonferroni correction) but not mature milk of allergic compared with nonallergic mothers. Only 4 of 51 mothers avoided egg, and 16 reported egg intake at least once a week and 31 once or twice a month. The sIgA antibody levels to OVA were independent of egg consumption.

In contrast, the IgA antibody levels to BLG were similar in allergic and nonallergic mothers through the lactation period. Most mothers (42/51) reported a daily intake of milk products, and the remaining 9 consumed milk products at least once a week. The levels of sIgA to BLG were not affected by milk consumption.

The sIgA antibody levels to cat were similar in milk from allergic and nonallergic mothers. The levels were also similar in mothers who were exposed to cat at home or outside the home (24/52) compared with mothers with no obvious exposure (28/52).

DISCUSSION

Low levels of IgA to OVA and cat allergens, but not to BLG, were commonly detected in human milk. In previous studies IgA antibodies to casein and BLG have been detected in all¹⁰ or most of the samples.³ We could not confirm these reports, even with use of a highly sensitive ELISA to detect antibodies against BLG. Thus only half the colostrum samples and even fewer of the mature milk samples were positive for IgA antibodies to BLG. The differences could be explained by the specificity of the system used by us because there is a high background binding of breast milk samples. Furthermore, the backgrounds vary considerably for different samples. If not corrected for, this may produce falsely positive results.

The levels of total IgA were similar in the allergic and nonallergic mothers,¹⁰ and the levels were higher in colostrum than in mature milk, as reported previously.^{8,11}

IgA antibodies to cat were present in most colostrum samples and also in several of the mature milk samples, both from allergic and nonallergic mothers. Furthermore, the levels were similar in milk from mothers who had or had not been in contact with cats. Cat allergen is ubiquitous in the Swedish environment, even in places where cats are not allowed.^{12,13} It is therefore difficult to assess the effects of a true lack of continuous exposure.

The levels of sIgA antibodies to OVA in colostrum were higher in nonallergic than in allergic mothers, supporting a previous report with another food antigen (ie, BLG¹⁴). Some of the mothers in our study did not have a regular intake of egg or milk products. However, because of the extensive use of egg and milk in food products, avoidance of these products is almost impossible. In contrast, similar levels of breast milk antibodies to BLG and OVA in atopic and nonatopic mothers were reported in another study.⁹ Many of the mothers in that study actively avoided milk and egg and were thus less exposed to the antigens.

The higher levels of antibodies against OVA but not BLG in nonallergic compared with allergic mothers are possibly explained by the higher antigenicity of the former antigen and less exposure. This possibility is supported by previous reports of higher levels of IgG antibodies to OVA than to BLG in sera from children^{15,16} and by the higher prevalence of sensitization to egg white than to cow's milk.¹⁷

Although the immune response to allergens is already primed before birth,¹⁸⁻²⁰ the postnatal environment is crucial for the type of immunity that will be established after infancy. The breast milk is part of the environment to which the baby is exposed^{3,21,22} and the composition partly depends on maternal immunity. For example, the levels of transforming growth factor- β , IL-10, and IL-6 in breast milk correlate with total sIgA levels.²³ These cytokines regulate IgA synthesis²⁴⁻²⁶ and they also regulate IgE antibody production.²⁷⁻²⁹ It is therefore tempting to speculate that the varying levels of these cytokines among allergic and nonallergic mothers could explain the different levels of OVA-specific IgA levels in the breast milk.

It has been suggested that sIgA in the gastrointestinal

tract may prevent the penetration and absorption of foreign antigens and that the absence of IgA antibodies in human milk against major allergens could be a risk factor for infant allergy.³ Furthermore, low levels of total colostrum IgA antibodies³ and IgA to BLG¹⁰ were associated with cow's milk allergy. We could not confirm this in an 18-month follow-up of the infants of the mothers in this study.³⁰

In conclusion, secretory IgA antibodies to cat were commonly detected in human milk and the levels were similar in allergic and nonallergic mothers. Thus even a fairly low exposure of the maternal mucosa (eg, by inhalant allergens) can induce antibody secretion into the milk. In contrast, allergic compared with nonallergic mothers had lower levels of IgA antibodies to OVA in colostrum, whereas the antibody levels to BLG were similar in the 2 groups. Differences in mode of antigen exposure and allergenicity may possibly explain the differences.

We thank Kerstin Hagersten and Ulrika Leek for their technical assistance and Ing-Marie Sandberg and Lena Lindell for the helpful assistance with the breast milk samples.

REFERENCES

- Goldman AS, Goldblum RM. Defence agents in human milk. In: Jensen RG, editor. Handbook of milk composition. San Diego: Academic Press; 1995. p 727-45.
- Björkstén B. Environmental risk factors for atopy. Vol 4. Berlin: Springer-Verlag; 1997.
- Machtinger S, Moss R. Cow's milk allergy in breast-fed infants: the role of allergen and maternal secretory IgA antibody. *J Allergy Clin Immunol* 1986;17:341-7.
- Hanson L, Jalil F, Ashraf R, Bernini S, Carlsson B, Cruz JR, et al. Characteristics of human milk antibodies and their effect in relation to the epidemiology of breast-feeding and infections in a developing country. *Adv Exp Med Biol* 1991;310:1-15.
- Björkstén B. Early immune responses to allergens. *Int Arch Allergy Immunol* 1997;113:80-2.
- Lilja G, Dannaeus A, Fälth-Magnusson K, Graff Lönnevig V, Johansson SGO, Kjellman N-IM, et al. Immune response of the atopic women and foetus: effects of high- and low-dose food allergen intake during late pregnancy. *Clin Allergy* 1988;18:131-42.
- Fälth-Magnusson K, Öman H, Kjellman N-IM. Maternal abstention from cow milk and egg in allergy risk pregnancies: effect on antibody production in the mother and the new-born. *Allergy* 1987;42:64-73.
- Savilahti E, Tainio VM, Salmenperä L, Arjomaa P, Kallio M, Perheentupa J, et al. Low colostral IgA associated with cow's milk allergy. *Acta Paediatr Scand* 1991;80:1207-13.
- Fälth-Magnusson K. Breast milk antibodies to foods in relation to maternal diet, maternal atopy and the development of atopic disease in the baby. *Int Arch Allergy App Immunol* 1989;90:297-300.
- Savilahti E, Tainio VM, Salmenperä L, Arjomaa P, Kallio M, Perheentupa J, et al. Levels of IgA and cow milk antibodies in breast milk vs the development of atopy in children: low colostral IgA associated with cow milk allergy. *Adv Exp Med Biol* 1991;310:417-25.
- Ladjeva I, Peterman JH, Mestecky J. IgA subclasses of human colostral antibodies specific for microbial and food antigens. *Clin Exp Immunol* 1989;78:85-90.
- Munir AK, Björkstén B, Einarsson R, Schou C, Ekstrand Tobin A, Warner A, et al. Cat (Fel d 1), dog (Can f 1), and cockroach allergens in homes of asthmatic children from three climatic zones in Sweden. *Allergy* 1994;49:508-16.
- Berge M, Munir A, Dreborg S. Concentrations of cat (Fel d 1), dog (Can f 1) and mite (Der f 1 and Der p 1) allergens in the clothing and school environment of Swedish school children with and without pets at home. *Podiatr Allergy Immunol* 1998;9:25-30.
- Renz H, Vestner R, Petzoldt S, Brehler C, Prinz H, Rieger CHL. Elevated concentrations of salivary secretory IgA anti cow's milk protein in new-borns at risk of allergy. *Int Arch Allergy Appl Immunol* 1990;92:247-53.
- Jennmalm MC, Björkstén B. Development of IgG subclass antibodies to ovalbumin, birch and cat during the first eight years of life in atopic and nonatopic children. *Pediatr Allergy Immunol* 1999;10:112-21.
- Hattevig G, Kjellman B, Sigurs N, Grodzinsky E, Hed H, Björkstén B. The effect of maternal avoidance of eggs, cow's milk and fish during lactation on the development of IgE, IgG and IgA antibodies in infants. *J Allergy Clin Immunol* 1990;85:108-15.
- Nilsson L. Risk factors for atopic disease in childhood [dissertation]. Linköping: Linköping University; 1998.
- Jones CA, Kilburn SA, Warner JA, Warner JO. Intrauterine environment and foetal allergic sensitization. *Clin Exp Allergy* 1998;28:655-9.
- Brown MA, Halonen MJ, Martínez F. Cutting the cord: is birth already too late for primary prevention of allergy? *Clin Exp Allergy* 1997;27:4-6.
- Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730-7.
- Björkstén B. Risk factor in early childhood for the development of atopic diseases. *Allergy* 1994;49:400-7.
- Hanson LA, Ahlstedt S, Carlsson B. Secretory IgA antibodies against cow's milk protein in human milk and their possible effect in mixed feeding. *Int Arch Appl Immunol* 1977;54:457-62.
- Fagerås-Böttcher M, Jennmalm MC, Garofalo RP, Björkstén B. Cytokines in breast milk from allergic and non-allergic mothers. *Pediatr Res* 2000;47:157-62.
- van Vlaessler P, Punnonen J, de Vries JE. Transforming growth factor- β directs IgA switching in human B cells. *J Immunol* 1992;148:2062-7.
- Fujihashi K, McGhee JR, Lue C, Beagley KW, Taga T, Hirano T, et al. Human appendix B cells naturally express receptors for and respond to interleukin 6 with selective IgA1 and IgA2 synthesis. *J Clin Invest* 1991;88:248-52.
- Defrance T, Vanbervliet B, Brière F, Durand I, Rousset F, Banchereau J. Interleukin 10 and transforming growth factor β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J Exp Med* 1992;175:671-82.
- Vercelli D, Jabara HH, Arai KI, Yokota T, Geha RS. Endogenous interleukin-6 plays an obligatory role in interleukin-4-dependent human IgE synthesis. *Eur Immunol* 1989;19:1419-24.
- Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* 1993;90:3730-4.
- Wu CY, Brinkmann V, Cox D, Heusser C, Delespesse G. Modulation of human IgE synthesis by transforming growth factor- β . *Clin Immunol Immunopathol* 1992;62:277-84.
- Duchén K, Casas R, Fagerås-Böttcher M, Yu G, Björkstén B. Human milk polyunsaturated long chain fatty acids (PUFA) and S-IgA antibodies and early childhood allergy. *Pediatr Allergy Immunol*. 2000;11. In press.