Intrauterine sensitization of allergen-specific IgE analyzed by a highly sensitive new allergen microarray

Norio Kamemura, MSc,[a] Hitomi Tada, MSc,[a] Naoki Shimojo, MD, PhD,[b] Yoshinori Morita, MD, PhD,[b] Yoichi Kohno, MD, PhD,[b] Takao Ichioka, MD,[c] Koichi Suzuki, MSc,[a] Kenji Kubota, MSc,[a] Mineyoshi Hiyoshi, PhD,[a] and Hiroshi Kido, MD, PhD[a] *Tokushima, Chiba, and Naruto, Japan*

Background: To design a rational allergy prevention program, it is important to determine whether allergic sensitization starts *in utero* under the maternal immune system.
Objective: To investigate the origin of allergen-specific IgE antibodies in cord blood (CB) and maternofetal transfer of immunoglobulins.
Methods: The levels of food and inhalant allergen-specific IgE, IgA, IgG, and IgG_4 antibodies in CB and maternal blood (MB) from 92 paired neonates and mothers were measured by using a novel allergen microarray of diamond-like-carbon–coated chip, with high-sensitivity detection of allergen-specific antibodies and allergen profiles.
Results: The levels of allergen-specific IgE antibodies against food and inhalant allergens and allergen profiles were identical in CB and newborn blood, but the levels and profiles, specifically against inhalant allergens, were different from those in MB. The level of allergen-specific IgA antibodies was below the detection levels in CB despite clear detection in MB. Therefore, contamination with MB in CB was excluded on the basis of extremely low levels of IgA antibodies in CB and the obvious mismatch of the allergen-specific IgE and IgA profiles between CB and MB. However, the levels of allergen-specific IgG and IgG_4 antibodies and their allergen profiles were almost identical in both MB and CB.
Conclusion: Allergen-specific levels of IgE and IgA antibodies and their allergen profiles analyzed by the diamond-like-carbon allergen chip indicate that IgE antibodies in CB are of fetal origin. Food-allergen specific IgE antibodies were detected more often than inhalant-allergen specific IgE antibodies in CB, the reason of which remains unclarified. (J Allergy Clin Immunol 2012;130:113-21.)

Key words: *Prenatal, allergen-specific IgE, IgA, IgG, and IgG_4, sensitization, cord blood, allergen chip*

Newborns sometimes show measurable amounts of IgE antibodies in cord blood (CB), and a relatively high level of total IgE is often regarded a prenatal risk factor for atopic propensity in the newborn.[1-5] The latter conclusion is supported by the detection of allergen-specific IgE[6,7] and allergen-specific T-cell memory[8-10] in CB and suggests that primary sensitization can occur transplacentally *in utero*. However, the timing of allergen sensitization is still controversial, with conflicting evidence suggesting transplacental priming[6] versus postnatal priming.[11,12]

These conflicting conclusions could be due to the following background including the methods of analysis: (1) High probability of maternal blood (MB) contamination during CB sampling or through small placental bleeding during late pregnancy or delivery. (2) Low-sensitivity detection of allergen-specific IgE levels and allergen-specific IgE profiles against various food and inhalant allergens in CB. Since the majority of total IgE in CB is nonspecific IgE, generally much higher than allergen-specific IgE levels,[11,13,14] the detection of allergen-specific IgE and its profiles of CB are difficult, highlighting the need for the development of new highly sensitive methods for the detection of allergen-specific antibodies. In a recent study,[15] we described a new microarray technique of high-density antigen immobilization using the carboxylated arms on the surface of a diamond-like-carbon (DLC)-coated chip, which had higher sensitivity in detecting allergen-specific IgE, IgA, IgG, and IgG_4 compared with the UniCAP system and allergen-specific immunoglobulin profiles against various food and inhalant allergens.

The present study is an extension to our previous study[15] and was designed to further examine the utility of the new method. Specifically, we used the DLC chip to detect allergen-specific IgE, IgA, IgG, and IgG_4 and determine the allergen profiling patterns in carefully sampled CB to avoid MB contamination. The new technique identified allergen-specific IgE antibodies in CB, which were of fetal origin. The results allowed analysis of the mechanism of allergen sensitization in the fetus and maternofetal transfer of immunoglobulins.

METHODS

Subjects

The study included 92 healthy paired pregnant women and their newborns recruited at Kawatetu-Chiba Hospital, Chiba University Hospital, and Health Insurance Naruto Hospital from January 2007 to May 2008 in Japan. At birth, CB was collected by needle puncture of the umbilical vein after careful

From [a]the Division of Enzyme Chemistry, Institute for Enzyme Research, University of Tokushima, Tokushima; [b]the Department of Pediatrics, Graduate School of Medicine, Chiba University, Chiba; and [c]the Health Insurance Naruto Hospital, Naruto.

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Corresponding author: Hiroshi Kido, MD, PhD, Division of Enzyme Chemistry, Institute for Enzyme Research, University of Tokushima, Kuramoto-cho 3-18-15, Tokushima 770-8503, Japan. E-mail: kido@ier.tokushima-u.ac.jp.

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Abbreviations used
BU: Binding unit
CB: Cord blood
DLC: Diamond-like-carbon
MB: Maternal blood
NB: Neonatal blood

cleaning of the umbilical cord to avoid MB contamination. Neonatal blood (NB) was obtained at the time of birth by Contact-Activated Lancets low flow (BD Microtainer, Franklin Lakes, NJ), and venous MB was obtained at 4 to 5 days after delivery. Blood samples were then centrifuged at 150 × *g* for 10 minutes to prepare serum. Serum was frozen at −30°C until analysis. All subjects provided written informed consent to participate in this study. This study was approved by the ethics committees of the Graduate School of Medicine, Chiba University, and Tokushima University Hospital.

Allergen chip assay

Allergen-specific IgE, IgA, IgG, and IgG_4 levels were measured in serum by the allergen diagnosis DLC chip as described in detail previously.[15] Briefly, carboxylated DLC film-coated glass slide (Gene slide) was purchased from Toyo Kohan Co (Tokyo, Japan). Natural allergens *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were purchased from Allergon (Ängelholm, Sweden). Purified single allergens as molecular allergens, such as ovomucoid, ovalbumin, conalbumin, α-casein, β-casein, and β-lactoalbumin, were purchased from Sigma-Aldrich (St Louis, Mo). Japanese cedar was purchased from Cosmo Bio Co (Tokyo, Japan) and house dust from GREER (Lenoir, NC). Human serum IgE (75/502), IgG, IgA, and IgM (67/086) used as internal standards on chip were from the National Institute for Biological Standards and Control (Hertfordshire, United Kingdom).

After the activation of carboxylated DLC slides and the fabrication of allergen microarray, the individual arrays were incubated with 20 μL of 1:2 to 1:50 diluted serum as the primary antibody, then reacted with a HiLyte Fluor 555 (Dojindo Molecular Technologies, Inc, Kumamoto, Japan)-labeled secondary antibody against each human IgE, IgA, IgG, and IgG_4, and the resulting images were analyzed as described previously.[15] On each allergen chip, various concentrations of human IgE, IgG, or IgA were spotted as internal standards. From the cubic equation of IgE, IgG, or IgA standard concentrations, the amounts of allergen-specific antibodies bound to allergen on the chips were calculated and expressed as binding unit (BU). The BUs of IgE, IgA, IgG, and IgG_4 were reported as BUe, BUa, BUg, and BUg4, respectively. The detection limit of allergen-specific IgE against various natural and molecular allergens in serum in the DLC chip was 10 BUe/mL, which corresponds to about 0.07 IU/mL of the UniCAP system, indicating about 4 to 8 times higher sensitivity for the DLC chip than for the UniCAP system. The UniCAP system has a limit of 0.35 IU/mL for IgE detection.[6,16] The detection limits for allergen-specific IgA, IgG, and IgG_4 were 0.25 BUa, 2.50 BUg, and 0.53 BUg4, respectively.

We compared the sensitivity of the DLC chip with that of the UniCAP system for allergen-specific IgE in CB, which contains a relatively high level of nonspecific IgE antibodies[11,13,14] (Table I). The UniCAP system did not detect allergen-specific IgE antibodies in all CB samples analyzed in our experiments, even in samples of fluorescence units (BUe/mL) of more than 18 to 22 times the detection limit (10 BUe/mL) on the DLC chip. However, the difference in the sensitivity between the DLC chip and the UniCAP system using MB samples was equivalent to that in allergic patients[15] described above.

Total IgA assay

Total IgA concentration was determined by using an ELISA kit (Bethyl Laboratories, Montgomery, Tex) according to the protocol provided by the manufacturer. The chromogen produced was measured at an absorbance of 450 nm by using a SpectraMax Plus384 autoreader (Molecular Devices Corp, Sunnyvale, Calif).

TABLE I. Comparison of assay sensitivity in detecting antigen-specific IgE in CB and MB against food allergens and inhalant allergens using the DLC chip system and the UniCAP system

	CB (1:1 dilution)		MB (1:1 dilution)	
Allergen	DLC chip (BUe/mL)	UniCAP (Ua/mL)	DLC chip (BUe/mL)	UniCAP (Ua/mL)
Food				
Egg white	30.35	ND*	77.41	0.545
	11.02	ND	23.65	ND
Ovomucoid	180.0	ND	134.8	0.960
	84.89	ND	64.66	ND
	13.30	ND	23.15	ND
Milk	221.4	ND	182.7	1.095
	30.90	ND	61.71	0.540
	18.05	ND	23.15	ND
Inhalant				
Cedar pollen	55.55	ND	90.98	0.960
	21.78	ND	32.20	ND
Df	54.01	ND	80.76	1.275
	47.38	ND	25.53	ND
Dp	63.04	ND	215.6	2.950
	26.48	ND	60.70	ND

CB serum (1:1 dilution) and MB serum (1:1 dilution) were used for the measurement of allergen-specific IgE levels on the UniCAP system and the DLC chip. Detection limit on the DLC chip: 10 BUe/mL.
Df, Dermatophagoides farinae; *Dp, Dermatophagoides pteronyssinus*; *Ua*, arbitrary unit.
**ND*, Not detectable of UniCAP assay: <.35.

Statistical analysis

Statistical analysis was conducted by using the Statistical Package for Social Sciences (version18.0; SPSS, Inc, Chicago, Ill). Most data sets showed skewed distribution, and thus Spearman's rank correlation test was used to assess the relationship between the different samples. A P value of ≤.05 was considered significant.

RESULTS

Allergen-specific serum IgE, IgA, IgG, and IgG_4 levels and their profiles in CB, NB, and MB

Allergen-specific IgE, IgA, IgG, and IgG_4 levels in CB, NB, and MB and their profiles were analyzed by using the allergen diagnosis DLC chips. The DLC chip detected more than 1 allergen-specific IgE of the tested 11 allergens in 83.7% of CB (n = 92). Fig 1 shows allergen-specific IgE, IgA, IgG, and IgG_4 profiles in representative paired CB, NB, and MB samples. MB contained high-reactive IgE levels against inhalant allergens *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* and moderate-reactive IgE levels against food allergens milk, α-casein, and β-casein and also inhalant allergens cedar pollen and house dust. CB, however, did not contain any reactive IgE levels against inhalant allergens *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cedar pollen, and house dust, but it had moderate-reactive IgE levels against food allergens milk, α-casein, β-casein, and ovomucoid. Although MB contained various allergen-specific IgA antibodies, CB did not show any reactive IgA. Almost identical levels of allergen-specific IgG and IgG_4 antibodies to each allergen and similar profile patterns were observed among MB, NB, and CB. The difference in the allergen-specific profiles of IgA and IgE between MB and CB or NB indicates no MB contamination in the paired CB samples and suggests that allergen-specific IgE antibodies in CB are derived from the fetus. The almost identical allergen-reactive

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