表1　患者背景

		治療群(N=18)	対照群(N=16)	P value
平均年齢(歳)		7．3±1．9	8±2．2	P=0.32[1)]
男女比(男:女)		14:4	12:4	P=0.85[1)]
アレルギー疾患の合併	気管支喘息	あり12:なし6	あり10:なし6	P=1.00[2)]
	アトピー性皮膚炎	あり14:なし4	あり14:なし2	P=0.66[2)]
平均卵白特異的IgE		15．1±3．3	22．8±2．8	P=0.43[1)]
粉末卵白の負荷試験による閾値(中央値;範囲(mg))		100(10－500)	100(10－300)	P=0.89[1)]
平均除去食品数		4．8±3．3	3．2±3．0	P=0.068[1)]

1)Mann-Whitney U test
2)Fisher's exact test

図1　rush OITにおけるQOLの変化

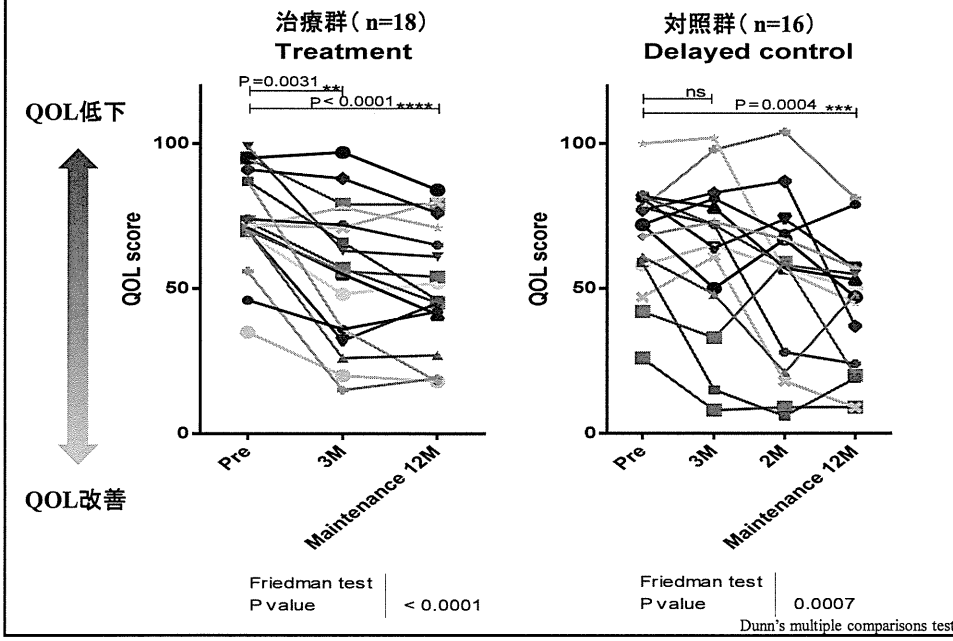

表2　rush OITの経過

		治療群(N=18)	対照群(N=16)	P value
平均免疫療法期間(日)		15.0±9.4	16.7±5.3	P=0.14
退院までの平均日数(日)		21.0±11.0	21.6±4.56	P=0.10
免疫療法中の誘発症状(中央値;範囲(回))	皮膚	3(0−6)	2(0−9)	P=0.44
	消化器	6(0−41)	4(0−39)	P=0.84
	呼吸器	0.5(0−12)	1(0−5)	P=0.87
	循環器	0(0−0)	0(0−1)	P=0.47
	grade3以上の誘発症状	0(0−5)	0(0−14)	P=0.39
維持期2ヵ月までの誘発症状(中央値;範囲(日))	皮膚	2.5(0−32)	1.5(0−30)	P=0.43
	消化器	1(0−36)	0.5(0−75)	P=0.35
	呼吸器	0.5(0−21)	1(0−11)	P=0.82
	grade3以上の誘発症状	0(0−1)	0(0−2)	P=0.85

Mann-Whitney U test

表3　QOLに関与する因子についての検討

		QOL改善群(N=20)	QOL不変、低下群(N=14)	P value
平均年齢(歳)		7.6±2.1	7.6±2.0	P=0.93[1)]
登録時閾値(中央値;範囲(mg))		100(10−500)	100(10−300)	P=0.48[1)]
合併する食物アレルギー	小麦除去	有5名、無15名	有3名、無8名	P=1.00[2)]
	乳除去	有9名、無11名	有6名、無5名	P=0.72[2)]
	小麦・乳除去	有2名、無18名	有3名、無8名	P=0.31[2)]
気管支喘息の合併		有11名、無9名	有11名、無3名	P=0.27[2)]
退院までの平均日数(日)		17.9±4.7	26.4±11.8	P=0.001[1)]
免疫療法中の症状出現回数(中央値;範囲(回))	皮膚	1(0−9)	1(0−5)	P=0.84[1)]
	消化器	2(0−31)	7(0−41)	P=0.043[1)]
	呼吸器	1(0−10)	1(0−4)	P=0.95[1)]
維持期2ヵ月までの症状出現日数	皮膚	1(0−40)	4(0−23)	P=0.53[1)]
	消化器	1(0−10)	0(0−36)	P=0.30[1)]
	呼吸器	1(0−21)	1(0−14)	P=0.50[1)]

※QOL質問票の総点が10点以上低下したものを改善群とした。　1)Mann-Whitney U test
2)Fisher's exact test

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「食物アレルギーにおける経口免疫療法の確立と治癒メカニズムの解明に関する研究」
分担研究報告書

経口免疫療法による特異抗体の変化：アレルゲンコンポーネントの解析

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研究要旨

本研究課題では、血清特異抗体のアレルゲン認識特異性を解析することにより、より簡便で患者への負担も少ない診断技術の開発と耐性獲得と特異抗体レパートリーとの関連を明らかにすることを目的とした。昨年度の研究において、２年後においても鶏卵経口負荷試験陽性反応を示した患者グループと１年後に耐性を獲得した患者グループでは、最初の経口負荷試験前における鶏卵卵白主要アレルゲンであるオボムコイドの第３ドメインに対する IgE 抗体価に差異があることを示し、また、鶏卵アレルギーにおける急速経口免疫療法の実施前後とその後のオボムコイド特異的 IgE 抗体の変動を予備的に解析した。本年度は、OM に特異的な IgE 抗体および卵白総タンパク質に体する IgE 抗体について、基礎研究で用いられる精製オボムコイドを抗原とした ELISA 法と臨床検査で用いられているイムノキャップ法とで測定し比較解析した。一方、患者末梢血白血球を用いた治癒メカニズムの解明研究に用いる高純度オボムコイドを新たに分離精製し大量調製した。さらに、牛乳アレルギーにおける IgE 抗体の分析に用いる牛乳カゼインコンポーネントを、大腸菌過剰発現系を用いて組換えタンパク質として大量に調製することを試みた。αs1-カゼイン、β-カゼインおよびκ-カゼインの各コンポーネントは正常に発現し、ヒスチジンタグを用いて高純度に精製することができた。今後、血清 IgE 抗体分析のための抗原としての活用が期待できる。

A．研究目的

経口免疫療法における耐性獲得の免疫学的機序の解明は幅広い臨床応用が期待できる。本研究課題では、血清特異抗体のアレルゲン認識特異性を解析することにより、より簡便で患者への負担も少ない診断技術の開発と耐性獲得と特異抗体レパートリーとの関連を明らかにすることを目的としている。昨年度までの研究により、鶏卵卵白の主要アレルゲンコンポーネントであるオボムコイド（OM）に対する特異 IgE 抗体価は、症例間でバラツキが見られるが、２つの変動パターンに分類が可能であり、さらに OM の限定分解フラグメントであるドメイン３に対する IgE 抗体も症例間で大きく差異があることを明らかにした。本年度は、鶏卵卵白の主要アレルゲンである OM とオボアルブミン（OVA）、および比較対象のために卵白総タンパク質のそれぞれに特異的な IgE 抗体について、主に基礎研究で用いられている精製 OM を抗原とした ELISA 法で測定し、臨床検査で用いられて

いるイムノキャップ法による測定結果と比較解析した。また、血清　抗体分析と平行して、別の分担者グループにより患者末梢血白血球を用いた治療メカニズムの解明を目指した研究が進んでおり、その際に必要となる高純度OM票品が大量に必要となる。そこで、新鮮卵白より酸沈殿法とイオン交換クロマトグラフィーを組み合わせて分離精製することとした。

牛乳アレルギーにおいては、カゼインと乳清タンパク質のいずれもアレルゲンとして同定されている。カゼインは複数のコンポーネントから構成される複合体としてミセル構造をとって牛乳中に分散しており、どのような形態で体内に吸収されてアレルギー症状を誘発するかは明らかではない。このようなアレルギー誘発のメカニズムを詳細に研究するためには高純度のコンポーネントが必要となる。そこで、まずカゼインミセルの主要コンポーネントであるαs1-カゼイン、β-カゼインおよびκ-カゼインを調製することとした。カゼインミセルは塩溶液には不溶性であり、各コンポーネントを相互に夾雑しないように分離することは容易ではない。そこで、相互の夾雑の心配がない組換えタンパク質としてカゼインコンポーネントを調製することとした。

B．研究方法

鶏卵の急速経口免疫療法を実施し、１年後の経過が終了した患者１１例について、登録時、維持量到達後、維持期２カ月後、維持期１２ヶ月後に採血し、血清を分離して凍結保存されていたサンプルを用いた。血清中のオボムコイドに対する特異IgE抗体は、精製抗原をコートしたマイクロプレートに希釈血清を添加し、結合した抗体を酵素標識二次抗体により検出する標準的なELISA法により測定した。また、抗体検査イムノキャップ法により測定したデータを東大病院小児科より入手し、比較解析した。

新鮮鶏卵より卵白を分離し、均質化した後、トリクロロ酢酸沈殿法によりOVA等を沈殿除去することによりOMを濃縮した。透析によりトリクロロ酢酸を除去し緩衝液で平衡化した後、SP-Sephadex C-50によるイオン交換クロマトグラフィーによりOMを分離精製した。カラムからの溶出画分におけるOMの純度をSDS-PAGEにより検定した。

牛乳カゼインのcDNAを泌乳期ウシ乳腺cDNAプールからPCR法により増幅し、大腸菌発現ベクターに挿入してチオレドキシン融合タンパク質として発現させた。発現したタンパク質を、抗カゼイン抗体を用いた免疫ブロット法で解析した。

C．研究結果

ELISA法による血清抗OM IgE抗体価は、急速経口免疫療法開始前（登録時）ではELISAの吸光度として0.03ー0.3程度であったが、維持量到達時には一例を除いて全て上昇し６例では0.3ー0.6程度まで顕著に上昇した（図１）。一方、イムノキャップ法では、７例において、最も高い抗OM IgE抗体価が20 UA/ml以下で、その多くでは期間を通して大きな変動は見られなかった（図２）。50ー300UA/ml程度のIgE抗体価を示した症例では、維持量到達時に２例で上昇し別の２例では低下した。また、卵白総タンパク質を抗原としてELISA法によっ

て IgE 抗体を測定した場合においても、ELISA 法では治療後に明確に上昇していた血清に関して、イムノキャップ法では僅かな上昇や１２ヶ月後に顕著に低下するなど、必ずしもイムノキャップ法での結果とは一致しなかった。

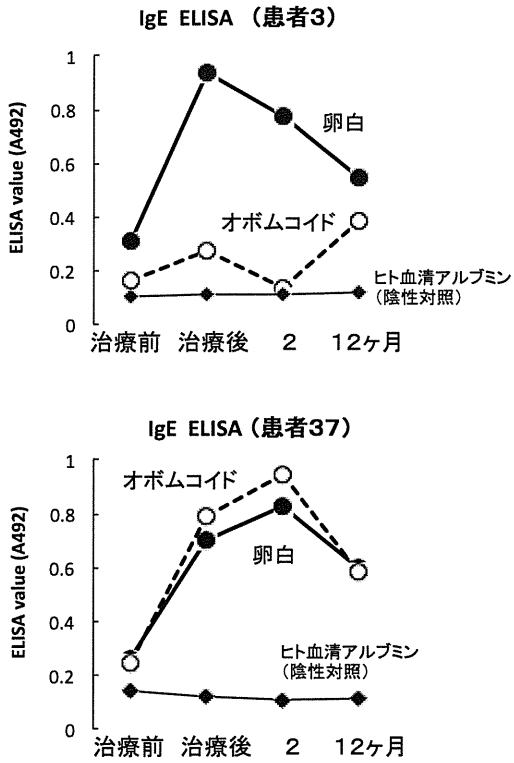

図１　ELISA 法による血清 IgE 抗体の変動の解析例　卵白、精製オボムコイド、陰性対照としてのヒト血清アルブミンを抗原として、経口免疫療法治療前後とその後の血清特異 IgE 抗体の変動を測定した。

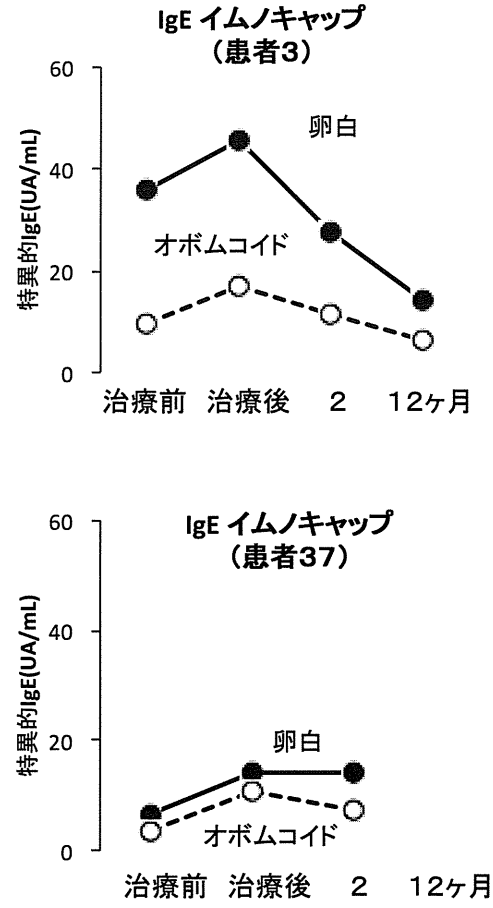

図２　イムノキャップ法による血清 IgE 抗体の変動の解析例　卵白およびオボムコイドに対する、経口免疫療法治療前後とその後の血清特異 IgE 抗体の変動を測定した。

新鮮鶏卵卵白より分離精製した OM は SDS-PAGE により単一のバンド（糖鎖の不均一性による一群のバンド）として検出され、OVA や他の卵白タンパク質の混入は、CBB 染色のレベルでは検出されなかった。抗-OM 抗体を用いた免疫ブロット法によって、この一群のバンドは明確に染色されたことから、純度の高い OM が分離精製できたと判断

された。

カゼインの３種のコンポーネントを大腸菌により融合タンパク質として発現させた。いずれのカゼインコンポーネントについても発現誘導処理によって、融合タンパク質の分子量とほぼ一致するタンパク質のバンド強度が明確に増強され、カゼインは大腸菌発現系において安定に発現することが明らかとなった。また、天然のカゼインコンポーネントに対して調製した抗カゼイン抗体を用いた免疫ブロットにより、αs1-、β-、κ-カゼインの各抗原タンパク質が単一のバンドとして検出され、組換え型カゼインも天然のカゼインと同等の抗原性を示すことが示された。

D．考察

イムノキャップ法では１UA/ml以下でほとんど検出されていない場合でもELISA法では明確に変動が測定できるなど、ELISA法とイムノキャップ法で抗-OM IgE抗体および抗-卵白タンパク質IgE抗体の測定値に大きな差異が見られた。ポリスチレンプレート（ELISA）とセスロース膜（イムノキャップ）というアレルゲンを固相化する担体の違いや、固相化されているアレルゲン（OMや卵白タンパク質）の量や密度の違いが考えられるが、今後、さらに詳細に比較解析する必要がある。また、耐性獲得や症状の誘発などの臨床的知見と血清IgE抗体の変動との関連性を解析するような臨床基礎研究においても、できればイムノキャップ法とELISA法を併用して、より多くの基礎データを収集する必要があると思われる。

鶏卵卵白より高純度のOMを調製することができた。今後、脱塩、濃縮や、用途によっては脱LPS処理等を行い、患者末梢血白血球を用いた治療メカニズムの解析に使用する予定である。

牛乳カゼインの３種のコンポーネントは特定の高次構造を持たず酵素分解を受けやすいため、大腸菌内で分解されることが懸念されたが、いずれも未分解の状態で安定に発現させることが可能であった。κ-カゼインについては、他の２つのコンポーネントに比べて発現効率が低く、大量に調製するにはより良い発現条件の検討が必要である。天然の牛乳カゼインからの各コンポーネントの分離精製が容易ではないため、これらの組換え型カゼインコンポーネントは、抗体分析における標準抗原として有用であると考えられた。

E．結論

ELISA法とイムノキャップ法ではOM特異的IgEの測定値が大きく異なっており、両方の分析法を併用しつつ、抗体価の変動解析を進める必要がある。

カゼインの３種のコンポーネントを大腸菌により融合タンパク質として発現させ組換えアレルゲンを調製できることが明らかとなり、今後、牛乳アレルギーにおけるコンポーネント別の特異抗体解析を進めることが可能となった。

F．研究発表

1. 論文発表

なし

2. 学会発表

なし

G．知的所有権の取得状況

なし

Ｈ２４厚生労働科学研究費補助金（難治性疾患等克服研究事業（免疫アレルギー疾患等予防・治療　研究事業））

分担研究報告書

新規アレルゲン特異的抗体測定法の開発

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研究要旨

牛乳の急速経口免疫療法に伴う治療開始前後の生体内の各種免疫グロブリン価の変化、特に血液と唾液中の特異抗体（IgE、IgG4、IgA）価の変動を高感度アレルゲンマイクロアレイ(DLCチップ)法で検討して、以下の事が明らかとなった。①牛乳抗原（Milk、α-Casein、β-Casein, β-Lactoglobulin）は、治療により低下傾向が見られた。IgEとは反対にIgG4、IgA価は、治療によって増加傾向が見られ、この効果は特にIgG4において著明で、IgAの増加の程度はIgG4に比べて軽度であった。唾液のIgAは極一部の患者で治療後に増加が確認された。

A. 研究目的

食物アレルギーの多くは自然寛解することから、基本的な治療方針はアレルゲン食品の除去をきちんと行って誤食の危険を避けて自然寛解を待つことである。しかしながら、学童期に入ってからの自然寛解率は低くまた学童期以降は社会生活における不利益が大きいことから、積極的に寛容を誘導する方法として経口免疫療法が近年注目されてきている。臨床的にはこれらの治療法は一定の効果が認められるが、その方法は未だ確立された方法もなく、治療の機序も不明である。

この研究では、牛乳の急速経口免疫療法に伴う各種抗原特異的抗体価（IgE、IgG4、IgA）を、治療の前後で測定して、急速な治療に伴う各種抗体価の変動を血液と唾液で評価し、各抗体価の変動の意義を解析した。

B. 方法

1）抗体価の測定に使用したアレルゲンチップは、ダイヤモンドライクカーボン(DLC)でガラス基板をコートしたチップ（DLCチップ）で[1,2]、これまでに使用されていたUniCAPに比べて抗原の種類によって違いがあるものの４－７倍高感度に測定ができ、しかも検体量が数μLで数十種類の抗原に対する抗体価を定量することができる特色を持つ。

このシステムは、内部標準を搭載していることから、内部標準抗体の種類を変えることで容易に各種抗体価を測定できることから、IgE以外に各種抗原特異的IgG4、IgAを測定した。本年度の研究では、牛乳の急速減感作療法であることから、牛乳の４抗原をチップに搭載して検証した。なお、DLCチップを用いてIgE、IgG4、IgA、saliva IgAを測定した抗原のレイアウトを図１に示す。このチップでは、コントロール抗原として鶏卵由来の抗原を搭載して同時に検証している。同一抗原を３点プロットしており、３点の測定値の平均値を求めている。この測定から得られる値は、Binding Unit (BU)で表示し、IgE、IgG4、IgAをそれぞれBUe、BUg4、BUaとして表した。今回の急速減感作療法のスケジュールを図２に示す。急速経口免疫療法開始前、免疫療法開始3か月後における血漿および唾液中のアレルゲン特異IgA, IgG4, IgEを測定した。なお、対照は無治療に割り付けられた患者群でエントリー時、エントリー３か月後の唾液および血漿中の抗体を測定した。

C. 研究結果

1）牛乳の急速経口免疫療法に伴う治療開始前後の生体内の各種免疫グロブリン価の変化を唾液と血液中の鶏卵と牛乳のアレルゲン成分特異抗体

（IgE、IgG4、IgA）価を高感度アレルゲンマイクロアレイ(DLCチップ)法でモニターした。図３にIgE抗体価の治療開始前後の変化を示す。
牛乳の主要抗原であるMilk、α-Casein、β-Casein, β-Lactoglobulin の抗体価は、治療によって低下傾向が見られたが、鶏卵成分に比べて牛乳成分では変化が軽度であった。一方、IgEとは反対にIgG4、IgAは、図４、５に示すように、治療で増加傾向が見られ、この効果は特にIgG4において著明で、IgAの増加の程度はIgG4に比べて軽度な傾向が見られた。なお、ここでも昨年実施した鶏卵成分に比べて牛乳成分では変化が軽度であった。唾液のIgAは、極一部の患者で治療後の増加が検出された。

D. 考察

牛乳の急速経口免疫療法によって、血液中の抗原特異的 IgE は低下を示し、これに伴って IgG4、IgA は増加傾向を示した。増加傾向を示す抗体の中でも IgA はその程度が軽度であった。以上のことから、アレルギーを引き起こす IgE 抗体は急速経口免疫療法で低下して、従来アレルギーの発症を抑制することが示唆されていた IgG4 は増加を示すことが明らかとなった。なお抗原特異的 IgG 全体の中で IgG4 の占める割合は低く、IgG4 以外の IgG サブクラスについても調査する必要のあることが示唆された。また IgA もアレルギーの発症を抑制することが示唆されるが、その増加は軽度でしかもゆっくりと増加する可能性があり、今後の経過を観察する必要がある。

E. 結論

１）血清及び唾液中の食物抗原特異的 IgE、IgG4、IgA を高感度に定量するアレルゲンマイクロアレイの測定法を確立した。
２）牛乳の経口免疫療法開始３か月後、IgE 抗体価は低下傾向を示したが、プラセボ群では大きな変化は無かった。一方 IgG4、IgA 価は治療で増加傾向を示し、この効果は IgG4 に著明で、IgA の増加の程度は軽度であった。

F. 健康危険情報

なし。

G. 研究発表

論文発表

1 Kamemura N, Tada H, Shimojo N, Morita Y, Kohno Y, Ichioka T, Suzuki K, Kubota K, Hiyoshi M and Kido H. Intrauterine sensitization of allergen-specific IgE analyzed by a highly sensitive new allergen microarray. ***J. Allergy Clin Immunol.*** 130(1): 113-121, 2012.

2. Morita H, Nomura I, Orihara K, Yoshida K, Akasawa A, Tachimoto H, Ohtsuka Y, Namai Y, Futamura M, Shoda T, Matsuda A, Kamemura N, Kido H, Takahashi T, Ohya Y, Saito H, Matsumoto K. Antigen-specific T-call responses in patients with non-IgE-mediated gastrointestinal food allergy are predominantly skewed to T(H)2. ***J. Allergy Clin Immunol.*** 15(2):133-142, 2013.

H. 知的財産権の出願・登録状況

なし

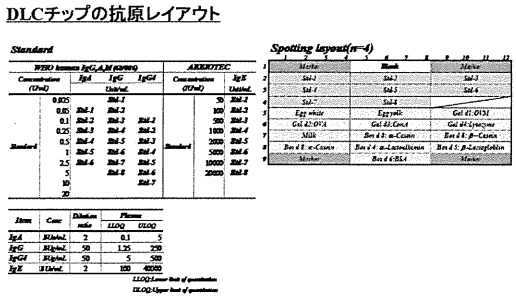

図1. DLC チップにおける抗原搭載のレイアウト

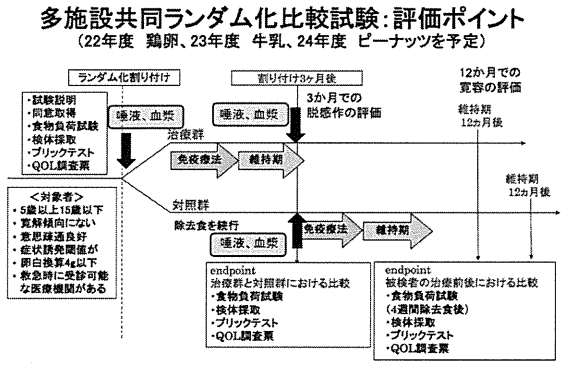

図2　急速経口減感作療法の実施計画

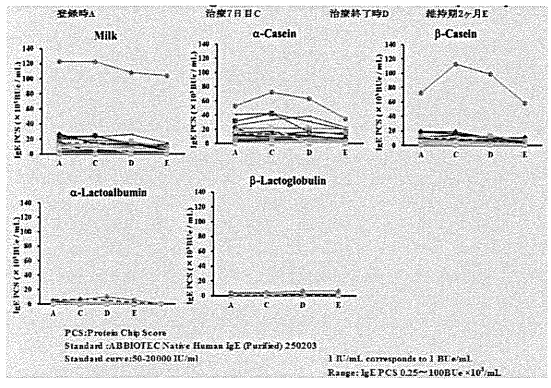

図3　牛乳の急速減感作療法における抗原特異的 IgE の変動比較

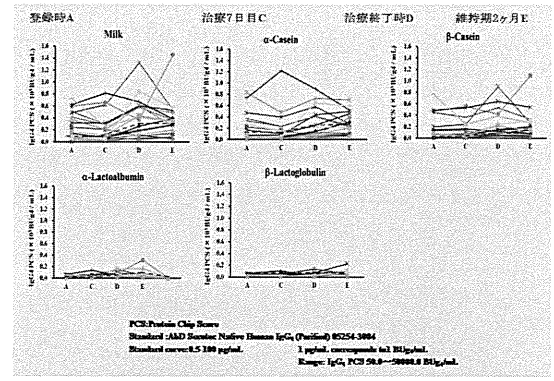

図4　牛乳の急速減感作療法における抗原特異的 IgG4 の変動比較

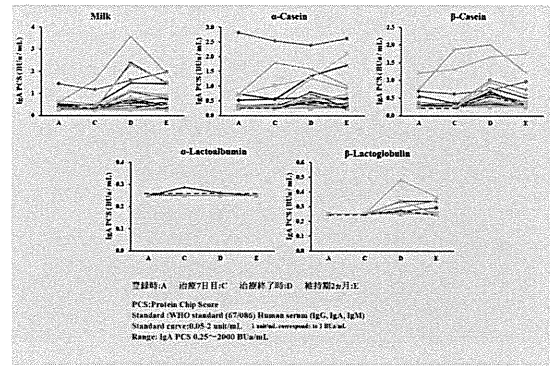

図5　牛乳の急速減感作療法における抗原特異的 IgA の変動比較

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kamemura N, Tada H, Shimojo N, Morita Y, Kohno Y, Ichioka T, Suzuki K, Kubota K, Hiyoshi M and Kido H.	Intrauterine sensitization of allergen-specific IgE analyzed by a highly-sensitive new allergen microarray.	J Allergy Clin Immunol	130(1)	113-121	2012
鈴木宏一、亀村典生、多田仁美、窪田賢司、澤淵貴子、木戸博	低侵襲性高感度マルチ抗原アレルギー診断チップの開発	アレルギーの臨床	32(430)	553-557	2012
木戸博、亀村典生、多田仁美、日吉峰麗	唾液を用いたアレルギー診断の新技術と今後の展望	BIO INDUSTRY	29(10)	42-47	2012
Morita H, Nomura I, Orihara K, Yoshida K, Akasawa A, Tachimoto H, Ohtsuka Y, Namai Y, Futamura M, Shoda T, Matsuda A, Kamemura N, Kido H, Takahashi T, Ohya Y, Saito H, Matsumoto K.	Antigen-specific T-cell responses in patients with non-IgE-mediated gastrointestinal food allergy are predominantly skewed to T(H)2.	J Allergy Clin Immunol	15(2)	133-142	2013
木戸博、多田仁美、亀村典生、窪田賢司、鈴木宏一	Diamond-Like Carbon を用いた新規高感度アレルゲンマイクロアレイの多項目抗原特異的IgE測定	アレルギー・免疫	20(1)	56-62	2013

IV. 研究成果の刊行物・別刷

Original article

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

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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;■■■:■■■-■■■.)

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

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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0091-6749/$36.00

doi:10.1016/j.jaci.2012.02.023

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.

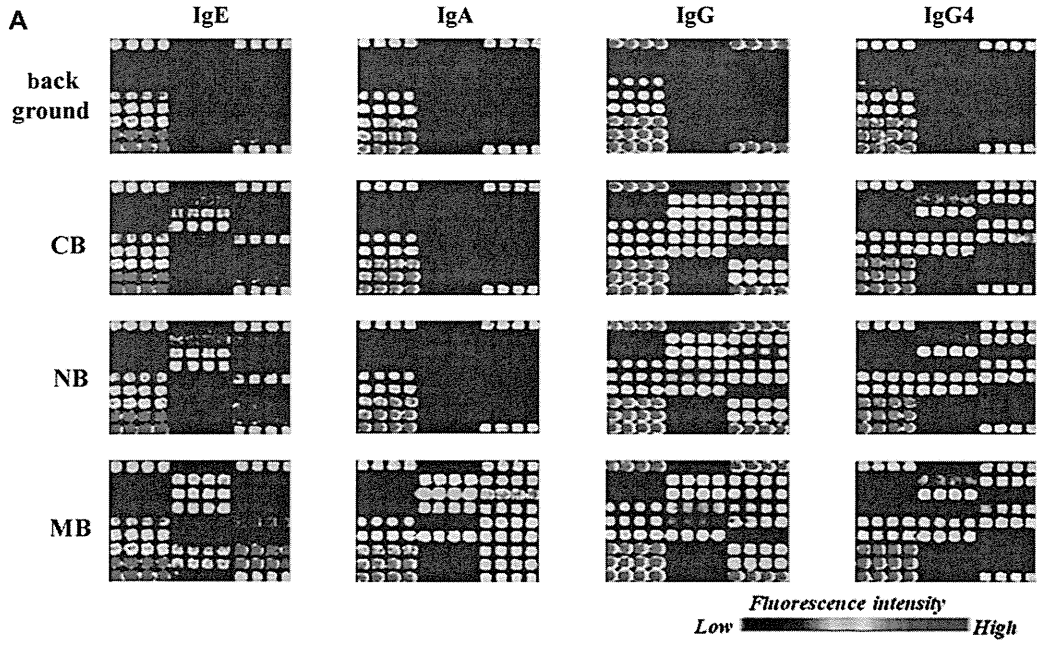
Statistical analysis

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



B

GD	Blank	GD
Blank	Milk	Egg white
Std	α-Casein	Egg yolk
	β-Casein	Ovalbumin
	α-Lactoalbumin	Ovomucoid
	β-Lactoglobulin	Conalbumin
	Cedar pollen	Dp
	House dust	Df
	Blank	GD

FIG 1. Multiallergen-specific profiling patterns of IgE, IgA, IgG, and IgG_4 in paired CB, NB, and MB analyzed by the DLC chip. **A,** Rainbow displays of fluorescence intensities of allergen-specific IgE, IgA, IgG, and IgG_4 reactivities against allergens of an array probed sera of paired CB, NB, and MB. **B,** Layout of the allergen DLC chip. *Df, Dermatophagoides farinae*; *Dp, Dermatophagoides pteronyssinus*; *GD,* guideline dot by standard serum; *Std,* standard calibration of human serum IgE from the National Institute for Biological Standards and Control. Each allergen was spotted in quadruple on the chip. Results are representative of 1 pair of 92 samples.

profiles of IgG and IgG_4 among CB, NB, and MB support the established finding of maternofetal transfer of IgG.[17] Similar findings were observed in the other 91 paired CB and MB samples.

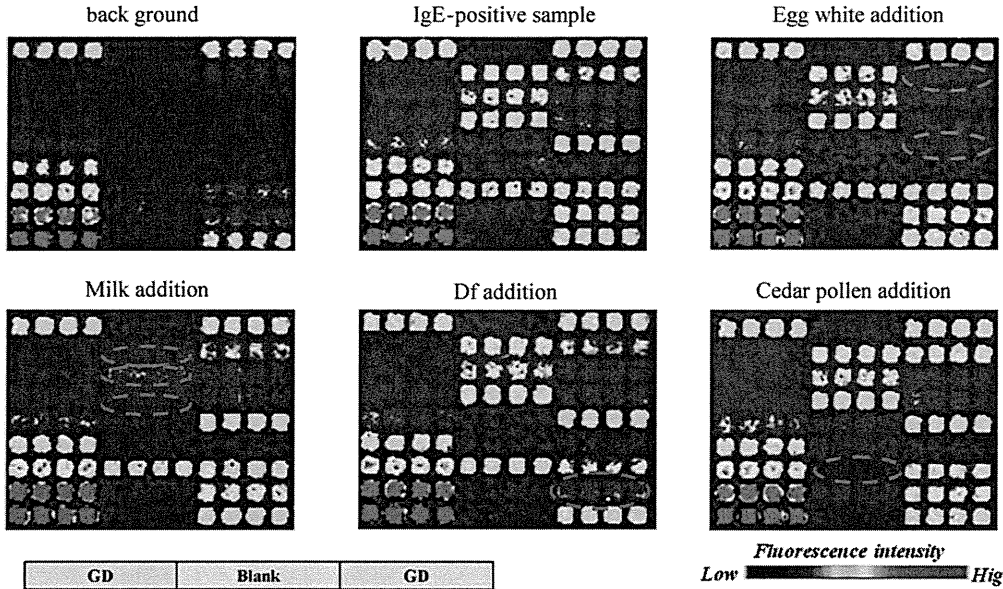
To evaluate the cross-reactivity of the antigen-IgE antibody reaction on the highly sensitive DLC chip, serum was preincubated with each allergen for 2 hours at 37°C followed by allergen-specific IgE detection on the chip (Fig 2). Each allergen selectively and almost completely adsorbed allergen-specific IgE antibodies without any interference or cross-reactivity by other antigen-antibody reactions. These results indicate that allergen-specific IgE was selectively detected on the DLC chip.

Total IgA levels in CB and MB

Since the total IgA level in CB is generally used as an indicator of transfer of MB,[18] we measured total IgA levels in CB and MB by using ELISA. Total IgA levels in all CB were within the minimal levels between 1.2 and 19.4 μg/mL (Fig 3) and no allergen-specific IgA was detected (Fig 1 and Table II). In contrast, total IgA levels in MB were between 0.8 and 3.5 mg/mL. Therefore, the total IgA levels in MB did not correlate with those in CB. These results indicate that MB contamination is below the detection level in CB collected by careful needle puncture of the umbilical vein.

Allergen-specific IgE, IgA, IgG, and IgG_4 levels in CB and MB

Allergen-specific IgE, IgA, IgG, and IgG_4 levels were analyzed in 92 paired CB and MB samples. The proportion of CB samples positive for allergen-specific IgE against each food allergen (using a cutoff value of 10 BUe) ranged from 6.5% to 69.6%, with the highest proportion for ovomucoid, while the proportion of samples positive for each inhalant allergen ranged between 6.5% and 28.3% (Table II). The proportions of MB samples positive for allergen-specific IgE against each food allergen were almost similar to those of CB. In contrast, the proportions of MB samples positive for allergen-specific IgE against inhalant allergens were considerably higher (between 72.8% and 84.8%) than those of CB. Specifically, the proportion of CB samples positive for allergen-specific IgE



GD	Blank	GD
Blank	Milk	Egg white
Std	α-Cascin	Egg yolk
	β-Cascin	Ovalbumin
	α-Lactoalbumin	Ovomucoid
	β-Lactoglobulin	Conalbumin
	Cedar pollen	Dp
	House dust	Df
	Blank	GD

FIG 2. Allergen-specific IgE adsorption for evaluating cross-reactivity of antigen-IgE antibody reaction on the DLC chip. MB sample containing allergen-specific IgE against both food and inhalant allergens was preincubated with egg white (5 μg/mL), milk (5 μg/mL), Df (500 μg/mL), or cedar pollen (500 μg/mL) at 37°C for 2 hours. After the reaction, each sample was centrifuged at 17,500 × *g* for 30 minutes to remove antigen-antibody complex, and then the supernatant was used for multiallergen profiling of IgE on the DLC chip. Bottom, layout of the allergen on the DLC chip. *Df, Dermatophagoides farinae; Dp, Dermatophagoides pteronyssinus*; *GD*, guideline dot; *Std*, standard calibration of human serum IgE.

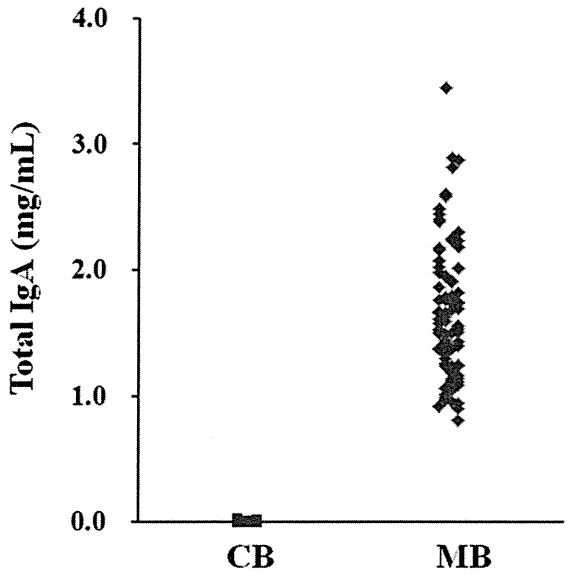


FIG 3. Total IgA levels in CB and MB. Total IgA levels in CB and MB (n = 92) were analyzed by ELISA.

with IgE-positive MB only against each food allergen was high and the mean value was 86.4%. The mean BU ratio for CB/MB in IgE-positive subjects only against the food allergens (ie, CB BUe/MB BUe; Table II) was 1.24 ± 0.60. In contrast, the proportion of CB samples positive for allergen-specific IgE in the IgE-positive MB subjects only against the inhalant allergens was lower (range, 9.0%-33.8%), with a mean value of 20.7%. The mean BU ratio for CB/MB in the IgE-positive subjects only against inhalant allergens (ie, CB BUe/MB BUe; Table II) was 0.54 ± 0.50. These results suggest a potentially greater sensitization against food allergens than against inhalant allergens *in utero*.

The levels of allergen-specific IgA against the allergens tested in CB were below the detection limits, but the proportion of MB samples positive for allergen-specific IgA against food and inhalant allergens ranged between 7.6% and 77.2%, with the highest value against milk (Table II). A fairly similar or identical trend was noted in CB and MB for allergen-specific IgG against food and inhalant allergens (Table III). The mean proportions of CB samples positive for allergen-specific IgG against food and inhalant allergens in subjects with IgG-positive MB only were 91.2% and 87.3%, respectively, and the mean BU ratios of CB/BM in the IgG-positive subjects only (CB BUg/MB BUg; Table III) against food and inhalant allergens were 1.08 ± 0.38 and 0.87 ± 0.24, respectively. The similar proportions of CB and MB samples and BU ratios around 1.0 indicate maternofetal transfer of allergen-specific IgG.

Although the mean proportion of allergen-specific IgG_4-positive CB in the IgG_4-positive MB subjects only against food

TABLE II. Allergen-specific IgE and IgA values in paired CB and MB samples, and BU ratio and correlation between CB- and MB-positive only

	CB (n = 92)		MB (n = 92)		CB/MB-positive only				
IgE positive in:	n	Percent*	n	Percent*	n	Percent†	BU ratio‡ (CB BUe/MB BUe ± SD)	Correlation between CB and MB (r_s)§	*P* value
Food									
α-Casein	28	30.4	25	27.2	18	72.0	1.09 ± 0.60	0.63	.005
β-Casein	16	17.4	15	16.3	11	73.3	1.10 ± 0.63	0.69	.002
α-Lactoalbumin	6	6.5	1	1.1	1	100	0.95 ± 0.00	NA	
β-Lactoglobulin	15	16.3	5	5.4	5	100	1.26 ± 0.65	0.30	.006
Ovalbumin	34	37.0	12	13.0	11	91.7	1.44 ± 0.70	0.44	.180
Ovomucoid	64	69.6	55	59.8	55	100	1.89 ± 0.82	0.89	<.001
Milk	18	19.6	22	23.9	12	54.5	1.14 ± 0.54	0.87	<.001
Egg white	23	25.0	8	8.7	8	100	1.06 ± 0.25	0.81	<.001
Mean						86.4	1.24 ± 0.60		
Inhalant									
Cedar pollen	6	6.5	67	72.8	6	9.0	0.34 ± 0.16	0.83	.042
Df	26	28.3	74	80.4	25	33.8	0.59 ± 0.58	0.06	.830
Dp	18	19.6	78	84.8	15	19.2	0.70 ± 0.74	0.21	.300
Mean						20.7	0.54 ± 0.50		

	CB (n = 92)		MB (n = 92)		CB/MB-positive only				
IgA positive in:	n	Percent*	n	Percent*	n	Percent†	BU ratio‡ (CB BUa/MB BUa ± SD)	Correlation between CB and MB (r_s)§	*P* value
Food									
α-Casein	0	NA	46	50.0	0	NA	NA	NA	
β-Casein	0	NA	50	54.3	0	NA	NA	NA	
α-Lactoalbumin	0	NA	7	7.6	0	NA	NA	NA	
β-Lactoglobulin	0	NA	15	16.3	0	NA	NA	NA	
Ovalbumin	0	NA	17	18.5	0	NA	NA	NA	
Ovomucoid	0	NA	19	20.7	0	NA	NA	NA	
Milk	0	NA	71	77.2	0	NA	NA	NA	
Egg white	0	NA	16	17.4	0	NA	NA	NA	
Mean									
Inhalant									
Cedar pollen	0	NA	21	22.8	0	NA	NA	NA	
Df	0	NA	35	38.0	0	NA	NA	NA	
Dp	0	NA	26	28.3	0	NA	NA	NA	
Mean									

Df, *Dermatophagoides farinae*; *Dp*, *Dermatophagoides pteronyssinus*; *NA*, not available due to lack of positive cases.
*Percentages of allergen-specific IgE- and IgA-positive samples.
†Percentages of allergen-specific IgE- and IgA-positive samples from MB-positive only.
‡BU ratios (CB BUe/MB BUe, CB BUa/MB BUa).
§Correlation coefficient analyzed Spearman's rank correlation test (r_s, *P* value) of allergen-specific IgE and IgA-positive CB and MB-positive only.

allergens was 96.0%, those against inhalant allergens was below the detection levels in the samples tested (Table III). The mean BU ratio between IgG_4-positive CB and IgG_4-positive MB subjects only (CB BUg4/MB BUg4) against food allergens was 0.99 ± 0.39. The results add support to the notion of maternofetal transfer of allergen-specific IgG_4.

Correlation of allergen-specific IgE, IgA, IgG, and IgG_4 between CB and MB

The results of correlation analysis of allergen-specific IgE, IgA, IgG, and IgG_4 levels (BU) between paired CB and MB samples (n = 92) determined by the DLC chip are shown in Table IV. There were strong correlations for allergen-specific IgE levels against food allergens between CB and MB with considerably high correlation coefficients (range, 0.53-0.94; $P < .001$), with the exception of weak correlation for α-lactoalbumin at 0.37 ($P < .001$). In contrast, the correlation coefficients for inhalant allergens were low (range, 0.01-0.30). In addition, there were strong correlations for allergen-specific IgE against crude allergens between CB and MB in the IgE-positive MB subjects only (Table II), such as milk, egg white, and cedar pollen, with high correlation coefficients of 0.87 ($P < .001$), 0.81 ($P < .001$), and 0.83 ($P < .042$), respectively. The correlation profiles of allergen-specific IgE against various food and inhalant allergens for CB and MB are depicted in Fig 4.

There were also significant and strong correlations for allergen-specific IgG against food and inhalant allergens between CB and MB ($r_s > 0.74$; $P < .001$), except allergen-specific IgG against cedar pollen and α-lactoalbumin (r_s, not available) and against β-lactoglobulin ($r_s = 0.58$; $P < .001$) (Table IV). There were also strong correlations for allergen-specific IgG_4 against food allergens between CB and MB ($r_s > 0.85$; $P < .001$), although that for allergen-specific IgG_4 against α-lactoalbumin was weaker ($r_s = 0.68$; $P < .001$). The correlation

TABLE III. Allergen-specific IgG and IgG_4 values in paired CB and MB samples, and BU ratio and correlation between CB- and MB-positive only

	CB (n = 92)		MB (n = 92)		CB/MB-positive only				
IgG positive in:	**n**	**Percent***	**n**	**Percent***	**n**	**Percent†**	**BU ratio‡ (CB BUg/MB BUg ± SD)**	**Correlation between CB and MB (r_s)§**	***P* value**
Food									
α-Casein	34	37.0	34	37.0	32	94.1	0.93 ± 0.30	0.75	<.001
β-Casein	7	7.6	8	8.7	7	87.5	0.88 ± 0.24	0.83	.021
α-Lactoalbumin	1	1.1	1	1.1	1	100	1.12 ± 0.00	NA	
β-Lactoglobulin	6	6.5	7	7.6	5	71.4	1.14 ± 0.44	0.90	.037
Ovalbumin	52	56.5	50	54.3	48	96.0	1.30 ± 0.60	0.80	<.001
Ovomucoid	47	51.1	46	50.0	45	97.8	1.18 ± 0.45	0.90	<.001
Milk	24	26.1	27	29.3	24	88.9	0.92 ± 0.26	0.53	.008
Egg white	52	56.5	52	56.5	49	94.2	1.15 ± 0.35	0.87	<.001
Mean						91.2	1.08 ± 0.38		
Inhalant									
Cedar pollen	0	0.0	1	1.1	0	NA	NA	NA	
Df	19	20.7	23	25.0	19	82.6	0.86 ± 0.20	0.71	.001
Dp	24	26.1	25	27.2	23	92.0	0.88 ± 0.28	0.83	<.001
Mean						87.3	0.87 ± 0.24		

	CB (n = 92)		MB (n = 92)		CB/MB-positive only				
IgG_4 positive in:	**n**	**Percent***	**n**	**Percent***	**n**	**Percent†**	**BU ratio‡ (CB BUg4/MB BUg4 ± SD)**	**Correlation between CB and MB (r_s)§**	***P* value**
Food									
α-Casein	34	37.0	33	35.9	33	100	0.93 ± 0.39	0.89	<.001
β-Casein	13	14.1	15	16.3	13	86.7	0.76 ± 0.27	0.95	<.001
α-Lactoalbumin	17	18.0	16	17.0	16	100	0.90 ± 0.42	0.92	<.001
β-Lactoglobulin	40	43.5	42	45.7	38	90.5	1.07 ± 0.42	0.93	<.001
Ovalbumin	68	73.9	67	72.8	67	100	1.19 ± 0.46	0.91	<.001
Ovomucoid	70	76.1	70	76.1	70	100	1.16 ± 0.48	0.93	<.001
Milk	24	26.1	25	27.2	23	92.0	0.76 ± 0.30	0.94	<.001
Egg white	66	71.7	67	72.8	66	98.5	1.15 ± 0.44	0.94	<.001
Mean						96.0	0.99 ± 0.39		
Inhalant									
Cedar pollen	0	NA	0	NA	0	NA	NA	NA	
Df	0	NA	0	NA	0	NA	NA	NA	
Dp	0	NA	0	NA	0	NA	NA	NA	
Mean									

Df, Dermatophagoides farinae; *Dp, Dermatophagoides pteronyssinus*; *NA*, not available due to lack of positive cases.
*Percentages of allergen-specific IgG- and IgG_4-positive samples.
†Percentages of allergen-specific IgG- and IgG_4-positive samples from MB-positive only.
‡BU ratios (CB BUe/MB BUe, CB BUa/MB BUa).
§Correlation coefficient analyzed Spearman's rank correlation test (r_s, *P* value) of allergen-specific IgE and IgA-positive CB and MB-positive only.

coefficients between CB and MB were not available for allergen-specific IgA against the allergens tested (Table II). Furthermore, the correlation coefficients for allergen-specific IgG_4 against inhalant allergens were not available because allergen-specific BUg4 values were below the detection levels (Tables III and IV). The correlation profiles for allergen-specific IgG and IgG_4 against each allergen between CB and MB are shown in Figs E1 and E2 in this article's Online Repository at www.jacionline.org.

DISCUSSION

We recently developed a new allergen diagnosis microarray with high sensitivity by using DLC-coated chips for profiling allergen-specific IgE, IgA, IgG, and IgG_4 against food and inhalant allergens. The DLC chip allows lowering the limit of detection of allergen-specific IgE in the UniCAP system to further dilution at 4- to 8-fold for each allergen,[15] and the detection limit of allergen-specific IgE in the DLC chip had about 5 times higher sensitivity than that in the UniCAP system in MB and serum of allergic patients. The present study demonstrated a larger difference in the detection sensitivity between the DLC chip and the UniCAP system in CB than in MB (Table I). The reason for the larger difference is not clear at this stage, but a relatively high level of nonspecific IgE in CB may disturb the detection of allergen-specific IgE on the UniCAP system but not on the DLC chip system. The latter immobilizes extremely high-density antigens on the surface of the DLC-coated chip[15] and maintains antigen-antibody reactivity even in the presence of high levels of nonspecific IgE. Our highly sensitive allergen-specific IgE detection system is suitable for the detection of low levels of allergen-specific IgE in CB compared with other previous methods.

The presence of IgE antibodies in CB has been analyzed extensively in the past 20 years since it is important in the design of allergy prevention strategies, particularly allergen avoidance

TABLE IV. Correlation of allergen-specific IgE, IgG, and IgG_4 BU between the 92 paired CB and MB

	Correlation between CB and MB					
	IgE		IgG		IgG_4	
Allergen	r_s	*P* value	r_s	*P* value	r_s	*P* value
Food						
α-Casein	0.69	<.001	0.92	<.001	0.94	<.001
β-Casein	0.70	<.001	0.74	<.001	0.85	<.001
α-Lactoalbumin	0.37	<.001	NA		0.68	<.001
β-Lactoglobulin	0.59	<.001	0.58	<.001	0.97	<.001
Ovalbumin	0.56	<.001	0.93	<.001	0.96	<.001
Ovomucoid	0.94	<.001	0.94	<.001	0.97	<.001
Milk	0.60	<.001	0.91	<.001	0.95	<.001
Egg white	0.53	<.001	0.94	<.001	0.97	<.001
Inhalant						
Cedar pollen	0.30	.004	NA		NA	
Df	0.01	.906	0.83	<.001	NA	
Dp	0.19	.073	0.83	<.001	NA	

Df, Dermatophagoides farinae; *Dp, Dermatophagoides pteronyssinus*; *NA*, not available due to lack of positive cases.

during pregnancy.[1-5,19-22] However, there is conflicting evidence on whether allergen-specific IgE in CB is a reflection of fetal immunity or the result of transfer of maternal IgE to the fetus. The controversy is probably related, at least in part, to the low sensitivity of the methods used for the detection of allergen-specific IgE in CB, and precise allergen-specific IgE profiling patterns against food and inhalant allergens are not available at present. The measurement of the total IgE level in CB is not recommended for allergy risk screening.[6] Furthermore, CB sampling by means of needle puncture of the umbilical vein is essential to avoid MB contamination. To deal with these problems, we collected CB by needle puncture of the umbilical vein and analyzed allergen-specific IgE and other immunoglobulins both in CB/NB and MB by using the newly developed highly sensitive allergen diagnosis DLC chip.

The allergen diagnosis DLC chip detected allergen-specific IgE against more than 1 of the allergens tested in 83.7% of CB from infants analyzed. The rate of detection was higher than those reported previously,[6,11] most likely due to the highly sensitive (Table I) and selective detection of allergen-specific IgE by the DLC chip (Fig 2). The representative data of allergen-specific IgE profiling patterns of CB and NB (Fig 1) showed characteristic patterns that were not identical to those in the paired MB. These results indicated lack of contamination of MB in CB and that IgE in CB is a product of the fetus. If IgE in CB is derived from MB through maternofetal transfer,[11] the allergen-specific profiling pattern of the CB should be similar or identical to that of the MB. The results of the DLC chip of no perfect match of the allergen profiles of CB and MB in the paired 92 samples tested support the conclusion that the allergen-specific IgE identified in CB are of fetal origin.

It has been reported that IgA does not cross the placental barrier and is not produced *in utero* in significant amounts.[23] In contrast, maternal IgG antibodies are transferred to the fetus across the placenta by a specific receptor-mediated mechanism.[24,25] The total IgA levels in CB are commonly used to estimate MB contamination and levels greater than 50 μg/L indicate MB contamination.[11,18] In the present study, the total IgA levels in CB of all our samples were less than 50 μg/mL (range, 1.2-19.4 μg/mL), indicating no or minimal MB contamination. The reliability of the data from the DLC chip was also confirmed by the allergen-specific profiles of IgA, IgG, and IgG_4 (Fig 1): the obvious mismatch of the allergen-specific IgA profile of CB and MB supports no maternofetal transfer of IgA. On the other hand, the similar allergen-specific IgG and IgG_4 profiles in CB and MB provide support for the maternofetal transfer of IgG.

The mean proportion of allergen-specific IgE-positive CB with IgE-positive MB against food allergens was 86.4%, which was about 4 times that with IgE-positive MB against inhalant allergens (20.7%), and their mean allergen-specific IgE BU ratios (CB BUe/MB BUe) for food and inhalant allergens were 1.24 and 0.54, respectively (Table II). These results may provide interpretation for the findings shown in Fig 4 and Table IV of higher levels of allergen-specific IgE (BUe) in CB against food allergens than those against inhalant allergens, and strong and significant correlations between CB and MB for food allergen-specific IgE levels, but weaker correlations for inhalant allergen-specific IgE. These data suggest that maternofetal transfer of food allergens is more frequent and easier than that of inhalant allergens, although previous studies showed crossing of food and inhalant allergens through the placenta in *ex vivo* models.[26,27]

The mechanisms of maternofetal transfer of allergens have been discussed extensively, including fetus allergen-uptake[6] of allergen-IgG complexes through the amniotic fluid by aspiration or permeation through the fetal skin[22,28] and through active transplacental transport.[29] Therefore, the presence of allergen-specific IgG levels in MB and CB may increase the risk of maternofetal allergen transfer and induction of allergen-specific IgE in CB.[30] Furthermore, it has been shown that the fetal immune system can produce IgE antibodies from week 11 of gestation,[13] and thus maternofetal transfer of allergen may trigger allergen-specific IgE production *in utero*. Once these food and inhalant allergens are transferred across the barrier, they may induce allergic sensitization *in utero* under the influence of maternal immune conditions.

In our experiments, however, the mean allergen-specific IgG BU ratios (CB BUg/MB BUg) for food and inhalant allergens were not significantly different at 1.08 and 0.87, respectively (Table III), and allergen-specific IgG levels do not necessarily explain the difference in the levels of IgE (BUe) in CB against food and inhalant allergens. At present, the reasons for the difference in the proportion of allergen-specific IgE-positive CB and the levels of IgE against food and inhalant allergens are not clear. To analyze this difference, further measurements should be conducted of food and inhalant allergen levels in CB and maternal circulation at the time of delivery.

Previous studies reported the presence of low (undetectable) levels of allergen-specific IgE in infant blood during the breast-feeding period at 6 months of age, compared with detectable levels of allergen-specific IgE in CB of some infants.[11,31] This observation might be due to the separation after birth from the source of allergens (ie, amniotic fluid and transplacental transport) and also from the maternal immune system. The findings of sequential appearance of first food-related and later in the preschool age of inhalant allergen-related IgE despite constant environmental exposure to the inhalant allergens by birth is a common knowledge. To study the mechanisms of age-dependent changes in the allergic phenotypes, simultaneous measurements of antigen-specific IgE, IgA, IgG_1, IgG_4, and IgG in serum, nasal secretion, and saliva by the DLC chip as well as measurements of cytokine levels in these samples might be helpful. The present

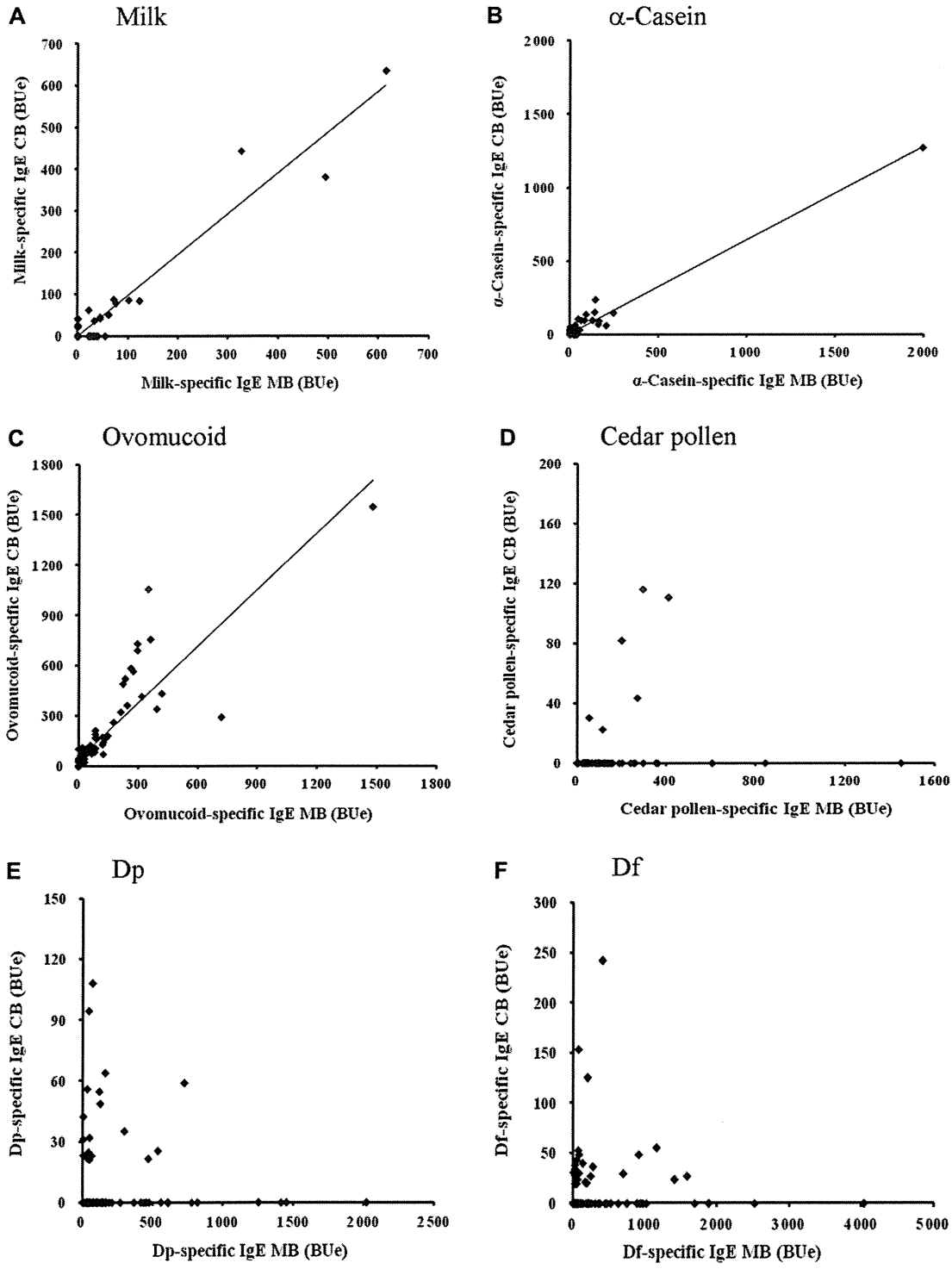


FIG 4. Comparison and correlation of allergen-specific IgE levels between CB and MB analyzed by the DLC chip. Allergen-specific IgE levels in CB and MB (n = 92) depicted in BUe. The cutoff value was 10 BUe. **A,** Milk. **B,** α-Casein. **C,** Ovomucoid. **D,** Cedar pollen. **E,** Dp. **F,** Df. Spearman's rank correlation test was used to assess the relation between the values of CB and MB. *Df, Dermatophagoides farinae; Dp, Dermatophagoides pteronyssinus.*

study found allergen-specific IgG against food and inhalant allergens but no allergen-specific IgG_4 against inhalant allergens in MB and CB. Further studies are also required on the relationship between allergen-specific IgE, IgG, and IgG_4 inductions in fetus and early infantile allergy against food and inhalant allergens.

Conclusions

Analysis using a highly sensitive DLC microarray for allergens demonstrated differences in allergen-specific IgE profiles in 92 paired MB and CB/NB samples. The finding clearly indicates that IgE levels in CB reflect *in utero* sensitization.