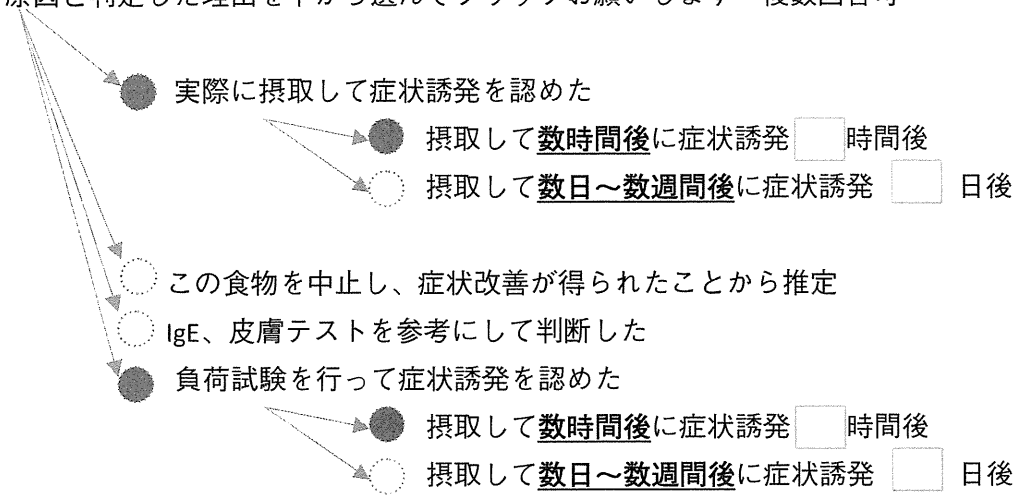
牛乳について

原因と判定した理由を下から選んでクリックお願いします　複数回答可



その詳細について　日付、摂取量、負荷試験誘発症状、負荷後何時間もしくは、何日後に症状が誘発されたのか、検査所見変化（発熱、CRP、好中球数上昇、好酸球減少、便の変化）などを、無理のない範囲でお書きください

2歳以上19歳以下 Page 8　合併症について

アレルギー疾患と炎症性腸疾患の合併についてお聞きいたします
重症度の判断は、記入していただいている先生方の基準や印象で選択してください

アレルギー疾患について

気管支喘息(BA)	○あり	●なし	○重症	○中等症	○軽症
アトピー性皮膚炎(AD)	●あり	○なし	●重症	○中等症	○軽症
食物アレルギー(FA)、即時型	○あり	●なし	○重症	○中等症	○軽症
FA 非即時型皮膚症状　あり	○し	●重症	○等症	○軽症	○

ボックス記載　発症が確認された日付もお書きください

炎症性腸疾患について

クローン病	○あり	●なし	○重症	○中等症	○軽症
潰瘍性大腸炎	●あり	○なし	●重症	○中等症	○軽症

ボックス記載　発症が確認された日付もお書きください

2歳以上19歳以下 Page 9　治療について

この年齢層の患者さんの治療は、年小児は食餌療法が中心になると思います。どんな食事内容として治療したのか、その有効性（反応性）についてお書きください
年長児ではステロイドなど抗炎症治療も中心となると存じます。全身性ステロイド、局所ステロイドに分けてお書きください
また、好酸球性食道炎では、まずPPIで症状、内視鏡像、組織像がどれだけ改善するかを確認します。

PPI使用あり　　なし

開始日
PPIの薬物名
使用用量
効果判定とその方法
　症状からの判定　改善●　症状一部持続○　不変○
　内視鏡マクロ所見　改善●　症状一部持続○　不変○　未施行○
　内視鏡ミクロ所見　改善●　症状一部持続○　不変○　未施行○

食餌療法　あり　　なし

開始日

反応性　良好　不良
内容と経過について；除去の方法と、症状の消失が得られたかについてお書きください

全身性ステロイドあり　なし

開始日

種類

最高用量

反応性　　良好　　不良

減量スピードについて

副作用の出現　　あり　　なし　　ボックス記載

局所ステロイドありなし

吸入ステロイド嚥下、エントコート（ブデソニド腸溶剤）、注腸ステロイドなど

開始日

種類

最高用量

反応性　　良好　　不良

減量スピードについて

その他の治療あり　　なし

使用開始日

治療内容

使用用量

2歳以上19歳以下 Page 10　特殊検査

特殊検査をオーダーされる場合、下から選んでクリックしてください
ポップアップが出ますので、よくお読みになって検体をご提出ください

リンパ球刺激試験	2012/8/17 提出	
GWAS 用　DNA 採血	2012/9/7 提出	
ケモカインアレイ	2012/8/17 提出	2012/9/1 提出
消化管組織マイクロアレイ		
便	2012/8/17 提出	

このページは下書きです
自由欄に書けるのが良い　結果などもコピーペーストするとよい
検査をしたその時の重症度でひもづけしたい　これをカレンダーで選べるようにしたい
マイクロアレイ採取　内視鏡組織所見と連結していること
このページは下書きです

● **GWAS 用　DNA 採血**（オーダーした場合クリック）

採取日西暦　　年　　月　　日

検査の概要 pdf　　同意書 pdf

オーダーされると、石村先生にメールが送られる

各主治医はスピッツ、同意書、倫理審査の件を石村先生と交渉していただく

● **ケモカインアレイ**（オーダーした場合クリック）

検査の概要 pdf　　同意書 pdf

採取日 1 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 2 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 3 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

結果貼り付け

● **消化管組織マイクロアレイ**（オーダーした場合クリック）

検査の概要 pdf　　同意書 pdf

採取 1 回目　○炎症あるとき　○炎症改善時○どちらとも言えない　西暦　年　月　日

採取日 2 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 3 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

この日の内視鏡所見も記入願います

結果貼り付け

● **リンパ球刺激試験**（オーダーした場合クリック）　この検査のみは前もって予約が必要です

検査の概要 pdf　　同意書 pdf

採取日 1 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 2 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 3 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

● （オーダーした場合クリック）検査名 [　　]

検査の概要 pdf 同意書 pdf

採取日 1 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 2 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 3 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

● （オーダーした場合クリック）検査名 [　　]

検査の概要 pdf 同意書 pdf

採取日 1 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 2 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

採取日 3 回目　○炎症あるとき　○炎症改善時　西暦　　年　　月　　日

ライフスタイル　住所、島かどうか　流通の特殊性がある場所か否かを知りたい

住居　都道府県、スクロール選択

- 都市部
- 田園地帯（自然が多くを占める）
- 中間帯
- 島しょ（北海道、本州、四国、九州、沖縄本島以外の島で暮らしている方）

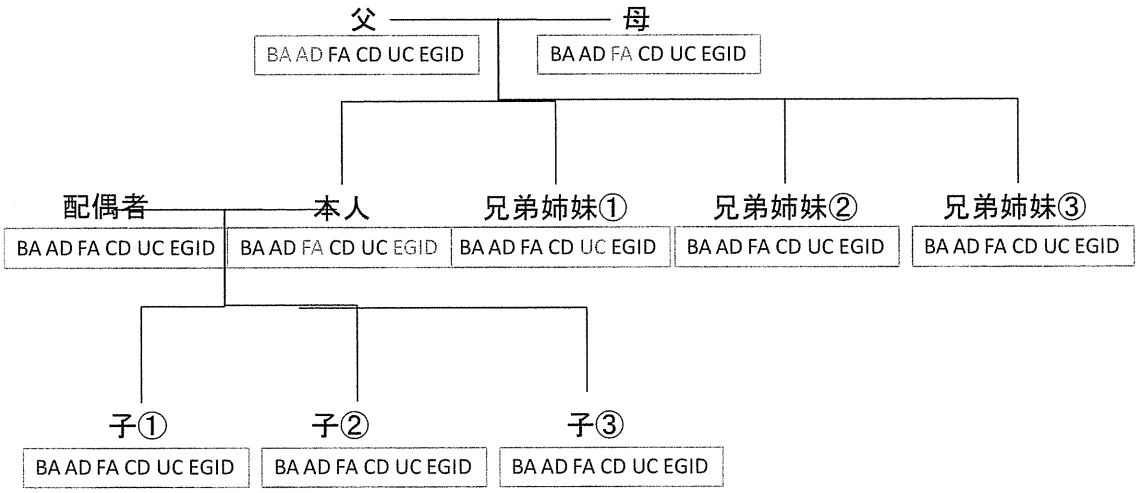
ボックス記載

遺伝歴

疾病がある場合、略語をクリックしてください

BA;気管支ぜんそく　AD;アトピー性皮膚炎　FA;食物アレルギー

CD;クローン病　UC;潰瘍性大腸炎 EGID; 好酸球性消化管疾患



ボックス記載

0-19 歳のリスクファクター質問項目

妊娠合併症

不明　　なし　　あり

妊娠中の母の牛乳、乳製品摂取

不明　完全除去　少なめに摂取していた　特に意識せず摂取していた　　乳製品を毎日摂っていた

出生体重 [　　] g

分娩形式

自然　　帝王切開　　吸引　　そのほか

出生時の特記事項

なし　あり　呼吸障害　細菌感染症　　その他

[　　]

感作は皮膚からか、消化管からかを知りたい

乳児湿疹　乳児期のアトピー性皮膚炎

下痢をしやすい　便秘傾向　消化管感染での入院歴

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
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著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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箕輪圭 新井勝大	下痢	独立行政法人国立成育医療研究センター病院編集、松井陽・奥山虎之編集主幹	国立成育医療研究センター病院小児臨床検査マニュアル	診断と治療社	東京	2013	55-58

Ⅳ．研究成果の刊行物・別刷

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http://dx.doi.org/10.1016/j.jaci.2012.08.040

Antigen-specific T-cell responses in patients with non–IgE-mediated gastrointestinal food allergy are predominantly skewed to T_H2

To the Editor:

IgE-mediated allergy is triggered by cross-linking of antigen-specific IgE antibodies on the cell surfaces of mast cells and basophils, followed by local accumulation and activation of inflammatory cells, including eosinophils and T_H2 cells. T_H2 cells produce such cytokines as IL-4, IL-5, and IL-13, which promote IgE production and eosinophilopoiesis and play central roles in the development of chronic allergic inflammation. On the other hand, non–IgE-mediated allergies, such as hypersensitivity pneumonitis, are considered mediated by cellular immunity, which has not been thought to involve antigen-specific T_H2 cells because IgE antibody would be detected if T_H2 cells were activated. Non–IgE-mediated gastrointestinal food allergies include food protein–induced enterocolitis syndrome (FPIES), food protein–induced proctocolitis, and food protein–induced enteropathy. The precise underlying mechanisms are almost unknown, except for a fundamental role of TNF-α,[1] presumably because this disease entity is relatively rare in incidence and is encountered during infancy in human subjects but not seen in experimental animals. Here, for the first time, we were able to detect antigen-specific T_H2 cell responses in infants with non–IgE-mediated gastrointestinal food allergies by analyzing 89 blood samples collected from all over Japan.

The antigen-specific lymphocyte stimulation test is a classic method for investigating antigen-specific T-cell proliferation and theoretically should be applicable to the study of gastrointestinal food allergies. However, a couple of previous studies demonstrated that the antigen-specific lymphocyte stimulation test was useful, whereas another study found no such usefulness.[2] We hypothesized that this controversy was due to contamination of the antigen preparations with LPS and tested this hypothesis. The limulus amebocyte lysate assay detected high concentrations of LPS in commercially available milk protein preparations, as previously reported (see Table E1 in this article's Online Repository at www.jacionline.org).[3] In addition, significant lymphoproliferative responses were found in the presence of as little as 10 pg/mL LPS (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org), and PBMCs from younger children showed more pronounced lymphoproliferation in response to LPS (see Fig E1, *B*). Therefore we attempted to remove contaminating LPS from milk protein preparations by passing them through a prepacked endotoxin affinity column. However, a high LPS concentration was detected even after that treatment (see Table E1), and therefore we obtained a special β-lactoglobulin preparation with very low contaminating LPS levels (kindly provided by Bean Stalk Snow, Tokyo, Japan). Further studies were performed by using these milk protein preparations, which contained LPS at a final concentration of less than 5 pg/mL.

Next, to elucidate what types of antigen-specific immune responses are induced in patients with gastrointestinal food allergies, we cultured PBMCs from patients and control subjects in the presence and absence of LPS-depleted milk component proteins. The study enrolled 65 patients with gastrointestinal food allergies, 12 patients with IgE-mediated cow's milk allergy (CMA) who showed only nongastrointestinal symptoms on ingestion of milk, and 12 control subjects who showed absolutely no symptoms on ingestion of milk. Table I[4] summarizes the clinical symptoms, clinical diagnosis, and demographic data for the 2 patient groups. None of the patients with gastrointestinal food allergies had detectable levels of IgE against milk proteins in sera. We were unable to recruit infants with IgE-mediated CMA who were age matched with the infants with non–IgE-mediated

TABLE I. Demographic characteristics of the patients

	IgE-mediated CMA		Gastrointestinal food allergies	
	No.		No.	
Age (mo)	12	38.0 (26.5-60.0)	65	2.0 (1.0-4.0)
Male/female sex	12	7/5	65	40/25
Day of onset	12	—	65	32.5 (7.0-115.5)
Symptoms at onset				
Vomiting	12	0% (0/12)	65	53.8% (35/65)
Bloody stool	12	0% (0/12)	65	47.7% (31/65)
Diarrhea	12	0% (0/12)	65	47.7% (31/65)
Failure to thrive	12	0% (0/12)	65	38.4% (22/65)
Lethargy	12	0% (0/12)	65	38.4% (22/65)
Fever	12	0% (0/12)	65	18.5% (12/65)
Eczema	12	100% (12/12)	65	7.7% (5/65)
Wheeze	12	33.3% (3/12)	65	0% (0/65)
Laboratory data				
Milk-specific IgE (IU/mL)	12	56.95 (11.74-90.8)	65	<0.34 (<0.34)
Peripheral blood eosinophils (%)		Not examined	53	7.7 (3.6-13.5)

Data are expressed as medians (interquartile ranges). The inclusion criteria were as follows: (1) gastrointestinal symptoms were present more than 2 hours after ingestion of milk and (2) 3 of Powell's criteria were fulfilled,[4] including (a) switch to therapeutic milk leading to resolution of symptoms, (b) differential diagnosis from other disorders, and (c) verified body weight gain. A definitive diagnosis based on the results of oral food challenge tests that were performed after complete resolution of the initial symptoms was achieved in 19 patients. Patients with gastrointestinal symptoms within 2 hours after ingestion of milk were excluded. On the basis of such symptoms as vomiting, diarrhea, and failure to thrive, the patient group (n = 65) consists of 34 patients with FPIES, 4 patients with food protein–induced enteropathy syndrome (enteropathy), and 27 patients with food protein–induced proctocolitis syndrome (proctocolitis). A definitive diagnosis based on the results of oral food challenge tests was achieved in 13 and 6 patients with FPIES and proctocolitis, respectively. None of the patients underwent endoscopic biopsy.

TABLE II. Antigen-specific lymphoproliferation and cytokine production profiles in patients with gastrointestinal food allergies, patients with IgE-mediated allergy, and control subjects

	Control subjects		IgE-mediated CMA		Gastrointestinal food allergies			
	No.		No.		No.		*P* value†	*P* value‡
Proliferation (SI)*	20	1.290 (0.830-1.738)	9	3.077 (2.484-3.492)	65	2.894 (2.004-7.147)	<.01	<.001
Cytokine (pg/mL)								
TNF-α	12	74.69 (58.44-144.8)	10	77.78 (58.04-141.4)	65	241.0 (89.21-729.6)	NS	<.05
IL-6	12	79.24 (36.36-193.8)	10	337.9 (57.43-1021)	65	1151 (157.0-4802)	NS	<.01
IL-1β	11	26.02 (6.880-46.47)	10	27.49 (6.548-65.04)	64	48.75 (11.7-136.1)	NS	NS
IL-2	12	4.15 (0.0-10.04)	10	12.31 (7.23-17.58)	58	16.32 (7.760-39.49)	NS	<.01
IL-3	12	0.0 (0.0-0.38)	10	0.40 (0.0-3.61)	62	4.22 (0.0-29.49)	NS	<.05
IL-4§	12	5.365 (2.895-6.358)	10	3.795 (2.033-7.788)	65	5.670 (2.775-12.06)	NS	NS
IL-5	12	2.080 (0.0-19.56)	10	46.59 (4.663-173.5)	65	63.66 (7.360-310.4)	NS	<.01
IL-10	12	9.285 (3.075-15.71)	10	56.17 (18.74-76.91)	65	57.92 (12.61-198.8)	NS	<.05
IL-13	12	21.61 (0.270-65.04)	10	82.56 (16.28-555.3)	65	291.7 (22.10-1417)	NS	<.01
IFN-γ	11	3.910 (0.0-67.06)	10	31.91 (3.635-102.0)	65	71.86 (5.49-303.4)	NS	NS
IL-17	12	0.0 (0.0-2.350)	10	7.635 (1.710-39.63)	65	7.150 (0.0-17.83)	NS	NS

PBMCs from each patient were stimulated separately with each of 5 different milk protein preparations, and the data show the highest concentration of each cytokine detected in response to the 5 different stimuli. Data are expressed as medians (interquartile ranges).

*The stimulation index *(SI)* was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm).

†Nonparametric test to compare control subjects and patients with IgE-mediated CMA.

‡Nonparametric test to compare control subjects and patients with gastrointestinal food allergies.

§According to the standard curve, the minimal detection limit was 5.88 pg/mL.

gastrointestinal food allergies. This study was approved by regional ethics committees, and written informed consent was obtained from the guardians of all patients and control subjects.

The details of the lymphoproliferation test and cytokine production assay are described in the Methods section in this article's Online Repository at www.jacionline.org. In brief, PBMCs from heparinized peripheral blood were suspended at a cell density of 1×10^6/mL in AIM-V medium (Gibco, Grand Island, NY) without serum. Lymphoproliferation was measured by using tritiated thymidine uptake during a 16-hour period after a 5-day stimulation with 100 μg/mL of each LPS-depleted milk protein preparation (α-lactalbumin, β-lactoglobulin, and α-, β- and κ-caseins). PBMCs were suspended at 1×10^6/mL in RPMI 1640 medium supplemented with 5% autologous plasma to investigate the antigen-specific cytokine production profiles. Culture supernatants were harvested at day 6 after stimulation with 100 μg/mL of each LPS-depleted milk protein preparation, and the cytokine production profiles were investigated by using the Luminex multiplex cytokine analysis kits (Millipore, Bedford, Mass) and ELISA (R&D Systems, Minneapolis, Minn).

In the first series of experiments, we investigated milk protein–specific lymphoproliferation in the control subjects, patients with IgE-mediated CMA, and patients with gastrointestinal food allergies. The lymphoproliferation level was similar in the patients with IgE-mediated CMA and those with gastrointestinal food allergies. Unlike in previous studies, however, the control subjects showed almost no proliferation (Table II). We presume that this was due to the extensive depletion of LPS contaminating the antigen preparations and the use of serum-free medium.

In the next experiments we investigated the cytokine production profiles in these subjects. TNF-α concentrations in the culture supernatants of milk protein–stimulated PBMCs from patients with gastrointestinal food allergies were significantly greater than those seen in patients with IgE-mediated CMA or control subjects. However, TNF-α levels in supernatants from patients with IgE-mediated CMA and control subjects were similar (Table II). Significantly higher concentrations of another proinflammatory cytokine, IL-6, were also seen only in the patients with gastrointestinal food allergies.

The concentrations of 3 T_H2 cytokines, IL-3, IL-5, and IL-13, in the supernatants of milk protein–stimulated PBMCs from patients with IgE-mediated CMA tended to be higher than those in the control subjects, but the differences did not reach statistical significance. In contrast, statistically significant and much higher concentrations of these T_H2 cytokines were found for the patients with gastrointestinal food allergies. Another T_H2 cytokine, IL-4, was undetectable in almost all subjects, and there were no differences among the 3 groups.

Concentrations of the T_H1 cytokine IFN-γ and the T_H17 cytokine IL-17 did not show statistically significant differences between any 2 groups.

The milk component that caused the most prominent tritiated thymidine uptake or the most prominent IL-2 or TNF-α production varied among the patients (see Fig E3 in this article's Online Repository at www.jacionline.org), suggesting that the lymphoproliferation and cytokine production observed in these assays were indeed antigen specific. In addition, the IL-5 concentration in the culture supernatant of cow's milk protein–stimulated PBMCs from patients with gastrointestinal food allergies correlated significantly with the peripheral blood eosinophil ratio at disease onset (see Fig E4 in this article's Online Repository at www.jacionline.org), suggesting that our *in vitro* assay reflects the *in vivo* conditions in these patients.

Collectively, T_H2 cytokines, including IL-3, IL-5, and IL-13, but not the T_H1 cytokine IFN-γ or the T_H17 cytokine IL-17 were significantly produced *in vitro* by milk protein–stimulated PBMCs from patients with gastrointestinal food allergies. The findings that tritiated thymidine uptake correlated significantly with IL-13 production (data not shown) along with the absence of milk-specific IgE antibody strongly suggest that the IL-13 detected in our assay was not produced by basophils in the PBMC fraction. IL-13 is a well-established mediator of intestinal

epithelial cell damage in patients with injuries and inflammatory diseases through activation of the tumor necrosis factor-like weak inducer of apoptosis-fibroblast growth factor-inducible molecule 14 (TWEAK-Fn14) axis.[5] Thus in addition to the previously known TNF-α, IL-13 might play a crucial role in the pathogenesis of gastrointestinal food allergies.

In conclusion, antigen-specific T-cell responses in patients with non–IgE-mediated gastrointestinal food allergy are predominantly skewed to T_H2. It remains unclear why antigen-specific IgE antibodies were not detected in these patients. Possible explanations are that neonatal B cells scarcely express IL-4/IL-13 receptors[6] or that production of IgE antibodies had just started but was still undetectable. This question warrants further study.

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Forkhead box protein 3 (*FOXP3*) hypermethylation is associated with diesel exhaust exposure and risk for childhood asthma

To the Editor:

Traffic-related air pollutants, such as diesel exhaust particles (DEP), significantly contribute to the pathogenesis of wheezing and asthma in early childhood.[1] These illnesses are characterized by chronic airway inflammation caused by a dysregulated immune system.[2] Attention has recently been directed toward regulatory T (Treg) cells because they are important in suppressing immune responses against nonspecific stimuli,[3] such as DEP. The suppressive phenotype of Treg cells is conferred by stable expression of forkhead box protein 3 (*FOXP3*).[3] Transcriptional silencing of *FOXP3* through hypermethylation of CpG islands in the promoter and intronic regions has been identified as a hallmark of committed Treg cells and human diseases, including asthma.[3,4] As such, Nadeau et al[4] reported increased *FOXP3* hypermethylation in blood DNA to be associated with diminished Treg cell function and increased asthma severity in children exposed to polycyclic aromatic hydrocarbons, a component of DEP. In this study we test the novel hypothesis that early (birth) and consistent exposure to high levels of traffic pollution alters *FOXP3* methylation status in DNA from saliva in a manner that correlates with DEP exposure, predicts wheezing/asthma in later life, or both. The oral cavity provides an important first line of defense against DEP exposure for children because mouth breathing is a common path of exposure.[5] Furthermore, other aerodigestive track tissues, such as buccal cells, have been successful in characterizing DNA methylation with respect to air pollutants and airway inflammation.[6,7] The ancillary goal is to establish a noninvasive, high-throughput, and quantitative assay for measuring risk of asthma linked to traffic-related air pollution.

TABLE I. Distribution of *FOXP3* percentage methylation in the sample population stratified by respiratory outcomes

Description	Mean	Minimum	Maximum	SD
Study sample	21.30	0.00	62.40	17.40
Wheezing phenotype				
Nonwheezers	17.00	0.00	55.10	16.20
Persistent wheezers*	35.84	18.10	58.35	15.20
Early transient wheezers*	24.16	0.32	61.30	17.90
Asthma status				
Nonasthmatic	19.50	0.00	62.40	16.90
Asthmatic†	32.70	1.10	58.40	17.90

*Significant difference between persistent wheezers ($P < .01$) and early transient wheezers ($P < .05$) compared with nonwheezers.
†Significant difference between asthmatic and nonasthmatic children ($P < .05$).

METHODS

Heparinized blood samples were stored at room temperature and transferred to the National Research Institute for Child Health and Development in Tokyo. The following procedures were performed no later than 24 hours after phlebotomy. PBMCs were obtained from peripheral blood by using Ficoll-Hypaque gradient sedimentation (Lymphocyte Separation Medium; ICN Biochemicals, Aurora, Ohio). The viability determined by using trypan blue dye exclusion (Sigma, St Louis, Mo) always exceeded 95%. PBMCs were suspended at a cell density of 1×10^6/mL in AIM-V medium (Gibco) without serum for lymphoproliferation, and in RPMI 1640 medium (GIBCO/Life Technologies, Gaithersburg, Md) in the presence of 5% autologous plasma for cytokine production assays.

Lymphoproliferation was measured based on tritiated thymidine (Amersham, Tokyo, Japan) uptake during a 16-hour period after 5 days of stimulation with 100 μg/mL of each LPS-depleted milk protein preparation (α-lactalbumin, Sigma; β-lactoglobulin, Bean Stalk Snow; and α-, β-, and κ-caseins, Sigma) at 37°C in a humidified 5% CO_2 atmosphere. Incorporated tritiated thymidine was counted with a liquid scintillation counter (TopCount NXT; PerkinElmer Life Sciences, Boston, Mass). The stimulation index was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm).

Culture supernatants were harvested at day 6, and the cytokine production profiles were investigated by using Luminex multiplex cytokine analysis kits (Millipore) and ELISA (R&D Systems).

The lymphoproliferation assays and cytokine production assays were performed in duplicates and triplicates, respectively.

There was a significant positive correlation between the IL-2 concentration in the PBMC culture supernatant and lymphoproliferation (stimulation index) after stimulation with κ-casein ($r = 0.269$, $P = .025$; see Fig E2). Similar tendencies were also found when PBMCs were stimulated with other milk protein preparations (data not shown).

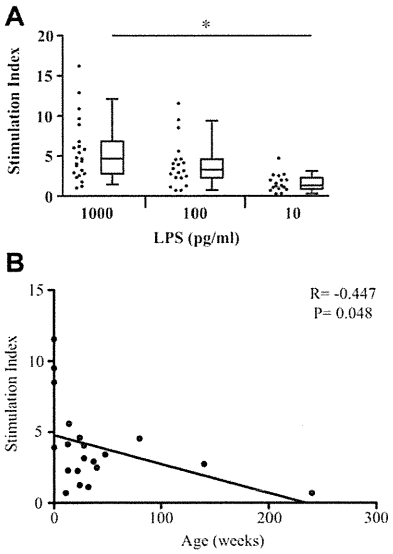

FIG E1. A, LPS at as little as 10 pg/mL can induce lymphoproliferation. PBMCs from young children (n = 60, 0-60 months of age) were stimulated with various concentrations of LPS (Sigma) for 5 days. Lymphoproliferation was measured by using tritiated thymidine uptake. The stimulation index was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm). *$P < .05$. **B,** LPS-induced lymphoproliferation was inversely associated with age. PBMCs from young children (n = 21, 0-240 weeks of age) were stimulated with 100 pg/mL LPS (Sigma) for 5 days. Lymphoproliferation was measured by using tritiated thymidine uptake. The stimulation index was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm).