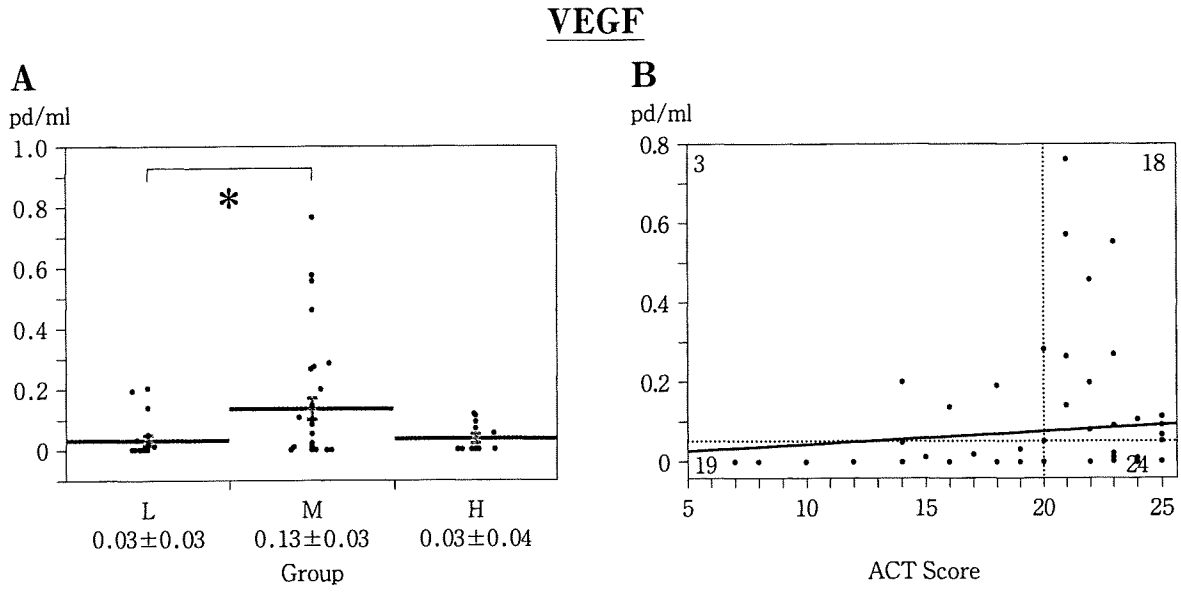

図5　EBC 中 VEGF 濃度と喘息コントロール状態の関連
A：L 群：ACT 20 点未満，M 群：ACT 20-24 点，H 群：ACT 25 点満点。L 群に比して M 群は有意に高値を示した。数値および，図中の平均線および bar は各群における VEGF 濃度の平均値±標準誤差を示す。
B：VEGF のカットオフ値 0.05 pg/ml および，ACT スコア 20 点を破線で示した。図内の数値は各象限に属する人数を記した。実線は近似式を示す：y=0.0033x−0.0090，r=0.101，p=0.4256

表4　各分子間の相関

	IP-10	VEGF	IL-1β	FeNO
IL-1ra	0.265	0.160	<0.0001**	0.0003**
	−0.141	0.178	0.738	0.436
IP-10	−	0.0013**	0.148	0.061
	−	0.394	−0.183	−0.235
VEGF	−	−	0.384	0.489
	−	−	−0.111	0.088
IL-1b	−	−	−	0.0249*
	−	−	−	0.280

各分子間の相関について，Pearson の積率相関係数を用いて解析した。
上段に有意確率を，下段に相関係数を示した。

ステロイド薬の用量を決定した場合，従来の喘息コントロール状態を指標とする場合と比べて少ない用量で同等以上のコントロールが得られると報告されている[14]。FeNO が気管支喘息の気道炎症のモニターに有用とされる病態生理学的背景として，喘息気道では，誘導型 NOS (inducible isotype of NOS：iNOS) の発現が，健常気道に比較して有意に亢進しているために，FeNO 上昇をきたすことが示唆されている[15]。細胞レベルでは，iNOS はマクロファージ，気道上皮細胞の他に，好酸球も発現しうることが報告されている一方[16]，好中球での発現は認めないとする報告がある[17]。実際，FeNO は気道の好酸球性炎症の程度と相関を認めることが，誘発喀痰[18,19]，粘膜生検[20,21]，気管支肺胞洗浄液 (Broncho-alveolar lavage fluid：BALF)[22] などの検体において報告されている。また，喘息患者への実験的ライノウイルス感染時にも FeNO が上昇するが，FeNO の上昇が大きいほど気道過敏性悪化が少ないことが報告されており，NO の増悪抑制的役割も示唆されている[23]。

今回のわれわれの結果では，ACT スコアと FeNO との有意な相関は認められなかった（**図**

1B)。既報においても，FeNOと喘息コントロール状態は相関しないとする報告が複数ある[24,25]。その背景としては，自覚症状と喘息状態との間に乖離がある可能性や，FeNOはステロイド治療早期に反応する'rapid response marker'であり[26]，自覚症状とFeNOの変動に時間的な解離がある可能性が考えられる。また，FeNOが喘息病態を反映しないサブセットが存在する可能性があり，好酸球性炎症の寄与が少ない喘息ではFeNOが低値となることや，FeNO高値でも無症状の場合があることが報告されている[27]。これらのことから，喘息管理においては，コントロール状態とFeNOの双方を考慮することが望ましいとされている[25]。しかしながら，今回の検討では，FeNOの閾値を55.7 ppbとした場合，コントロール不良状態を予測する感度は0.36と低かったものの，陰性的中率，すなわちFeNO低値であれば，コントロール状態良好である確率は0.72であり，単独の指標としても臨床的な有用性があることが示唆された（**表2**）。

今回，さらにEBCについて網羅的に液性因子濃度を解析した。EBCは，呼気により気道分岐部において発生した乱流によって，エアロゾルとなって呼気中に遊離した気道上皮被覆液を水蒸気とともに回収したものである。ある程度肺胞レベルの情報も反映するが，中枢側の気道情報の寄与が多いとされている[28]。採取は非侵襲的に行うことができるが，FeNO測定よりも時間を要し，15分程度の安静換気で2-3 mlのEBCを回収する。ただし，水蒸気による希釈が生じるため，蛋白濃度は薄く，濃縮処置が必要である。今回のわれわれの検討でも，濃縮処理を行うことで**表3**に示すような測定が可能であった。

喘息気道由来のEBCについては，種々のサイトカイン，ケモカイン，脂質メディエーターが測定されている[26]。しかしながら，網羅的な解析については，プロテインアレイを用いた1報があるのみであり[29]，定量性のあるビーズアレイシステムを用い，27分子と多くの炎症性マーカーを検討した報告はない。

今回のわれわれの検討では，IL-1β，IL-1raはコントロール不良群で低値傾向であり，喘息コントロール不良マーカーである事が示唆された（**図2, 3**）。IL-1βは，喘息気道上皮での発現亢進が報告されており[30]，線維芽細胞活性化作用を持つことから，気道リモデリングとの関連も示唆されている。EBCでの報告は1報にみられるのみであり[29]，プロテインアレイを用いて検討されているが，健常人との有意差は見出されていない。IL-1raはIL-1βのナチュラルアンタゴニストであるが，IL-1βと並行して上昇することが知られている。今回の検討でもIL-1β濃度と高い相関を示し，生体内でのフィードバック機構が働いていることが示唆された（**表4**）。今回の検討では，IL-1β，IL-1raともに，コントロール不良状態を予測する感度は低かったが，IL-1βは93.8 %と測定可能率が高く，陰性的中率，すなわち低値であれば，コントロール良好である確率は約0.7であり，臨床的有用性が示唆された（**表2**）。

IP-10はTh1に特異的に発現しているCXCR3のリガンドであり，Th1の選択的集積に関与するケモカインとされている。さらに*In vitro*では，IP-10は好酸球に発現しているCCR3に拮抗作用を持ち，好酸球集積を抑制する事が報告されており[31]，アレルギー性炎症を抑制する分子である可能性が想定されてきた。一方，喘息患者気道でIP-10発現が上昇していること[32]，マウスにおけるIP-10の強制発現では喘息病態が悪化する事[33]も報告されており，その位置づけは明確には定まっていない。喘息におけるEBC中IP-10濃度の検討は少ないが，健常者との比較では，未治療喘息患者において上昇している事が報告されている[29]。

今回の検討では，コントロール良好群で有意にIP-10濃度が高く，コントロール良好マーカーである事が示唆された（**図4**）。この背景として，IP-10はTh1指向性ケモカインとして，相対的に局所のTh2バランスを緩和し，結果的に喘息のコントロールが良好となった可能性が想定された。IP-10の測定可能率は34.4 %であり，閾値を0.78 pg/mlとした場合，コントロール良好状態を予測する感度は0.36と低かったが，特異度，陽性的中率は高く，測定可能であって高値をとれば，コントロール状態良好である確率は0.94であり，臨床的な有用性があるものと考えられた（**表2**）。さらに，喘息患者の気道粘膜生検において，経口ステロイド薬の使用でIP-10発現が上昇することが

報告されており[34]，今回の結果からはステロイド薬の効果のバイオマーカーとなっている可能性も示唆された。

VEGFに関しては，今回のわれわれの検討では，コントロール良好群で有意に濃度が高く，コントロール良好マーカーである事が示唆された（**図5**）。しかしながらVEGF発現は，喘息患者の気道粘膜，喀痰中，BALF中で健常者に比べ亢進している事[35]，喀痰中VEGF濃度は1秒量と負の相関を示すこと[36]が報告されている。さらにVEGFを気道に強制発現したマウスの実験系ではアレルギー性炎症は増悪することも報告されており[37]，喘息の増悪因子である事を示唆する報告が多い。一方で，喫煙者や間質性肺炎のBALF中で低下する事が報告されており[38]，VEGF発現低下は産生細胞である上皮障害を反映している可能性も示唆されており，今回の結果とは合致する解釈である。今回の検討では，コントロール良好状態を予測する感度は低かったが，特異度，陽性的中率は約0.8と高く，VEGF高値であればコントロール良好である確率は0.86であった（**表2**）。

近年の診療ガイドラインでは，気管支喘息の長期管理方針の決定にあたり，コントロール状態の把握が必須となっている。コントロール状態を評価する方法としては，ACTなどの症状アンケート，ピークフローモニタリング（PEFM），そして呼吸機能検査が用いられている。症状アンケートの長所は簡便性であるが，短所は自覚症状と気道閉塞の程度に乖離がある症例ではPEFMのような客観的指標との乖離が生ずる可能性があり，%PEFやPEF変動との相関が弱い事も報告されている[39]。一方PEFMの長所は，気道閉塞の状態を経時的かつ客観的に把握しうる点であるが，短所としてわが国の成人における認知率が29％と低く[40]，連日の手技を完遂できる症例が限られているのが現状である。

コントロール状態の指標と異なる病態の評価軸が，気道炎症モニタリングである。FeNOはPEFの日内変動と相関していること[41]，EBC中のTNF-αとTGF-βが，PEFの週内変動と相関している事が報告されており[29]，手技が煩雑なPEFMの代替指標となりうることが示唆されている。さらに，コントロール状態ではなく，FeNOを指標とした管理の有用性も示唆されてきている[14]。

今回の検討では，症状アンケートと，気道炎症マーカーであるFeNOおよびEBC中IL-1ra，IL-1β，IP-10，VEGF値との間に一定の関連があることを見出し，臨床的に長期管理に有用なマーカーの候補を抽出した。今後は，これらの指標がPEFMの諸指標といかに相関しているか，これらのマーカーを指標とした管理が臨床的に有用かどうかについてが，検討課題と考える。

結　論

気管支喘息管理において，従来の喘息コントロール状態に加えて，病態の根幹にある気道炎症をモニタリングする有用性が示唆されている。非侵襲的な気道炎症モニタリングの手法として，FeNO，EBC中の液性因子濃度を網羅的に検討し，コントロール不良マーカーとしてFeNO，EBC中IL-1β，IL-1ra濃度を抽出した。これらの指標は陰性的中率が約0.7であり，低値であればコントロール良好である可能性が高いことが示唆された。また，コントロール良好マーカーとしてIP-10，VEGFを抽出した。これらの指標は陽性的中率が約0.9であり，高値であればコントロール良好である可能性が高いことが示唆された。

以上から，気管支喘息の気道炎症モニタリングにおいて，FeNOやEBCの解析が可能であり，コントロール状態との関連があることから，一定の臨床的有用性が示された。これらの指標がPEFMなどの気道閉塞の指標といかに相関しているか，またこれらを指標とした管理が臨床的に有用かどうかについてが，今後の検討課題と考える。

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The relationships between the levels of cytokines and chemokines in exhaled breath condensate and the control status of asthma

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Background : The usefulness and importance of the monitoring of airway inflammation in asthma control has been recently suggested and establishment of non-invasive techniques are required in clinical practice. We tested the usefulness of measuring of fraction of exhaled NO (FeNO) and the levels of chemokines/cytokines in exhaled breath condensate (EBC). We comprehensively analyzed 27 targets in EBC from asthma patients and determined the relationships between asthma control and the levels of these targets.

Method : By using beads array system Luminex, we simultaneously measured the concentrations of 15 cytokines, 7 chemokines, and 5 growth factors in EBC from 64 patients under standard treatment. The levels of fraction of exhaled NO (FeNO) were also determined. The status of asthma control was analyzed by Asthma Control Test (ACT).

Results : The levels of FeNO, IL-1β, and IL-1ra tended to be higher in poor control group and there were significant positive co-relationships between these markers. These markers show~0.7 negative predictive value to predict poor control status. In contrast, the levels of IP-10, VEGF tended to be lower in poor control group and there was significant co-relationships between these markers. The level of IP-10 was also significantly co-related with ACT score. These markers show~0.9 high positive predictive value to predict good control status.

Conclusions : Beads array system was applicable to the analysis of EBC and the clinical utility of FeNO and EBC anaysis was suggested. The elevated levels of FeNO, IL-1β and IL-1ra were related to poor asthma control and the elevated levels of VEGF and IP10 were related to good control. These results also suggested the different airway inflammatory profile between good and poor control asthma patients.

Key words : Bronchial asthma, Exhaled breath condensate, FeNO (Fraction of exhaled Nitric Oxide), IP-10 (Interferon-γ-inducible 10 KD protein), IL-1β

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特集II 気管支喘息のモニタリングをめぐって

呼気ガス*

長瀬洋之**
大田　健**

Key Words：exhaled nitric oxide, asthma, predictive marker

はじめに

気管支喘息の病態において，気道炎症が中心的役割を果たしていることが明らかとなっている．しかしながら，現状のガイドラインにおける気管支喘息管理は，主に症状やピークフローなどの喘息コントロール状態を指標としている．より中心的に病態を反映する指標として，気道炎症の程度を客観的に評価できるマーカーの確立が期待されている．喀痰中好酸球，呼気凝縮液などが研究目的で検討されているが，結果が即時には得られにくい指標であり，日常臨床ではいまだに実用化されていない，一方呼気ガスは，もっとも非侵襲的に検討でき，結果が即時に得られる指標として期待されている．

呼気分析は，呼気臭を診断に応用したギリシャ時代にはじまる．呼気ガスは窒素，酸素，水，一酸化窒素(NO)，一酸化炭素(CO)をはじめとして，1,000以上の揮発性ガスを含むとされている．気管支喘息においては，NO, CO, H_2O_2や脂質メディエーターについて検討されているが，もっとも広く検討され，臨床応用に近いと考えられる指標は，呼気中一酸化窒素濃度(FeNO)である．気管支喘息においては，1993年にはじめてFeNO値の上昇が示唆された．

表1　FeNO測定における留意点

1. FeNOは流速依存性である．
2. 鼻腔由来NOの混入を避ける．
   呼気時に5 cmH_2Oの抵抗，ノーズクリップは使用しない．
3. 喫煙でFeNOは低下．
   1時間以内に喫煙しない/喫煙歴を記録する．
4. スパイロメトリー後はFeNO値は一過性に低下．
   FeNO値測定後にスパイロメトリーを行う．
5. 硝酸塩を含む食物(レタスなど)摂取で上昇．
   基本的には禁食が望ましい．

気道におけるNOは，喘息中枢気道に発現している誘導型NO合成酵素(inducible isotype of nitric oxide synthase；iNOS)が，L-アルギニンからNOを合成することで産生される．非喘息気道ではiNOSの発現は少ないため，喘息気道に特異的な炎症マーカーとして期待されている．また，気管支喘息患者の呼気凝縮液のpHは低いことが報告されているが，低pHではNO_2^-から非酵素的にNOに変換されうることも報告されている．本稿では，気管支喘息におけるFeNOモニタリングの方法，臨床応用について述べたい．

FeNO測定に影響を与える因子とその対処(表1)

FeNO値の測定法については，ATS/ERSが推奨する測定方法を発表しており[1]，ある程度標準化

* The usefulness of exhaled nitric oxide measurements in diagnosis and treatment of asthma.

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表2　FeNOの正常上限値

論文	症例数	上限値(ppb)
9)	405名 小児	25.2 (上限95%)
7)	1,131名 喘息症例を除外した非喫煙成人	24～54 (上限97.5%)
10)	528名 選択せず	41.1 (上限95%)
11)	204名 喘息症例を除外した非喫煙成人	19.7 (上限95%)

(文献[12]より引用改変)

表3　喘息診断のためのFeNOカットオフ値

論文	症例数	カットオフ(ppb)	感度(%)	特異度(%)
13)	96名 小児	9.7	86	92
14)	51名 新患	20	88	79
15)	101名 消防士	47	42	96
16)	喘息34名 健常28名	31	72	76
17)	記載なし	18.5	69	71

されているため，これに則って説明したい．

まず，FeNO値は流速依存性であり，呼気流速が速くなるとFeNO値は低下する[2]．測定値のレンジが広くとれて，なおかつ，速すぎず遅すぎず，実行可能な呼気流速として50m*l*/sが推奨されている．

鼻腔はNOの豊富な産生源であるため，鼻腔気の口腔内へのもれこみは，FeNO値を上昇させる[3]．そのため，呼出時に抵抗をかけ，鼻腔からの漏れ込みを防止する必要があり，5 cmH_2Oの抵抗値が推奨されている．また，呼吸機能検査で用いられるノーズクリップは鼻腔からの出口を閉塞し，口腔内への漏れ込みを生ずる可能性があるため使用しない．また，吸気は経口的に行う．測定機器のうち，もっともコンパクトで普及が期待されているNIOX MINO®では，経口的に吸気しないと測定できないようになっており，呼気抵抗や呼気流速も上記の条件を満たすように設定できる．

喫煙はFeNO値を低下させる．喫煙の急性効果としては，5分後にFeNO値は約20%低下し，15分後には回復するとされているため[4]，測定前約1時間程度は喫煙しない．また，禁煙1週間後にはFeNO値は上昇することから，喫煙は長期的にFeNO値のベースライン値を抑制することが示されており，喫煙者では低値に出ることに留意する．

FeNO値はスパイロメトリー施行5分後には10ppb程度低下し，15～30分後には回復すること，β_2刺激薬吸入後は上昇傾向を示すことが報告されているため[5]，呼吸機能検査前にFeNO値を測定することが望ましい．

食事の影響としては，レタスなどの硝酸塩を含む食物でFeNO値が上昇する可能性があり，鼻腔由来のNO値へは摂食後3時間程度影響をおよぼすため[6]，基本的には禁食が望ましい．これらのポイントを表1にまとめた．

FeNO値の気管支喘息診断における有用性

健常人におけるFeNO値は表2に示すように，20～40ppbとされている．また，Ln(FeNO)＝−0.0026＋(0.013×身長(cm))＋(0.010×年齢(歳))のような近似式も作られており，身長，年齢に伴い，FeNO値は増加するが，近似式のあてはまりは悪く，個人差が大きいことも示唆されている[7]．

喘息診断におけるFeNOのカットオフ値が表3に示すように検討されており，20～40ppbとされ，感度69～88%，特異度71～96%と報告されている．

FeNO値の気管支喘息管理への応用

吸入ステロイド薬の使用量を増減する際に，喘息症状に基づいて行った場合とFeNO値に基づいて行った場合を比較した検討がある[8]．喘息症状としては，夜間覚醒，短時間作用型β_2刺激薬の使用回数，ピークフロー値の変動，1秒量について基準を設け，1つでも満たさない場合に，吸入ステロイド量が増量される．一方，FeNO群では，15ppbを下回るまで吸入ステロイドを増量し，15ppbを下回った場合は，減量を続ける．なお，FeNO値は250m*l*/sの呼気流速で測定されており，標準的な50m*l*/sでは，35ppb程度に相当する．吸

表4　標準的長期管理指標にFeNO値を加える有効性

論文	試験内容	症例数	カットオフ (ppb)	症状	吸入ステロイド量
18)	症状 vs. 症状＋FeNO値	85名 小児	30	FeNO群で気道過敏性低下	有意差なし
19)	症状＋β_2刺激薬使用＋1秒量 vs. 上のすべて＋FeNO値	47名 小児	20	FeNO群でMEF改善	FeNO群で多い
20)	症状 vs. 症状＋FeNO値	118名	26	有意差なし	FeNO群で少ない

表5　吸入ステロイド薬の減量/中止の成功予測におけるFeNO値測定の有用性

論文	試験内容	症例数	増悪予測基準	
21)	吸入ステロイド減量	40名 小児	FeNO ＞22ppb	感度78.6% 特異度68.6%
22)	吸入ステロイド中止	78名	ΔFeNO ＞60%	感度50% 特異度65%
23)	吸入ステロイド中止	40名 小児	中止4週後FeNO ＞49ppb	感度71% 特異度93%

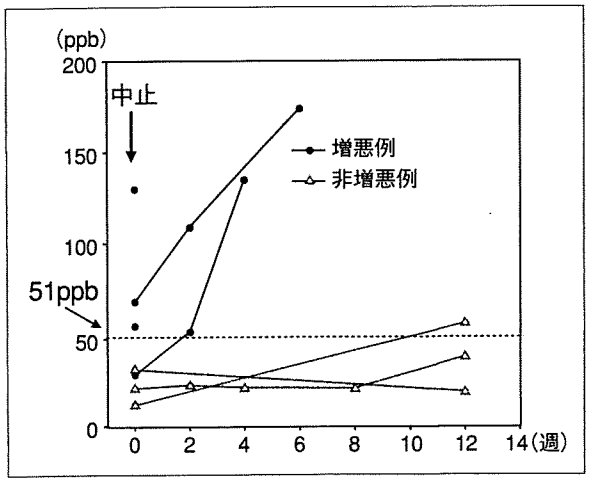

図1　吸入ステロイド薬中止前後のFeNO値

入ステロイド薬は，フルチカゾンが用いられ，100～1,000μg/日の間で1年間かけて投与量が調節された．その結果決定された吸入ステロイド量はコントロール群では567μg/日で，一方FeNO群では292μg/日と有意に少量であった．また，その量を維持量としてさらに1年間経過観察したところ，FeNO群では維持量が少ないにもかかわらず，コントロール群と発作頻度の有意差を認めなかった．この試験のほかにも，標準的な長期管理指標にFeNO値を加える有用性について検討した報告が複数ある(表4)．FeNO値を指標に加えることによって，最終的な吸入ステロイド量が同等でも，気道過敏性の改善を得たとする報告や，より少ない吸入ステロイド量で同等のコントロールを得た報告があり，FeNO値測定の有用性が示唆されている．

さらに，表5に示すように，吸入ステロイド薬の減量や中止が成功するかどうかの予測因子として，FeNO値の有用性が検討されている．減量成功については，22ppbをカットオフ値とした場合，良好な感度と特異度で予測できることが報告されている．中止に関しては，FeNO値が中止前から60%以上増加することや，中止4週後のFeNO値が49ppbを超えることなどで，増悪を予測できる可能性が報告されている．

また，上記の検討では，吸入ステロイド薬中止後，増悪までの日数の中間値は，成人では17日，小児では36日と報告されている．そこで，われわれは3か月以上吸入ステロイド薬単剤でトータルコントロールを達している症例を対象とし，吸入ステロイド中止時に加えて2週後にもFeNO値を測定した(図1)．中止時または2週後のどちらかでFeNO値51ppbを超えていればその後増悪することが示唆され，症状増悪前に吸入ステロイド中止を回避する指標となることが示唆された．

おわりに

近日，スウェーデン製のコンパクトなNIOX MINO®が入手可能となっている．米国のFDAで

承認されており，わが国でも将来的には保険適応が目指されている．本体(75万円)の耐用回数は1,500回で，300回使用可能なセンサーキット(45万円)を装着すると使用開始可能であり，1回あたりの測定コストは2,000円程度である．測定結果は，呼出後約100秒で得られ，日常診療での応用も期待できるスピードである．呼気中一酸化窒素濃度(FeNO)値は気管支喘息の診断，管理に有用で，非侵襲的かつ即時に結果が得られる指標として，今後の実臨床への応用が期待されるところである．

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Functional Analysis of the Thymic Stromal Lymphopoietin Variants in Human Bronchial Epithelial Cells

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Thymic stromal lymphopoietin (TSLP) is an IL-7–like cytokine that triggers dendritic cell–mediated T helper (Th)2 inflammatory responses, and is implicated in the pathogenesis of allergic diseases in humans. Two *TSLP* splice variants have been reported. To find functional genetic variants that might contribute to disease, we conducted analyses of single nucleotide polymorphisms (SNPs) of the *TSLP* gene in human bronchial epithelial cells. We surveyed SNPs on the *TSLP* gene by sequencing genomic DNA from 36 subjects, and characterized the linkage disequilibrium of the gene. We examined whether the SNPs have functional effects on mRNA expression or protein production using real-time PCR, reporter gene analysis, and enzyme-linked immunosorbent assay. We identified a total of 23 polymorphisms in the *TSLP* gene. The long form of *TSLP*, which is associated with allergic inflammation, was highly induced by poly(I:C) (double-stranded RNA) stimulation in normal human bronchial epithelial cells (NHBE) ($P = 0.0060$). The SNP rs3806933 (−847C > T) in the promoter region of long-form *TSLP* was found to create a binding site for the transcription factor activating protein (AP)-1, and *in vitro* functional analyses demonstrated that the SNP enhanced AP-1 binding to the regulatory element. The functional variant increased promoter-reporter activity of long-form *TSLP* in response to poly(I:C) stimulation in NHBE. Functional genetic polymorphism of the *TSLP* gene appears to contribute to Th2-polarized immunity through higher TSLP production by bronchial epithelial cells in response to viral respiratory infections.

Keywords: bronchial epithelial cells; dsRNA; polymorphisms; splicing variants; *TSLP*

CLINICAL RELEVANCE

We identified a single nucleotide polymorphism that creates a binding site for activating protein-1 and affects the transcriptional efficiency of the long-form TSLP induced by poly(I:C) in bronchial epithelial cells. The variant may be involved in the pathogenesis of T helper (Th)2-mediated diseases.

Dendritic cells (DCs) play a crucial role in the pathogenesis of allergic diseases, and thymic stromal lymphopoietin (TSLP) activates $CD11c^+$ DCs and induces production of T helper (Th)2-attracting chemokines (1–3). TSLP-activated DCs prime naïve T cells to produce the inflammatory cytokines IL-4, IL-5, and IL-13, while down-regulating IFN-γ and IL-10 (2–5). Recent murine studies have shown a central role for TSLP in the development of allergic asthma (6, 7). Lung-specific expression of a *TSLP* transgene induces allergic airway inflammation characterized by infiltration of Th2 cells, goblet cell hyperplasia and increased serum immunoglobulin (Ig)E levels (6). *TSLP receptor* (*TSLPR*) knockout (KO) mice exhibit strong Th1 responses, with high levels of IL-12 and IFN-γ, but low production of IL-4, -5, -10, -13 and IgE (7). Furthermore, *TSLPR* KO mice failed to develop an inflammatory lung response to an inhaled antigen (7). In humans, epithelial cells trigger DC-mediated inflammation by producing TSLP in allergic diseases (1), and *TSLP* expression in asthmatic airways is increased and correlated with both the expression of Th2-attracting chemokines and disease severity (8). These findings prompted us to search for a functional *TSLP* polymorphism as a candidate genetic factor for involvement in respiratory diseases such as asthma.

Recent studies have shown higher expression of *TSLP* mRNA at host environmental interfaces such as human primary skin keratinocytes, bronchial epithelial cells, and lung fibroblasts (1, 2). Respiratory viral infections can influence both the development and severity of asthma, and frequently cause acute exacerbation of the disease (9, 10). However, the cellular and molecular mechanisms of the interactions between viral infection and allergic inflammation remain unclear (10). The epithelial cell is a first defense line in the pathogenesis of viral respiratory infections, which can initiate innate immune responses (9). Toll-like receptor (TLR)3 has been shown to recognize double-stranded (ds)RNA and mediate antiviral activity against rhinovirus infection of human bronchial epithelial cells (11–13). dsRNA produced by RNA viruses such as rhinoviruses and respiratory syncytial viruses during replication in infected cells is a potent stimulus for innate antiviral immune responses, and

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polyriboinosinic:polyribocytidylic acid [poly(I:C)] is thought to mimic the effects of dsRNA (14). Airway epithelial cells play an essential role in the innate host defense against infection (15) and highly express TLR2, TLR3, TLR5, and TLR6 (16). It was only recently found that the inflammatory mediators IL-1β and TNF-α regulate human *TSLP* gene expression, and human *TSLP* mRNA levels also increase after exposure to TLR2, TLR3, TLR8, and TLR9 ligands in airway bronchial epithelial cells (17, 18). Another study has shown that TSLP is released by small airway epithelial cells in response to bacterial peptideglycan, lipoteichoic acid, and poly(I:C) (19).

In this study, we examined mRNA expression of two *TSLP* splicing variants separately, and found that the long form of *TSLP* was highly induced by poly(I:C) stimulation in normal human bronchial epithelial cells (NHBE). We also found that a promoter polymorphism (rs3806933) created a binding site for the transcription factor activating protein (AP)-1 and enhanced AP-1 binding to the regulatory element. The functional variant also increased promoter-reporter activity of long-form *TSLP* in response to poly(I:C) stimulation in NHBE.

MATERIALS AND METHODS

Additional details are provided in the online supplement.

Screening for Polymorphisms

To identify single nucleotide polymorphisms (SNPs) in the human *TSLP* gene, we sequenced all exons, including 4.1 kb of the 5′ flanking region and a 1-kb continuous 3′ flanking region of the last exon, except for regions with interspersed repeats, from 24 subjects with asthma and 12 control volunteers. The 16 primer sets listed in the online supplement (*see* Table E1) were designed on the basis of genomic sequences from the GenBank database (accession number AC008572.6). Sequences were assembled and polymorphisms were identified using the SEQUENCHER program (Gene Codes Corporation, Ann Arbor, MI).

Statistical Analysis

Pairwise linkage disequilibrium (LD) was calculated as r^2 by using Haploview 3.2 (Massachusetts Institute of Technology, Cambridge, MA; http://www.broad.mit.edu/mpg/haploview/). Genotype distribution among 36 subjects in this study was compared with genotype frequencies in the public JSNP 550typed database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/) and the International HapMap project (http://www.hapmap.org/index.html.en), by the contingency χ^2-test. Comparisons in reporter assays, mRNA expression analysis, and protein expression analysis were performed with Student's *t* test or the Mann-Whitney U-test. Statistical significance was defined at the standard 5% level.

Cells, Reagents, and Stimulation

NHBE, normal human lung fibroblasts (NHLF), and bronchial smooth muscle cells (BSMC) were purchased and maintained using medium kits (BulletKit; Cambrex, East Rutherford, NJ). IL-1β, TNF-α, IL-4, and IL-13 were purchased from Peprotech EC, Ltd. (London, UK). Cells were stimulated with 10 μg/ml poly(I:C) (InvivoGen, La Jolla, CA), 100 ng/ml lipopolysaccharide (LPS) (InvivoGen), 1 μg/ml macrophage-activating lipopeptide (MALP)-2 (Alexis, Lausen, Switzerland), 10 ng/ml IL-1β, 10 ng /ml TNF-α, 100 ng/ml IL-4, and/or IL-13. RNaseA was purchased from Roche Diagnostics (Basel, Switzerland). Phosphate-buffered saline (PBS) was used as the vehicle.

Quantitative Real-Time RT-PCR and Enzyme-Linked Immunosorbent Assay

The expression of *TSLP* was determined by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) using SYBR Premix Ex Taq (Takara, Shiga, Japan). In all experiments, the amounts of cDNA were standardized by quantification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). TSLP in culture supernatants was measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN). The mean lower detection limit of the ELISA kit was approximately 31.3 pg/ml according to the manufacturer's protocol. However, in the present work, we calculated the lower detection limit from the standard curve, and the sensitivity of the TSLP ELISA kit was 15.6 pg/ml.

Determination of Transcriptional Initiation Sites

To determine the 5′ arrangement of the *TSLP* mRNA, 5′-RACE was performed by using the SMART RACE cDNA amplification kit (Clontech, Mountain View, CA). We extracted total RNA from poly(I:C)-activated NHBE and used it for SMART cDNA synthesis. We obtained the major PCR products, and after isolation and subcloning, eight clones were identified and sequenced.

Luciferase Assay

The promoter and last exon fragments from human genomic DNA were amplified by PCR. PCR products were subcloned into the reporter gene pGL3-promoter vector (Promega, Madison, WI). TRANSFAC Professional 10.3 (http://www.biobase.de/pages/) was used to predict putative transcription factor-binding sites. To confirm AP-1–mediated promoter activity of the long-form *TSLP*, NHBE were transiently transfected with a *TSLP* long-form promoter-driven luciferase reporter plasmid with expression vectors for AP-1 (*c-jun* and *c-fos*) or a mock vector.

Electrophoretic Mobility Shift Assay and Biotinylated Oligonucleotide Precipitation Assay

We prepared nuclear extracts from NHBE as previously described (17). Electrophoretic mobility shift assays (EMSAs) were performed using Gel Shift Assay Systems (Promega). Binding affinity of transcription factors to oligonucleotides was measured *in vitro*. NHBE cell lysates interacting with the oligonucleotides were precipitated by avidin-sepharose, and the bound proteins were separated on SDS-polyacrylamide gels. AP-1 was detected by immunoblotting with anti–AP-1 antibodies (Ab-2) (Oncogene Research Products, San Diego, CA).

RESULTS

Identification of Genetic Polymorphisms in the *TSLP* Gene and Linkage Disequilibrium

We found 23 genetic variants in the *TSLP* region by resequencing DNA samples from the 36 subjects. A total of seven polymorphisms had estimated minor allele frequencies (MAF) of greater than 5% (Table 1), and we calculated r^2 as the statistical value for pairwise LD among these seven variants (Table E2). Allele frequencies of SNPs based on the sequencing of the 36 DNA samples did not differ from those in the general Japanese population, which were obtained from the public JSNP 550typed database and International HapMap project (Table E3) (data not shown).

The SNPs rs3806932, rs3806933, rs2289276, rs11466741, rs2289277, and rs10073816 were in strong LD ($r^2 \geq 0.87$), and most of them were located in the putative promoter regulatory regions of the two splice variants (Figures 1A and 1B). We next estimated the frequencies of the haplotypes and identified three common haplotypes in the 36 subjects (Figure 1C), covering more than 97% of the population.

Poly(I:C)-Induced Expression of Long Splice Form of *TSLP* in NHBE

The gene *TSLP* contains four exons, and a public database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene) noted two alternative splice variants of *TSLP*: short-form and long-form *TSLP*. The splice variants use different initiation methionine codons for protein translation. Recent expression studies did not examine mRNA expression of the two splicing variants separately (17–19). We investigated whether the splice variants

TABLE 1. LOCATIONS AND ALLELE FREQUENCIES OF POLYMORPHISMS IN *TSLP*

SNP No.		SNP Long Form*	Location Long Form	SNP Short Form*	Location Short Form	Allele Frequency (%)	rs No.†
1	A > G	−3693	5′ flanking site	−5394	5′ flanking site	1	13186909
2	AGTG > del	−3641	5′ flanking site	−5342	5′ flanking site	1	—
3	T > A	−3492	5′ flanking site	−5193	5′ flanking site	1	—
4	G > A	−3404	5′ flanking site	−5105	5′ flanking site	3	17551370
5	A > C	−2590	5′ flanking site	−4291	5′ flanking site	4	10455025
6	A > G	−1914	5′ flanking site	−3615	5′ flanking site	29	3806932
7	A > G	−1070	5′ flanking site	−2771	5′ flanking site	1	—
8	C > T	−847	5′ flanking site	−2548	5′ flanking site	29	3806933
9	C > T	−82	exon 1	−1783	5′ flanking site	26	2289276
10	C > T	414	intron 1	−1288	5′ flanking site	4	1898671
11	C > A	591	intron 1	−1111	5′ flanking site	3	10062929
12	C > T	1117	intron 2	−585	5′ flanking site	26	11466741
13	G > A	1164	intron 2	−538	5′ flanking site	1	—
14	C > G	1479	intron 2	−223	exon 1	29	2289277
15*	C > G	1560	intron 2	−142	exon 1	22	2289278
16	C > T	1908	intron 3	207	intron 1	3	—
17	A > C	4403	exon 4	2702	exon 2	1	—
18	G > A	4740	exon 4	3039	exon 2	1	—
19	A > G	4997	exon 4	3296	exon 2	3	11466749
20	G > A	5306	exon 4	3605	exon 2	3	11466750
21	G > A	5901	exon 4	4200	exon 2	29	10073816
22	C > G	6143	3′ flanking site	4442	3′ flanking site	3	11466754
23	A > C	6339	3′ flanking site	4638	3′ flanking site	3	—

Definition of abbreviations: SNP, single nucleotide polymorphism; TSLP, thymic stromal lymphopoietin.
* Numbering according to the genomic sequence of *TSLP* (accession number AC008572.6). Position 1 is the A of the initiation codon.
† Number from the dbSNP of NCBI (http://www.ncbi.nlm.nih.gov/SNP/).

of *TSLP* mRNA were up-regulated by dsRNA and other TLR ligands separately in cultured NHBE, NHLF, and BSMC by examining expression patterns of variants by isoform-specific real-time PCR using cDNA panels of various human tissues (Figure E1A). The long splice form of *TSLP* was not expressed in every examined tissue, whereas the expression of the short splice form was consistently observed in all of the tissues (Figure E1A). We next investigated the *TSLP* expression in lung-derived primary cells, including NHBE, NHLF, and BSMC (Figure 2A). Although the short form of *TSLP* was expressed in NHBE, NHLF, and BSMC, the long form of *TSLP* was not spontaneously detected in NHBE and was barely expressed in NHLF and BSMC (Figure 2A). Respiratory infections are well recognized as major triggers of exacerbation of asthma in children and adults (9, 10). We further assessed whether the long form of *TSLP* could be induced by some pathogen-associated microbial patterns recognized by TLRs and other pattern-recognition receptors. In NHBE, the long form was strongly and temporarily induced within 4 hours by stimulation with poly(I:C) but not by LPS or macrophage-

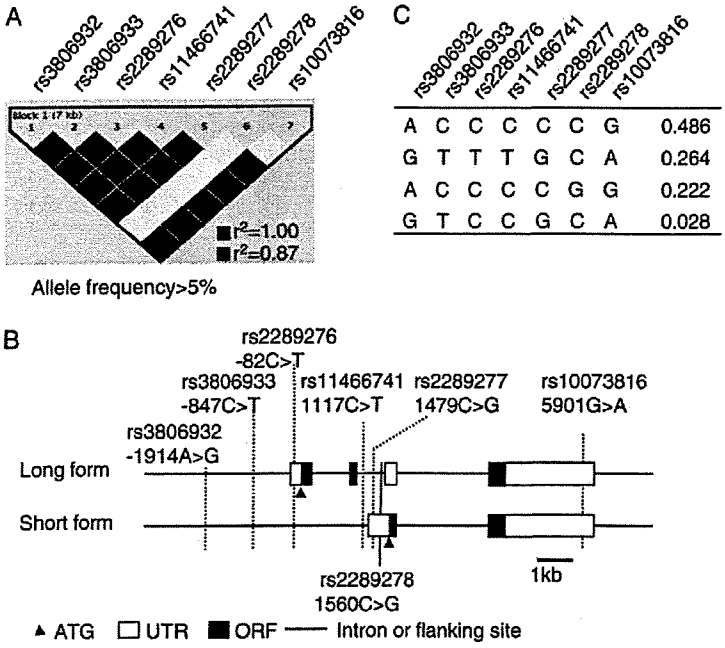

Figure 1. Single nucleotide polymorphisms (SNPs) and pairwise linkage disequilibrium (LD) map of the *thymic stromal lymphopoietin* (*TSLP*) gene. (*A*) LD structure at the *TSLP* locus using SNPs with allele frequencies greater than 5%. Pairwise r^2 values for all combinations of SNP pairs are shown in *grayscale*. (*B*) A graphic overview of polymorphisms with allele frequencies greater than 5% identified in relation to the exon/intron structure of the human *TSLP* gene. The translation start site (ATG), untranslated region (UTR), and open reading frame (ORF) are shown by *solid triangles*, *open boxes*, and *solid boxes*, respectively. (C) Haplotype frequencies for *TSLP* SNPs.

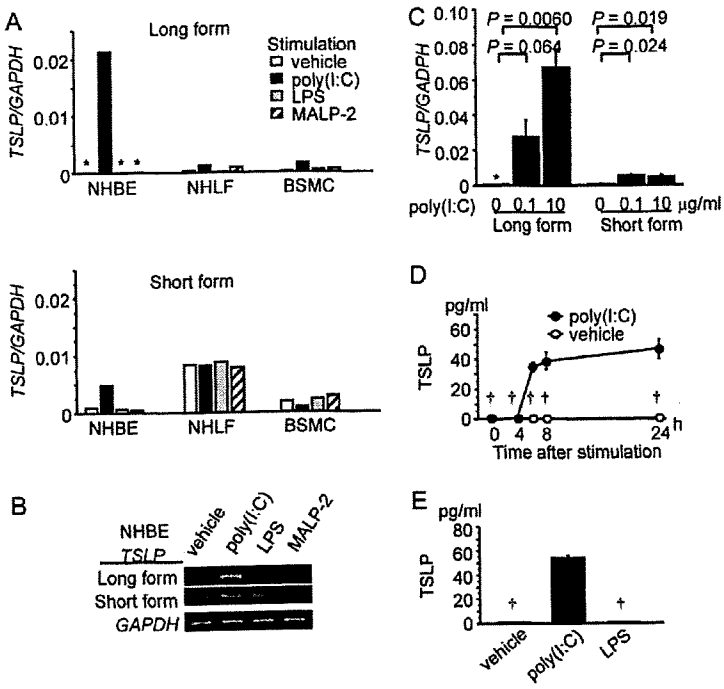

Figure 2. Induction of *TSLP* expression and TSLP protein production in normal human bronchial epithelial cells (NHBE) in response to pathogen-associated microbial patterns. (*A*) Quantitative RT-PCR assay of the long and short splice forms of *TSLP*. NHBE, normal human lung fibroblasts (NHLF), and bronchial smooth muscle cells (BSMC) were stimulated for 4 hours. PBS was used as vehicle. * Not detectable. (*B*) Representative agarose gel showing RT-PCR products from the long and short splice forms of *TSLP* in NHBE stimulated for 4 hours. (*C*) NHBE derived from four individuals were stimulated with indicated doses of poly(I:C) for 4 hours. The expression levels were normalized with *GAPDH* expression. Data represent mean ± SEM of duplicate samples and are representative of two independent experiments. *P* values were obtained by Student's *t* test. (*D* and *E*) ELISA of TSLP in culture supernatants of NHBE stimulated for the indicated times (*D*), and for 24 hours (*E*). A dagger (†) indicates that the concentration was below the detection threshold (< 15.6 pg/ml). Data represent mean ± SD of triplicate samples.

activating lipopeptide 2 (MALP-2), and the short splice form was scarcely induced (Figures 2A, 2B, and E1B). We confirmed the significant induction of the long splice form after stimulation with poly(I:C) using NHBE derived from four individuals (Figures 2C and E1C), and treatment of the poly(I:C) preparation with RNaseA abolished the *TSLP* induction (Figure E1D). The genotypes and Ct values compared with *GAPDH* of four individuals are shown in Table E4.

Recent studies have shown that proinflammatory and Th2 cytokines play important roles in the induction of *TSLP* expression by bronchial epithelial cells or keratinocytes during allergic inflammation (19, 20). We next examined whether treatment of NHBE cells with proinflammatory cytokines such as IL-1β or TNF-α would enhance the long-form *TSLP* gene expression. The long form of *TSLP* was induced by IL-1β and TNF-α in NHBE cells; however, the induction levels were lower than that by poly(I:C) (Figure E2A). Neither of these cytokines influenced the expression level of short-form *TSLP* (Figure E2A). We further examined effects of Th2 cytokines on the *TSLP* mRNA expression, and IL-4 and IL-13 synergistically enhanced the long-form expression of the *TSLP* gene by NHBE cells in response to poly(I:C) (Figure E2B). Expression of short-form *TSLP* was not induced by these Th2 cytokines (Figure E2B). The results imply the importance of the regulation of long-form *TSLP* expression in patients during allergic inflammation.

To examine the effect of the induction of mRNA expression of *TSLP* in NHBE, we further measured TSLP protein products in the culture supernatant of NHBE by ELISA, and found that the TSLP protein was upregulated within 6 hours after poly(I:C) stimulation (Figure 2D). However, no protein induction of TSLP was detected after LPS stimulation (Figure 2E).

Determination of the Transcriptional Start Sites of the Long and Short Forms of *TSLP*

To identify the transcriptional start sites of the two splicing forms of the *TSLP* gene, we performed a rapid amplification of cDNA ends (RACE) procedure using mRNA from NHBE cells. The locations of the *TSLP*-specific primers used in 5′-RACE cDNA amplification for the long and short splice forms are indicated in Figure E3A. The major products were obtained by using long- and short-form-specific primers, respectively (Figure E3B). The 5′-ends of these clones are shown in Figure E3C. In these clones, we could not find any novel exon.

Regulatory Effect of SNP on Expression of the Long Splice Form of *TSLP* in Response to poly(I:C)

To clarify whether the SNPs in the *TSLP* promoter region affected the expression of the two splice variants, we constructed plasmid clones containing the NF-κB regulatory region, which has been previously reported, promoter SNPs, and the exon 1 SNP of the short- and long-form *TSLP* genes. Each construct included a possible combination of the promoter and exon 1 SNPs of the two splice variants and a luciferase gene transcriptional unit in the 5′-to-3′ direction (-1914A-847C-82C [Major] and -1914G-847T-82T [minor] for the long form, and -3615A-2548C-1783C-585C-223C [Major] and -3615G-2548T-1783T-585T-223G [minor] for the short-form) (Figure 3A). The effects of the promoter sequences were examined in NHBE. The reporter activity of the clones containing the promoter and exon 1 SNPs of long-form *TSLP* was enhanced by stimulation with poly(I:C), and the clone containing the -1914G-847T-82T [minor] haplotype showed significantly greater transcriptional activity than the other haplotype, -1914A-847C-82C [Major] (Figure 3A). In contrast, the reporter activities of clones of short-form *TSLP* had no effect on transcriptional activity under culture conditions with and without poly(I:C) (Figure 3A). These results implied that the SNP in the promoter region of the long form of *TSLP* was able to enhance the poly(I:C)-dependent transcriptional activity. We next conducted deletion construct work to verify that the region studied actually contained the important transcriptional regions for the long-form *TSLP* gene (Figure 3B). The deletion construct also had transcriptional activity. We also found that

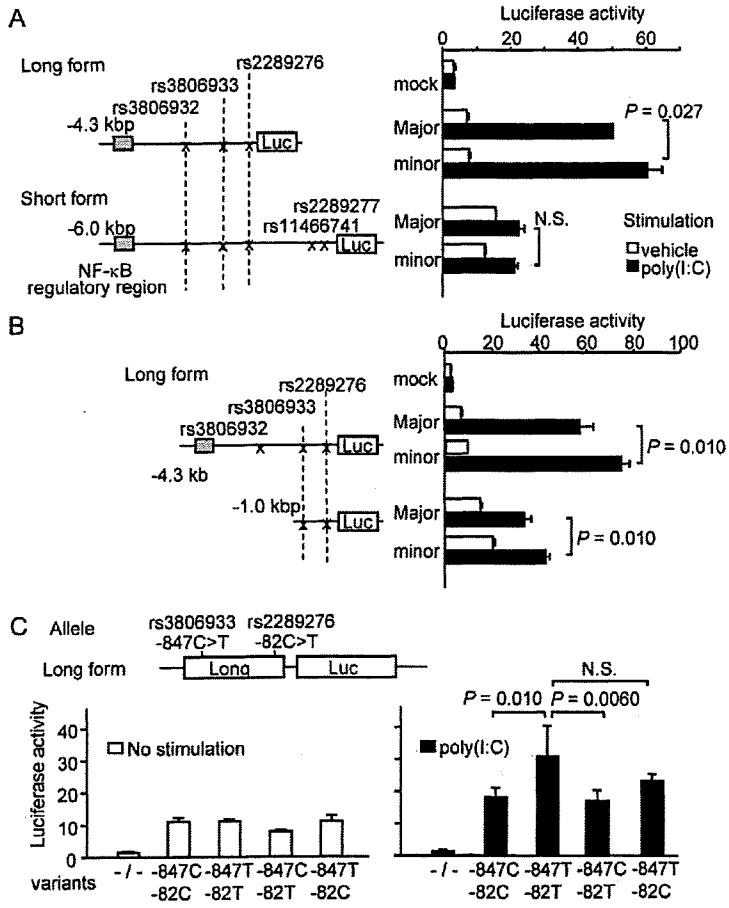

Figure 3. Splicing form- and allele-dependent transcriptional regulation of *TSLP* in NHBE stimulated with or without poly(I:C) for 4 hours. Data represent mean ± SD. (*A*) Transcriptional regulatory activities of haplotypes of long and short splice forms of *TSLP* in NHBE. The *gray box* indicates the NF-κB regulatory region. Data from three experiments in triplicate. $P = 0.027$, by Student's *t* test. (*B*) Identification of effects of SNPs in promoter region of long-form *TSLP* gene on the transcriptional activities. Data from three experiments in triplicate. $P = 0.010$ and $P = 0.010$, by Student's *t* test. (*C*) Schematic representation of the *TSLP* reporter constructs and relative luciferase activities of four haplotypes of the long splice form of *TSLP*. $P = 0.010$ and $P = 0.0060$, by Student's *t* test. Representative data from three experiments done six times.

the clone containing the -847T-82T [minor] haplotype showed significantly greater transcriptional activity than the other haplotype, -847C-82C [Major] in both clones with and without the NF-κB regulatory region (Figure 3B).

To identify the critical SNP responsible for the difference in transcriptional activity of the long splice form, we generated two other constructs, -847C-82T and -847T-82C (Figure 3C). The effects on transcriptional activity were examined in NHBE with and without poly(I:C) stimulation. No obvious transcriptional activity in these constructs was noted without stimulation, whereas, after poly(I:C) stimulation, C base substitution for the -847T base on the minor allele (-847T-82T to -847C-82T) resulted in significantly impaired transcriptional activity of the long splice form (Figure 3C). The impaired activity of the -847C-82T fragment was similar to that of the major allele (-847C-82C) (Figure 3C). These results suggested that -847C > T (rs3806933) played a functional role in transcriptional regulation of the long form of *TSLP*.

To assess the possibility of a regulatory role of the 3′-untranslated region (UTR) SNP in the expression level of *TSLP*, we examined the effect of the SNP on the stability of *TSLP* mRNA (Figure E4A). There was no difference among the stability profiles of the 3′-UTR SNP (rs10073816) in response to poly(I:C) stimulation (Figure E4B).

Transcription Factor Binding to the Regulatory SNP

We subsequently looked for nuclear factors that might bind to oligonucleotides corresponding to the genomic sequences of the promoter alleles of *TSLP*. We predicted a potential allelic difference in the cis-acting regulatory function in transcription by a bioinformatics approach (TRANSFAC). The sequence containing the -847T SNP (rs3806933) on the promoter region created a new consensus sequence corresponding to the putative binding element to AP-1 (Figure 4A).

Nuclear factor-kappa B (NF-κB) and AP-1 are transcription factors crucial for inducing the expression of various cytokines dependent on TLR3 (21, 22). Using nuclear extracts from NHBE stimulated with poly(I:C), we observed nuclear transfer of NF-κB and AP-1 within 1 h by EMSA (Figure 4B). We next examined the binding of AP-1 protein to the sequences containing the -847C > T (rs3806933) by a precipitation assay. Significant binding of AP-1 protein to the -847T oligonucleotide was detected in response to poly(I:C) stimulation; however, only faint binding activity to -847C oligonucleotides was seen with or without poly(I:C) stimulation (Figure 4C). Since the activation of total nuclear AP-1 protein without stimulation was low (Figures 4B and 4C), it appeared that the binding of AP-1 to -847T oligonucleotides was dependent on the activation of AP-1 protein. To further elucidate how AP-1 was involved in the critical SNP responsible for the difference in transcriptional activity of the long splice form, we analyzed the effects of AP-1 overexpression on the *TSLP* promoter activity, using an *in vitro* luciferase assay. The clone containing the -847T-82T haplotype showed greater transcriptional activity than those containing the -847C-82C haplotype when assayed under overexpression of AP-1 in the presence and absence of poly(I:C) (Figure 4D). Thus, AP-1 was shown to be involved in activation of the long-form *TSLP* promoter through the SNP -847C > T (rs3806933).

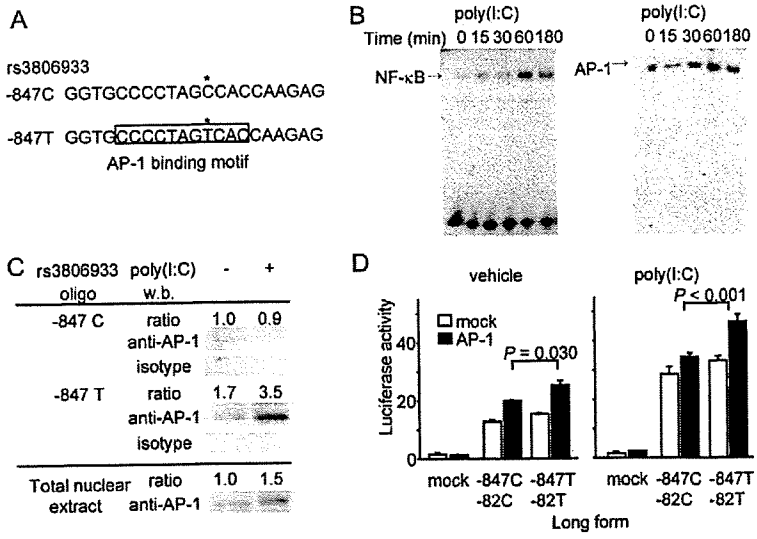

Figure 4. Differential transcription factors binding to regulatory SNP -847C > T (rs3806933). (*A*) The DNA sequences of transcription factor-binding motifs around SNPs in the *TSLP* long isoform. The positions of potential activating protein (AP)-1–binding sites are shown in the *open boxes*, and the *asterisks* represent the SNP. (*B*) NF-κB and AP-1 activation in NHBE stimulated with poly(I:C) *in vitro*. The *arrows* indicate the induced NF-κB (*left*) and AP-1 (*right*) complexes. (C) Binding affinity of transcription factors to oligonucleotides *in vitro*. Arbitrary densitometric units are shown above each band. Three independent experiments were performed with similar results. (*D*) AP-1–mediated promoter activity of the long-form *TSLP*. NHBE were transiently transfected with a *TSLP* long form promoter-driven luciferase reporter plasmid with expression vectors for AP-1 (*c-jun* and *c-fos*) or a mock vector. After 24 hours, NHBE were stimulated with or without poly(I:C) for 4 hours. Promoter activity was measured by luciferase assay.

DISCUSSION

The crucial role of the cytokine TSLP in the development of dermatitis and airway inflammation has been recognized. Of the two splice variants of the *TSLP* gene, only long-form *TSLP* was markedly induced by poly(I:C)/dsRNA stimulation in normal human airway epithelial cells and detected as TSLP protein with ELISA. Furthermore, we identified a functional SNP in the promoter region of the long-form *TSLP* that had an allele-specific effect on expression through varying affinity for the transcription factor AP-1.

Human *TSLP* is highly expressed by airway epithelial cells during allergic inflammation and potently activates immature CD11c$^+$ myeloid DCs (1). TSLP-activated DCs induce naïve CD4$^+$ T cell differentiation into Th2 cells that produce allergy-related cytokines (1–5, 8). TSLP induces human myeloid DCs to express OX40L, a member of the TNF family, and OX40L on TSLP-activated DCs triggers Th2 cell polarization, but OX40L loses the ability to polarize Th2 cells in the presence of IL-12 (23). Asthma is characterized by a Th2-type inflammation, and individuals with asthma are more susceptible to rhinovirus (RV) infections than normal individuals, having longer duration of lower respiratory tract symptoms when infected with RVs (24–26). A defective type 1 response to RVs in individuals with atopic asthma has been reported (27). When peripheral blood mononuclear cells were exposed to an RV and assessed for type 1 and type 2 cytokine production, IFN-γ and IL-12 concentrations were significantly higher in cells from normal individuals than in those from subjects with atopic asthma (27). RVs are RNA viruses that secrete a substantial level of dsRNA right after infection at the respiratory epithelium (11), and the -847T allele might cause overinduction of long-form *TSLP* transcripts via dsRNA stimulation of respiratory epithelial cells. Although little is known about what makes epithelial cells produce TSLP, the dominance of OX40L induced by TSLP over reduced IL-12 production in subjects with asthma may provide an explanation for Th2-dominant inflammation in asthma exacerbation. Overproduction of long-form *TSLP* caused by promoter polymorphisms in bronchial epithelial cells may lead to redundant Th2 responses and influence the host response to viral infection.

It is well accepted that the epithelium is an important therapeutic target for the treatment of asthma (15). Targeting TSLP itself is thought to be therapeutically efficacious as a new treatment for allergic disease (2, 28). Further studies to explore the roles of TSLP in respiratory viral infection and allergic inflammation would contribute to selecting patients most likely to respond.

Glucocorticosteroids are the most common anti-inflammatory drugs and are used as the first-line therapy for controlling asthma (29). These drugs bind to glucocorticoid receptors and inhibit the NF-κB– and AP-1–mediated transcription of various proinflammatory molecules through the glucocorticoid receptor–binding sites of their gene promoters (29, 30). Activation of NF-κB is critical for inflammation-induced expression of *TSLP* in airway epithelial cells, and an NF-κB–binding site was identified 3.7 kb upstream from the start of long-form *TSLP* transcription (17). In this study, we could not find any polymorphism around the NF-κB binding sequence. The promoter region of long-form *TSLP* containing -847T showed greater binding activity to AP-1 after dsRNA stimulation in human bronchial epithelial cells. We also found that the clone containing -847T showed greater transcriptional activity than those containing -847C when assayed under overexpression of AP-1. AP-1 is a prominent transcription factor in airway diseases and regulates the expression of multiple inflammatory proteins (30–32). These results suggest that the promoter *TSLP* SNP upregulates the microbe-induced production of TSLP by bronchial epithelial cells through enhancement of AP-1 binding to the promoter. AP-1 expression is enhanced in the asthmatic airway, and it is reduced after glucocorticoid therapy (32, 33). It is possible that the polymorphism of the *TSLP* gene-producing AP-1 site is related to the drug responsiveness.

In summary, we have demonstrated that the expression of long-form *TSLP*, which is a crucial cytokine for the induction of inflammatory Th2 responses, is highly induced by dsRNA in bronchial epithelial cells, as well as a functional promoter SNP that has an allele-specific effect on expression through altering affinity for the AP-1. These genetic factors of *TSLP* may influence the host response to viral infection, and knowledge of them will contribute to a better understanding of the pathogenesis of Th2-mediated respiratory diseases such as bronchial asthma.

Conflict of Interest Statement: M.T., Y.N., T.H., and M.H. applied for a patent regarding TSLP functional SNP in Japan. (Oct 31, 2006. No.2006-296561). S.F.Z. has stock in Amgen. None of the other authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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How to Cope with Allergic Diseases at Schools in Japan

—From the standpoint of a pediatric allergist—

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Abstract

The number of school children with allergic diseases is increasing, and various cares are considered necessary to secure the safe school lives of such children. Medical specialists and school officials developed a school life management certificate for children with allergic diseases, which serves as a tool for communication between medical facilities and schools in cases where such children need special care. Children with asthma require special attention during exercise, in dusty environments, when in contact with animals, during out-of-school activities involving overnight stay(s). Concerns for children with atopic dermatitis include stimulants that worsen skin eczema, such as perspiration, chlorine in swimming pools, and ultraviolet rays. School lunches are the most serious concern for children with food allergies. Even classes handling foodstuffs may cause health hazards, thus necessitating caution in this arena. Food allergies are the most frequent cause of anaphylaxis, but it should be noted that anaphylaxis may also occur after a combination of food and exercise or even after exercise alone. It is important that schools are aware of children with reliever drugs for anaphylaxis and asthmatic attacks.

Key words School, Allergic diseases, Bronchial asthma, Atopic dermatitis, Food allergy, Anaphylaxis

Introduction

The Research Study Committee on Allergic Diseases of the Ministry of Education, Culture, Sports, Science and Technology reported on the prevalence rates of various allergic diseases among approximately 12 million school children at elementary, junior high, and senior high schools throughout Japan in 2004. According to this report, the prevalence was 5.7% for bronchial asthma, 5.5% for atopic dermatitis, 2.6% for food allergies, 0.14% for anaphylaxis, 9.2% for allergic rhinitis, and 3.5% for allergic conjunctivitis. Although it is presumed that these prevalence rates failed to cover mild cases of atopic dermatitis and allergic rhinitis or conjunctivitis, children who have some type of allergic disease seem to account for more than 20% of all school children, even when overlapping cases of multiple allergic diseases are discounted. It is likely that every class has some children with allergic diseases, but the understanding of and countermeasures against these diseases by schools remain insufficient.

This paper describes how children with allergic diseases should be cared at school, and discusses the direction of future efforts in Japan.

Bronchial Asthma

Problematic aspects of school life for children with bronchial asthma include 1) exercise in physical education class and club activities, 2) contact with animals and activities in dusty

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environments, and 3) out-of-school activities requiring overnight stay(s). In regard to the first item, the phenomenon of exercise-induced asthma becomes clinically apparent as the severity of asthma increases. In particular, intense exercise that continues for a prolonged period of time, including running and soccer, is more likely to induce asthma. Concerning the second item, some asthmatic children also are allergic to animals and dusty environments, such as may occur when sweeping the classroom, may cause asthmatic attacks. Third, out-of-school activities involving overnight stay(s) are often associated with asthmatic attacks induced by mites or dusty environments.

Asthmatic children are usually able to participate in activities just like healthy children when they are not having an attack. However, once asthmatic attacks occur, they can create an emergency situation. Asthma is better controlled than previously due to improved drug treatments including inhaled steroid therapy. However, some patients can remain stable only with intensive treatment. It is important for school teachers to cooperate with parents in matters such as the evaluation of symptom control, possible interference with school life, and the needed response to asthmatic attacks. The use of a spacer with a bronchodilator inhaler (β_2-agonist) is required in emergency situations, but basically the decision is left to the patient. It is also necessary for the school to know whether the patient generally uses medicine during an asthmatic attack at school.

Atopic Dermatitis

The aspects of school life problematic to children with atopic dermatitis include 1) physical education classes involving swimming or prolonged activities under ultraviolet light, 2) contact with animals, and 3) post-perspiration status. In terms of the first item, the chlorine disinfectant used in swimming pools and strong ultraviolet rays are known to aggravate atopic dermatitis. The second item is problematic because children with atopic dermatitis sometimes have concomitant animal allergies. Third, perspiration is a factor that can aggravate the symptoms of atopic dermatitis, and is a great concern particularly in Japanese summer. If a shower is available after exercising, perspiration-induced aggravation of symptoms can be prevented to a considerable degree.

There has been a remarkable progress in the management of atopic dermatitis, through the use of topical treatment with steroids or tacrolimus ointment. If the patient has symptoms that cause difficulties in school life, it is important to talk with his or her parent(s) and encourage the implementation of appropriate treatment. Caution is necessary because the patient may become the target of bullying or avoid attending school.

Food Allergy

Most cases of food allergy in school-aged children are of the immediate type. Disease types of food allergy among school children are classified as follows: immediate-type allergy, oral allergy syndrome, and food-dependent exercise-induced anaphylaxis. In school-aged children, food allergies seldom lead to the aggravation of atopic dermatitis. Various agents cause food allergies, most commonly cow's milk, hen's eggs, and wheat. Buckwheat, peanuts, crustaceans, and fruits can also be causative agents. Food allergies in school children should be diagnosed on the basis of objective symptoms and the results of oral food challenge tests. Elimination of the causative agent should not be advocated simply because the patient is positive for IgE antibody against foods. This indicates the need for an appropriate response from healthcare professionals to food allergies and a proper understanding of the allergy by the parents/guardians.

The most concern in school life is the school lunch service, which is common in Japan. The presence or absence of specific changes in the school-provided lunch for children with food allergies is mainly dependent on the prefectural or municipal government. Essentially, every school child is to be provided with the same lunch. However, it is common in schools for a child in the class to bring a bag lunch because of his or her food allergy while others in the class have the lunch provided by the school. Improvement in the response to food allergies should be started by promoting an understanding of food allergies among school teachers. With this background, the range of available responses to food allergies should be determined according to the actual situation of each school lunch center and kitchen. It is true that school lunch-related health hazards