スパイロメトリーによる１秒量、努力性肺活量(FVC; forced vital capacity)、フローボリューム(FV; flow-volume)曲線が有用である。

i) 可逆性気流制限

１秒量は、気道閉塞を評価するゴールドスタンダードであり、FV 曲線は、末梢気道の状態を把握する良い指標となる。また PEF は、１秒量とともに気道閉塞を検出することができ、喘息の日常管理に有用である。

ii) 気道過敏性の亢進

気道の過敏性の評価には、アセチルコリンやその誘導体のメサコリン、あるいはヒスタミンといった気道収縮薬による気道過敏性試験を施行する。方法は、気道収縮薬の吸入により、１秒量の低下を指標とする標準法と、呼吸抵抗の上昇を指標とするアストグラフ法が用いられている。標準法では、１秒量が 20%以上の低下を示す気道収縮薬の最低濃度（閾値）か、反応曲線から１秒量を 20%低下させる濃度である PC20 を求めて評価する。喘息患者では気道過敏性試験でより低濃度の閾値、あるいは PC20 を示すことになる。特に咳のみや胸痛のみを主訴とする cough variant asthma や chest pain variant asthma の診断には、必須の検査である。

c) その他の検査所見（表２の項目４、5、６）

i) アトピー素因

アトピー型では、血清総 IgE 値の上昇がみられ、同時に抗原特異的 IgE 抗体も陽性である。抗原特異的 IgE 抗体は、皮膚反応試験（プリックテスト、皮内テストなど）か、血清反応試験(RAST; radioallergosorbent test や CAP 法、MAST 法など)により検出される。感度の点では皮膚反応試験が優れているが、最近は、より簡便な血清を用いた検査が好まれる傾向にある。問診においては、アレルギー疾患の家族歴や既往歴、生活環境として住宅環境、本人や同居者の喫煙、室内環境(空調、掃除、カーペット、建築年数、間取りや日当たりなど)、ペットの有無、職業と職場環境などが重要である。最も頻度の高い抗原は、吸入性抗原の室内塵(HD; house dust)やヒョウヒダニ(dermatophagoides)、通称チリダニ(house dust mite)である。また職業性喘息が疑われる場合には、抗原特異性 IgE 抗体の検索を症例毎に疑わしい抗原を用いて（時には研究室で調整して）行なう必要がある。

ii) 気道炎症の存在

気道炎症を臨床的にモニターするための指標は、十分に確立されていない。

血算では、好酸球の増多（500/mm^3 以上）のみられることが多い。

喀痰は通常漿液性で気泡に富み、好酸球の増多や剥離した気道上皮からなるクレオラ体を認める。喀痰がでない場合には、高張食塩水による誘発喀痰を採取して

検査することも有用である。また、将来的に普及することが予想される呼気中一酸化窒素(NO)の測定では、NOの上昇を認める。

d) 鑑別診断疾患の除外

最初から喘息と決めつけることなく、他の疾患の可能性を考慮し、鑑別診断を行なうことが重要である(表3)。

高齢化社会を迎え、うっ血性心不全による心臓喘息といわれる状態との鑑別、またその原因として、急性心筋梗塞の有無にまで思いを巡らす必要がある。また中年以降の喫煙者では、慢性閉塞性肺疾患(COPD)との鑑別、あるいは合併の有無を明らかにする。急性発症の呼吸困難と言う点では、緊急な対応を必要とする気胸と肺血栓塞栓症を見逃してはならない。また喘息には気道感染の併発が高率にみられることも考慮することが必要である。喘息を合併するアレルギー性呼吸器疾患では、通常の喘息治療でコントロールされ難い場合が多く、副腎ステロイド薬の全身投与を必要とする難治性喘息では特に、アレルギー性気管支肺アスペルギルス症やアレルギー性肉芽腫性血管炎(Churg-Strauss 症候群)などとの鑑別が必要である。

表3 鑑別すべき疾患

1. 上気道疾患：喉頭炎、喉頭蓋炎、vocal cord dysfunction(VCD)
2. 中枢気道疾患：気管内腫瘍、気道異物、気管軟化症、気管支結核、
   サルコイドーシス
3. 気管支～肺胞領域の疾患：COPD、びまん性汎細気管支炎、肺線維症、過敏性肺炎
4. 循環器疾患：うっ血性心不全、肺血栓塞栓症
5. アンギオテンシン変換酵素阻害薬などの薬物による咳
6. その他の原因：自然気胸、迷走神経刺激症状、過換気症候群、心因性咳嗽
7. アレルギー性呼吸器疾患：アレルギー性気管支肺アスペルギルス症、アレルギー性肉芽腫性血管炎(Churg-Strauss症候群)、好酸球性肺炎

4．治療

診断がつけば、JGL2006 に沿った治療を実行する。

a）概要

i）長期管理

喘息は、発作につながる可逆性の気道閉塞と気道過敏性とともに慢性の気道炎症とその結果引き起こされる気道傷害から成り立つ疾患である。したがって、治療する場合には、発作あるいは喘息症状だけではなく、背景にある気道炎症も標的として考え治療を組み立てることが、発作を起こさないことにつながる。すなわち、JGL2006 を規範として、まず患者毎に喘息の重症度を判定し(表 4 と表 5)、症状に対する治療と炎症を抑え症状を予防する治療(長期管理)の両面から、適切な薬物治療を実行することが基本となる(表 6)。

症状の治療には即効性の気管支拡張薬、長期管理としては、吸入ステロイド薬を基本薬として継続し、必要に応じて他の薬剤を併用して無症状の状態を維持するのである。喘息の治療を担当する医師側には、JGL2006 に沿った治療を喘息の病態を理解した上で実行することが望まれる。患者側には、薬剤の服用を遵守し(アドヒアランスを堅持し)、喘息の原因への曝露を回避することが要求される。良い生活環境にはフローリング、週 3 回以上の掃除、寝具の衛生管理が重要とされている。

表 4 未治療での喘息重症度の分類 (成人)

重症度[1]		ステップ 1 軽症間欠型	ステップ 2 軽症持続型	ステップ 3 中等症持続型	ステップ 4 重症持続型
喘息症状の特徴	頻度	週 1 回未満	週 1 回以上だが毎日ではない	毎日	毎日
	強度	症状は軽度で短い	月 1 回以上日常生活や睡眠が妨げられる	週 1 回以上日常生活や睡眠が妨げられる	日常生活に制限
				短時間作用性吸入 β_2 刺激薬頓用がほとんど毎日必要	治療下でもしばしば増悪
	夜間症状	月に 2 回未満	月 2 回以上	週 1 回以上	しばしば
PEF $FEV_{1.0}$[2]	%$FEV_{1.0}$, %PEF	80%以上	80%以上	60%以上80%未満	60%未満
	変動	20%未満	20～30%	30%を超える	30%を超える

1）いずれか 1 つが認められればそのステップと判断する。

2）症状からの判断は重症例や長期罹患例で重症度を過小評価する場合がある。呼吸機能は気道閉塞の程度を客観的に示し、その変動は気道過敏性と関連する。%$FEV_{1.0}$=($FEV_{1.0}$測定値/$FEV_{1.0}$予測値)×100、%PEF=(PEF測定値/PEF予測値または自己最良値)×100

表5 現在の治療を考慮した喘息重症度

現在の治療における患者の症状	現在の治療ステップ			
	ステップ1	ステップ2	ステップ3	ステップ4
ステップ1：軽症間欠型相当 ●症状が週1回未満 ●症状は軽度で短い ●夜間症状は月に1～2回	軽症間欠型	軽症持続型	中等症持続型	重症持続型
ステップ2：軽症持続型相当 ●症状は週1回以上，しかし毎日ではない ●月1回以上日常生活や睡眠が妨げられる ●夜間症状が月2回以上	軽症持続型	中等症持続型	重症持続型	重症持続型
ステップ3：中等症持続型相当 ●症状が毎日ある ●短時間作用性吸入 β_2刺激薬がほとんど毎日必要 ●週1回以上日常生活や睡眠が妨げられる ●夜間症状が週1回以上	中等症持続型	重症持続型	重症持続型	重症持続型
ステップ4：重症持続型相当 ●治療下でもしばしば増悪 ●症状が毎日 ●日常生活に制限 ●しばしば夜間症状	重症持続型	重症持続型	重症持続型	最重症持続型

表6 JGL2006: 成人喘息の長期管理

	ステップ1	ステップ2	ステップ3	ステップ4
長期管理薬 ●：通用 ○：考慮	○喘息症状がやや多いとき（たとえば月に1～2回），血中・喀痰中に好酸球増加のあるときは下記のいずれか1剤の投与を考慮 ・吸入ステロイド薬（低用量） ・テオフィリン徐放製剤 ・ロイコトリエン受容体拮抗薬 ・DSCG ・抗アレルギー薬[*]	●吸入ステロイド薬（低用量）通用 ●上記で不十分な場合は，下記のいずれか1剤を併用[*] ・テオフィリン徐放製剤 ・ロイコトリエン受容体拮抗薬 ・長時間作用性β_2刺激薬（吸入/貼付/経口） ○DSCGや抗アレルギー薬の併用可	●吸入ステロイド薬（中用量）通用 ●下記のいずれか1剤，あるいは複数を併用[*] ・テオフィリン徐放製剤 ・ロイコトリエン受容体拮抗薬 ・長時間作用性β_2刺激薬（吸入/貼付/経口） ○Th2サイトカイン阻害薬の併用可	●吸入ステロイド薬（高用量）通用 ●下記の複数を併用[*] ・テオフィリン徐放製剤 ・ロイコトリエン受容体拮抗薬 ・長時間作用性β_2刺激薬（吸入/貼付/経口） ○Th2サイトカイン阻害薬の併用可 ●上記のすべてでも管理不良の場合 ・経口ステロイド薬の追加[*]
発作時	短時間作用性吸入 β_2刺激薬[*]	短時間作用性吸入 β_2刺激薬[*]	短時間作用性吸入 β_2刺激薬[*]	短時間作用性吸入 β_2刺激薬[*]

ステップアップ：現行の治療でコントロールできないときは次のステップに進む。
ステップダウン：治療の目標が達成されたら，少なくとも3ヵ月以上の安定を確認してから治療内容を減らしてもよい。以後もコントロール維持に必要な治療は続ける。

ii) 発作への対応

長期管理を実行していても、発作が出現することもあり、発作に対する適切な対応も長期管理とともに非常に重要である。とくに喘息死をゼロにするためには、長期管理による予防効果だけではなく、死亡の直接の原因である発作に対して、適切に対応することが必須である。

発作は、時と場所を選ばず出現するので、患者自身での対応を指導することが必要である。とくに医療機関を受診しなければならないと判断する基準を明らかにして指導することが重要である。JGL2006 では、表 7 のように発作強度を分類しており、発作のために横になれない状態(中等度の発作)であれば医療機関を受診することを推奨している。とくに発作が重症化した経験のある患者、アドヒアランスの悪い患者では、担当医が CS の経口薬(例えばプレドニン)を渡しておき、30mg を目安に家庭で内服して受診するよう指導することも推奨されている。基本的には、通常の発作に対する家庭での治療をしても発作が収まらないときは、医療機関を受診し、もっと積極的で有効性の高い治療を施行しなければならないという認識を患者に持たせるよう指導する。

「発作に対する家庭での対応は、まず発作の強さを判定することから始まります。苦しくても横になれれば軽度の発作で、主治医の処方した吸入β2刺激薬の吸入あるいは経口の発作止めを頓服して下さい。目安として、吸入は 1 時間で 15〜20 分毎に動悸を感じない限り継続、経口薬は 30 分後に 1 回追加可能です。それでも収まらないときや明らかに悪化するときは 1 時間にこだわらず、受診することをお勧めします。また苦しくて横になれない中等度や話が困難な高度の発作では、ただちに気管支拡張薬を服用して受診して下さい。中等度でも気管内挿管歴や入院歴がある場合、高用量吸入ステロイド薬や経口ステロイド薬を継続投与されている場合には、家庭で経口ステロイド薬を主治医の指示に従い内服し、直ぐに受診して下さい。」という内容の話をして指導することになる。このような内容を口頭で指導するだけでなく、記載した行動計画表(アクションプラン)を作成し手渡すことも、JGL2006 の家庭での対応を実行するうえで必要である。

患者の受診後、その予後を左右する上で重要なのが医療機関での対応である(表 8)。とくに中等度よりも重症の高度(話すのが困難で動けない)や重篤・エマージェンシー(意識障害、呼吸停止)に相当する場合は、救急隊、入院設備のある病院あるいは院内での救命救急部との連携が必要となる。そして適切な治療の実行には、各患者の平素の治療内容、発作時に施行する治療内容や治療に当たっての注意点を記した診療カード(ぜん息カード、図９、１０頁)の作成が有用であると考えられる。カードに含まれる内容としては、処方されている治療薬、推奨される発作時の対応に加えて、喘息の発症時期、治療歴、入院歴、アスピリン喘息の有無、薬剤アレルギーの有無などである。

表7 喘息症状・発作強度の分類（成人）

発作強度[1]	呼吸困難	動作	検査値[3]			
			%PEF	Spo_2	Pao_2	$Paco_2$
喘鳴/胸苦しい	急ぐと苦しい 動くと苦しい	ほぼ普通	80%超	96%以上	正常	45mmHg未満
軽度（小発作）	苦しいが横になれる	やや困難				
中等度（中発作）	苦しくて横になれない	かなり困難 かろうじて歩ける	60～80%	91～95%	60mmHg超	45mmHg未満
高度（大発作）	苦しくて動けない	歩行不能 会話困難	60%未満	90%以下	60mmHg以下	45mmHg以上
重篤[2]	呼吸減弱 チアノーゼ 呼吸停止	会話不能 体動不能 錯乱，意識障害，失禁	測定不能	90%以下	60mmHg以下	45mmHg以上

1）発作強度は主に呼吸困難の程度で判定し，他の項目は参考事項とする。異なった発作強度の症状が混在するときは発作強度の重い方をとる。

2）高度よりさらに症状が強いもの，すなわち，呼吸の減弱あるいは停止，あるいは会話不能，意識障害，失禁などを伴うものは重篤と位置づけられ，エマージェンシーとしての対処を要する。

3）気管支拡張薬投与後の測定値を参考とする。

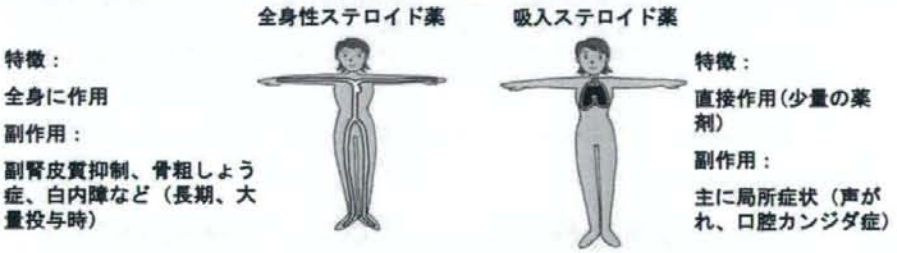
表8 喘息発作の強度に対応した管理法（成人）

治療		自宅治療可，救急外来入院，ICU管理[3]
β_2刺激薬吸入，頓用[4] テオフィリン薬頓用	喘鳴/胸苦しい	自宅治療可
β_2刺激薬吸入，頓用[4] テオフィリン薬頓用	軽度(小発作)	自宅治療可
β_2刺激薬ネブライザー吸入反復[5] エピネフリン皮下注（ボスミン®）[6] アミノフィリン点滴静注[7] ステロイド薬点滴静注[8] 酸素[9] 抗コリン薬吸入考慮	中等度(中発作)	救急外来 ・1時間で症状が改善すれば帰宅 ・2～4時間で反応不十分 ・1～2時間で反応なし 入院治療→高度喘息症状治療へ
エピネフリン皮下注（ボスミン®）[6] アミノフィリン持続点滴[10] ステロイド薬点滴静注反復[8] 酸素[11] β_2刺激薬ネブライザー吸入反復[5]	高度(大発作)	救急外来 1時間以内に反応なければ入院治療 悪化すれば重篤症状の治療へ
上記治療継続 症状，呼吸機能悪化で挿管[12] 酸素吸入にもかかわらずPao_2 50mmHg以下および／または意識障害を伴う急激な$Paco_2$の上昇 人工呼吸[3] 気管支洗浄 全身麻酔（イソフルラン・セボフルラン・エンフルランなどによる）を考慮	重篤	直ちに入院，ICU管理[3]

b) 吸入ステロイド薬の安全性

我が国では、副腎皮質ステロイド薬(CS)は怖い薬として位置付けられ、吸入薬についてもこれまでの恐怖感が、医師と患者の両方に根強く残っている。すなわち、CS は、まず経口薬や注射薬が種々の疾患で使用され、いろいろな副作用が出現することから良く効く反面怖い薬と言う認識が定着してしまっている。喘息の治療で使用される吸入ステロイド薬(ICS)は、CS の中では最後に登場した剤型であるが、すでに 20 年以上にわたり喘息の治療に用いられ、その効果と安全性から喘息の治療に革命を起こしたと言っても過言ではない(図 11)。

図11 吸入ステロイド薬について



局所抗炎症作用が強力。常用量では副腎機能の副作用はほとんど見られない。吸入薬のステロイド用量は、経口薬に比較して1/1000である。
肝臓において初回通過で90%が代謝される。
局所副作用としては、嗄声、咽頭の異常感、口腔のカンジダ症などがあるがうがいによって多くの場合が防げる。
小児の発育においても、長期の追跡結果では健常な子どもの発育（身長）と差がない。

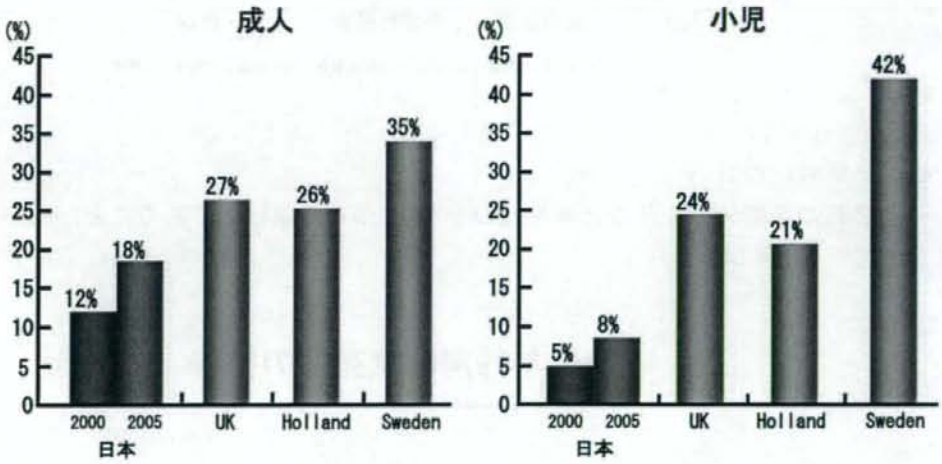
喘息は、気道の炎症を特徴とする慢性疾患であり、ICS を長期に投与することが治療の基本となる疾患である。副作用についてもしっかり研究され、ICS の常用量では、CS の全身投与でみられる副腎機能の抑制、骨粗鬆症、糖尿病、消化性潰瘍、免疫不全、異常脂肪沈着などはみられず、また懸念された小児の成長障害、胎児の奇形の発生、気道上皮細胞への悪影響などもみられていない。JGL2006 で推奨されている常用量である限り、咽頭のカンジダ症や嗄声が一般的な副作用で、重篤なものはみられていない。

c) 課題

i) 喘息の臨床に関する実態

我が国での喘息の実態調査として、国際的に共通の質問表を用いた電話による疫学調査が、2000年(AIRJ2000)と2005年(AIRJ2005)に実施された。その結果によると、成人におけるICSの使用頻度は、2000年は12%、2005年は18%で、これは2000年の英国(27%)、オランダ(26%)、スウェーデン(35%)と比べていずれも低値であった(図12)。すなわち、ICSを第一選択薬として推奨している喘息治療のガイドラインが十分に実行されていないことを示唆する結果であった。また小児では一層低頻度であることも示された。

図12 吸入ステロイド薬の使用頻度



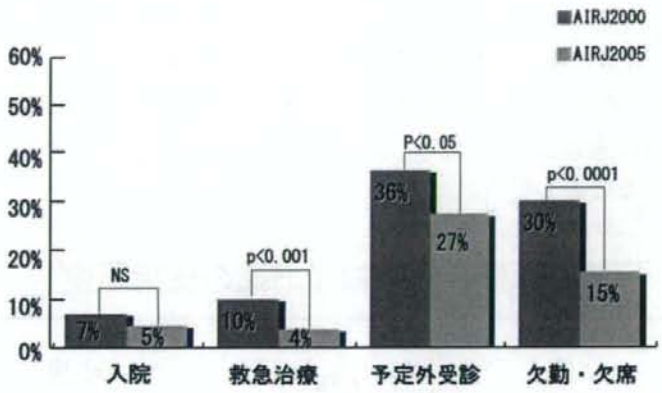
足立 満ほか：アレルギー 2002:51:411-420 (一部改変)

さらに注目されることは、ICSの使用頻度が2000年から2005年にかけて6%増加した結果、救急治療、予定外受診、欠勤・欠席の経験率が有意に減少し(図13)、吸入ステロイドを用いることの臨床効果を表しており、JGL2006に沿ったICSによる長期管理の有効性を強く支持している。

また、AIRJ2000の結果から、患者の自己管理を評価する上で重視されているピークフローメーターの使用が、理想とは程遠く、週1回以上使用している成人患者がわずか6%に過ぎないことが明らかとなった(図8、9頁)。すなわち、喘息の状態を客観的に評価するために、より簡便な方法を考えることの必要性が示された。

成人

図13 最近1年間の入院、救急治療、予定外受診、欠勤・欠席の経験率



AIRJ2005の数値はAIRJ2000との患者背景の差を考慮して調整した数値

ii) 高齢者への対応

喘息死の年齢分布を見ると喘息死の 90%以上が 60 歳以上であることが明らかとなっている(図 14)。

図14 年齢別喘息死数の推移

厚生労働省人口動態統計

この事実から、JGL2006 を実行するにあたり高齢者への対応をとくに意識することが必要である。高齢者では、喫煙者であれば COPD を合併していることが稀ではない。そして COPD の合併例では、COPD に対する治療も考慮する。

禁煙は喘息では ICS の効果が喫煙により抑制されることを阻止するが、COPD では治療においてさらに重要であり、これまでのところ疾患の進行を抑制する唯一の方法といっても過言ではない。したがって禁煙の実行が一層重要となる。

薬物療法では、抗コリン薬を含む複数の気管支拡張薬の使用が COPD の閉塞性換気障害に有効である。発作時には表３の鑑別すべき診断により注意を払い、また喘息に加えてこれらの疾患を併発している可能性もあることを忘れずに対応することが必要となる。さらに、薬剤の代謝も加齢や併用薬により変化するので、その点についても注意が必要である。

喘息死ゼロ作戦においては、いかに高齢者の喘息への対応を適切に行なえるようにするかが重要な課題として位置付けられると考えられる。

iii) JGL2006 の普及と実行に関する方策

JGL2006 の普及を妨げることとして、ガイドラインが複雑すぎることが挙げられている。表２～８にまとめられた内容を完全に記憶して日常の臨床で実行するのは、非専門医に限らずたとえ専門医であっても困難であると考えられる。また ICS を基本薬として用いる長期管理を実行するには、まず喘息の病態に長期管理を必要とする気道の慢性炎症が重要な役割を演じていることの理解を深め、炎症に対して ICS が最も有効で、しかも ICS は長年にわたる臨床的な検討の結果、安全性が極めて高いことの理解を広めることが必須であると考えられる。したがって、ゼロ作戦の戦略には、喘息の理解を深める教材、ガイドラインの実行を可能にする簡便なプログラム、治療効果として患者の状態を客観的にかつ簡便に評価するための方法などの道具立てが必要と考えられる。

研究成果の主たる別冊

Transduction of Phosphatase and Tensin Homolog Deleted on Chromosome 10 into Eosinophils Attenuates Survival, Chemotaxis, and Airway Inflammation[1]

Tetsuya Adachi,[2] Satoko Hanaka, Tomoko Masuda, Hisanao Yoshihara, Hiroyuki Nagase, and Ken Ohta

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is part of a complex signaling system that affects a variety of important cell functions. PTEN antagonizes the action of PI3K by dephosphorylating the signaling lipid phosphatidylinositol 3,4,5-triphosphate. In the present study, we used a TAT fusion protein transduction system to elucidate the role of PTEN in eosinophils and airway inflammation. A small region of the HIV TAT protein (YGRKKRRQRRR), a protein transduction domain known to enter mammalian cells efficiently, was fused to the N terminus of PTEN. Flow cytometric analysis of annexin V- and propidium iodide-stained cells was used to assess eosinophil survival. A chemotaxis assay was performed using a Boyden chamber. Cell analysis in bronchoalveolar lavage fluid and histological examinations were performed using OVA-challenged A/J mice. We found that TAT-PTEN was successfully internalized into eosinophils and functioned as a phosphatase in situ. TAT-PTEN, but not a TAT-GFP control protein, blocked the ability of IL-5 to prevent the apoptosis of eosinophils from allergic subjects. The eotaxin-induced eosinophil chemotaxis was inhibited by TAT-PTEN in a dose-dependent manner. Intranasal pretreatment with TAT-PTEN, but not TAT-GFP, significantly inhibited the OVA-induced eosinophil infiltration in bronchoalveolar lavage fluid. Histological examination of the lung, including H&E and Alcian blue/periodic acid-Schiff staining, revealed that TAT-PTEN, but not TAT-GFP, abrogated eosinophilic inflammation and mucus production. Our results suggest that PTEN negatively regulates eosinophil survival, chemotaxis, and allergic inflammation. The pharmacological targeting of PTEN may constitute a new strategy for the treatment of eosinophilic disorders. *The Journal of Immunology,* **2007, 179: 8105–8111.**

The pathogenesis of asthma is characterized by the infiltration of tissues by inflammatory cells such as eosinophils, mast cells, and T cells. Several mediators released by these cells cause epithelial damage, leading to enhanced bronchial hyperresponsiveness and airway obstruction (1). Although eosinophils have been considered as the most important cells in this process, the results of a clinical trial using an anti-IL-5 Ab has raised questions about the role of eosinophils in bronchial hyperresponsiveness (2). A subsequent study demonstrated that treating asthmatics with anti-IL-5 Ab reduces airway eosinophil numbers and the deposition of extracellular matrix proteins in the bronchial subepithelial basement membrane, suggesting the involvement of eosinophils in airway remodeling (3). In support of this finding, eosinophil-deficient mice exhibit decreased subepithelial fibrosis and smooth muscle hyperplasia (4). Thus, the targeting of eosinophils is considered to be an attractive strategy for treating asthma.

Phosphatase and tensin homolog on chromosome 10 (PTEN)[3] is a lipid and protein tyrosine phosphatase that dephosphorylates phosphotyrosine as well as the D3 position of phosphatidylinositol 3,4,5-triphosphate and phosphatidylinositol 3,4-diphosphate. The tumor suppressor gene *PTEN* is located at 10q23 and its mutation is detected in several neoplasms, including glioblastoma and breast and prostate cancers (5). The transduction of PTEN into tumor cell lines results in cell cycle arrest or apoptosis, suggesting that PTEN controls cell proliferation and survival (6–10). In *Dictyostelium* and mouse neutrophils, it has been demonstrated that the coordination of PI3K and PTEN is required for proper chemotaxis (11–13). The role of PTEN in immunity has also been investigated using PTEN-deficient mice. Suzuki et al. (14) have generated T cell-specific PTEN-deficient ($Pten^{flox/-}$) mice in which the T cells exhibit autoreactivity, enhanced proliferation, and inhibition of apoptosis. Similar phenomena are observed in the B cells derived from B cell-specific PTEN-deficient ($bPten^{flox/flox}$) mice (15). These results indicate that PTEN negatively regulates most cellular functions in the immune system. However, little is know about the role of PTEN in eosinophil function.

To elucidate the importance of intracellular signaling molecules in eosinophils, several approaches have been attempted. Although the application of pharmacological inhibitors is a common technique for targeting molecules of interest, its nonspecificity is frequently problematic. In contrast to proliferating cells, eosinophils have low turnover rates; hence, the validity of using antisense oligonucleotides or small interfering RNAs to assess signaling in eosinophils remains debatable. The overexpression of wild-type or dominant-negative protein by plasmid

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[3] Abbreviations used in this paper: PTEN, phosphatase and tensin homolog on chromosome 10; AB, Alcian blue; BALF, bronchoalveolar lavage fluid; PAS, periodic acid-Schiff; ROCK, Rho-associated coiled-coil-forming protein kinase; VEGF, vascular endothelial growth factor.

transfection is not possible because eosinophils are short-lived and terminally differentiated cells. To overcome these problems, we used a TAT fusion protein system that facilitates cellular internalization of the protein (16). It has been shown that the dominant-negative forms of Ras or PI3K efficiently block IL-5 signaling and corresponding functions in eosinophils (17–19).

In the present study, we generated TAT-PTEN to investigate the role of PTEN in eosinophils and airway inflammation. TAT-PTEN, but not a TAT-GFP control protein, blocked the ability of IL-5 to prevent apoptosis in the eosinophils obtained from allergic subjects. The eotaxin-induced eosinophil chemotaxis was inhibited by TAT-PTEN in a dose-dependent manner. Intranasal pretreatment with TAT-PTEN, but not TAT-GFP, significantly inhibited the OVA-induced eosinophilic inflammation in a murine model of asthma.

Materials and Methods

Generation of the TAT-PTEN construct

The TAT-GFP expression vector was provided by Dr. S. Dowdy (20). The expression cassette cosmid containing the wild-type *PTEN* (21) under the control of the CAG promoter (22) was supplied by the Riken Laboratory (Tsukuba, Japan). A cDNA fragment encoding *TAT-PTEN* was amplified by PCR (each cycle was conducted at 94°C for 40 s, 56°C for 30 s, and 72°C for 1 min, for a total of 35 cycles) using an AmpliTaq Gold DNA polymerase (Applied Biosystems) from the *PTEN*-containing cosmid with the forward primer that included the *TAT* sequence at the 5′ region (5′-TACGGTCGTAAGAAACGTCGCCAGCGTCGCCGTATGACAGCCATCATCAAAGAGATCGT-3′, *TAT* sequence is underlined) and the reverse primer (5′-TCAGACTTTTGTAATTTGTGTATGCTG-3′). The PCR product was subcloned into a pCRII-TOPO cloning vector (Invitrogen Life Technologies). To extend the His tag sequences at the 5′ region, an additional PCR (each cycle was conducted at 94°C for 40 s, 58°C for 30 s, and 72°C for 1 min, for a total of 35 cycles) was performed with the pCRII-TAT-PTEN construct as a template and the following primers (forward, 5′-ATGGGCAGCAGACATCATCATCATCATCACAGCAGCGGCTACGGTCGTAAGAAACGTC GCCAGCGT-3′, *His* sequence is underlined; and reverse, 5′-TCAGACTTTTGTAATTTGTGTATGCTG-3′). The final PCR product was cloned into the pCRII-TOPO vector and the sequence of the obtained construct (pCRII-His-TAT-PTEN) was confirmed using an Applied Biosystems PRISM 3700 Genetic Analyzer.

Purification of the TAT-PTEN protein

The polyhistidine-tagged *TAT-PTEN* gene was expressed in TOP10-competent *Escherichia coli* cells. TAT-PTEN was purified by sonication (50 kHz, amplitude: 20) in buffer Z (8 M urea, 20 mM HEPES (pH 8.0), 100 mM NaCl, 1 mM PMSF, and 1 μM pepstatin A) containing 20% glycerol. Cell lysates were resolved by centrifugation, absorbed onto a Ni Sepharose High Performance column (GE Healthcare Bio-Sciences), washed, and then sequentially eluted with 50, 100, and 250 mM imidazole in buffer Z/20% glycerol. Urea and imidazole were removed from the resultant protein solution in a dialysis buffer (20 mM HEPES (pH 7.4), 150 mM NaCl, 10 mM 2-ME, 1 mM PMSF, and 20% glycerol) using a low-volume 10,000 MWCO Slide-A-Lyser dialysis cassette (Pierce). The protein concentration was measured using a DC protein assay (Bio-Rad). Expression of the TAT-PTEN protein was confirmed by SDS-PAGE and Western blotting. The phosphoinositide phosphatase activity of the protein was determined using a PTEN malachite green assay kit (Upstate Biotechnology) with prepared phospholipid vesicles (0.1 mM diC_8PIP_3, 0.5 mM DOPS, 20 mM HEPES (pH 7.4), 1 mM EGTA, and 4.2% ammonium molybdate in 4 N HCl). Each fusion protein was flash frozen at −80°C.

Eosinophil purification

Peripheral venous blood was obtained from subjects with and without atopic characteristics. The geometric means of serum IgE from normal and atopic donors were 162 and 1479 IU/ml, respectively. Eosinophils were isolated by sedimentation with 6% dextran, followed by centrifugation on 1.088 Percoll (GE Healthcare Bio-Sciences) density gradients as modified from the method of Hansel et al. (23). The cells were further purified by negative selection using anti-CD16 immunomagnetic beads and a MACS system (Miltenyi Biotec). The eosinophils (>99% purity) were then suspended in HBSS with 1% FCS in tubes coated with 3% human serum albumin.

Preparation of cytosolic cell extracts

Eosinophils were incubated with and without TAT-PTEN or TAT-GFP for the indicated times at 37°C. The cells were lysed in a lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM Na_3VO_4, 1 mM NaF, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 1% Triton X-100, 10% glycerol, and 1 μg/ml aprotinin, leupeptin, and pepstain). After 20 min on ice, detergent-insoluble materials were removed by 12,000 × *g* centrifugation at 4°C. The whole cell lysates were boiled in 2× Laemmli reducing buffer for 4 min.

In some experiments, the cells were stimulated with 100 ng/ml IL-5 (R&D Systems) or 100 nM eotaxin (R&D Systems) for 3 min; the reaction was then terminated by adding nine volumes of ice-cold HBSS containing 1 mM Na_3VO_4. The cells were lysed using the Bio-Plex Cell Lysis Kit (Bio-Rad) for further quantification of phosphorylated and total Akt performed using Bio-Plex Phosphoprotein Panel (Bio-Rad) and Luminex 200 (Luminex). Data are shown as a percentage of mean fluorescence intensity of unstimulated control.

Gel electrophoresis and Western blotting

SDS-PAGE was performed using Ready Gels J (Bio-Rad). The concentration of the polyacrylamide was 7.5–10%. The electrophoresed gel was blotted onto Hybond ECL membranes (GE Healthcare Bio-Sciences). Blots were incubated in a blocking buffer containing 10% BSA in TBST buffer (20 mM Tris-HCl, 137 mM NaCl (pH 7.6), and 0.05% Tween 20) for 1 h, followed by incubation in the primary Ab (0.1 μg/ml) for 1–2 h (mouse monoclonal anti-His Ab obtained from GE Healthcare Bio-Sciences and rabbit polyclonal anti-PTEN and anti-GFP Abs obtained from Santa Cruz Biotechnology). After washing three times in TBST buffer, the blots were incubated for 30 min with a HRP-conjugated secondary Ab (0.04 μg/ml) directed against the primary Ab. The blots were developed with an ECL substrate according to the manufacturer's instructions (GE Healthcare Bio-Sciences). In some experiments, blots were reprobed with another Ab after stripping in a buffer of 62.5 mM Tris-HCl (pH 6.7), 100 mM 2-ME, and 2% SDS at 50°C for 30 min.

Cellular uptake of TAT fusion proteins

TAT-PTEN and TAT-GFP were labeled with FITC (Pierce) according to the manufacturer's instructions, and excess FITC was removed by dialysis using the same cassette as that described above for protein purification. Eosinophils resuspended in HBSS with 1% FCS were incubated with 1 μM of the FITC-conjugated TAT-PTEN or TAT-GFP for 30 min at 37°C. The cells were washed with HBSS with 1% FCS and placed on glass slides using Shandon Cytospin 3 (Thermo Scientific). The intracellular distribution of TAT fusion proteins was visualized under a laser confocal microscope (LSM510; Zeiss).

Survival assay

An apoptosis detection kit (MBL) was used to quantitatively determine eosinophils undergoing apoptosis, by virtue of their ability to bind to annexin V and exclude propidium iodide. Purified eosinophils were resuspended at 0.5×10^6 cells/ml in RPMI 1640 medium supplemented with 10% FCS. After incubation with TAT-PTEN or TAT-GFP, the eosinophils were further cultured with 1 ng/ml IL-5 (R&D Systems) in 24-well tissue culture plates for 24 h at 37°C. The cells were washed twice in cold PBS and stained with annexin V and propidium iodide according to the manufacturer's instructions. Eosinophil apoptosis was analyzed using a FACScan cytometer (BD Biosciences), gating on the live cell population.

Chemotaxis assay

Chemotaxis of eosinophils was conducted in duplicate using 5-μm polyvinylpyrrolidone-free polycarbonate membranes (Nucleopore) in Boyden chambers (NeuroProbe). Human eotaxin was diluted in HBSS containing 0.02% BSA and placed in the lower wells (100 μl) at a concentration of 10 nM. After incubation of the eosinophils with and without the TAT fusion proteins for 30 min, 100-μl aliquots of the cell suspension at 2×10^6 cells/ml were placed in the upper chambers. The loaded chambers were incubated at 37°C in humidified air containing 5% CO_2 for 1 h. The membrane was then removed, fixed, and stained for 3 min in May-Grünwald solution. The cells that migrated and adhered to the lower surface of the membrane were counted from 10 fields under the light microscope. The chemotactic response to the buffer (<40/10 fields) was subtracted from that induced with eotaxin with or without the inhibitors. The data are shown as a percentage of that induced with eotaxin alone.

Bronchoalveolar lavage fluid (BALF) cell analysis and histological examination in mice

Care and use of the animals followed the guidelines of the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. Specific pathogen-free male A/J mice (10–12 wk old) were purchased from SLC. The mice were initially immunized four times with 10 μg of OVA plus 2 mg of alum on days 0, 28, 35, and 49. After the sensitization, the mice were intranasally challenged with 40 μl of 10 mg/ml OVA from days 50–53. The mice were divided into four groups for the intranasal administration of pretreatment and challenge: 1) PBS plus PBS, 2) PBS plus OVA, 3) TAT-PTEN (3 μmol/mouse) plus OVA, and 4) TAT-GFP (3 μmol/mouse) plus OVA. On day 54, BALF was obtained from the mice by intubating and washing the lungs with 1 ml of saline until 5 ml of fluid was recovered. The cells were pelleted from the lavage fluid, resuspended in 1 ml of saline, and placed on glass slides for counting and fixation using Shandon Cytospin 3 (Thermo Scientific). The slides were then stained with Diff-Quik (Sysmex) and cell differentiation was assessed microscopically. The supernatant of the BALF was concentrated 10-fold by freeze-drying to examine the cytokine/chemokine levels. The measurement of cytokine/chemokine was performed using Bio-Plex Cytokine Panel (Bio-Rad) and Luminex 200. The lungs were fully inflated using 10 cm of H_2O pressure and fixed with 20% formaldehyde for H&E and Alcian blue/periodic acid-Schiff (AB/PAS) staining.

Statistical analysis

Results are expressed as means ± SEM. The data were analyzed for statistical significance using the Mann-Whitney *U* test and ANOVA. Post hoc analysis was performed using the Bonferroni/Dunn test.

Results

Cellular internalization and in situ activity of TAT-PTEN

Dowdy and colleagues (20) have described how TAT fusion proteins were internalized into mammalian cells within 30 min in a concentration-dependent manner. Thus, we constructed TAT-PTEN to examine the role of PTEN in eosinophil function and allergic inflammation (Fig. 1). Initially, we incubated eosinophils with 1 μM TAT-PTEN or TAT-GFP for 15 s, 5 min, and 30 min at 37°C to assess the cellular uptake of the proteins. After the incubation, the eosinophils were washed with cold HBSS and lysed. The lysates were subjected to electrophoresis and Western blotting with anti-PTEN or anti-GFP Ab and then reprobed with anti-His Ab. As shown in Fig. 2*A*, the blots with both anti-PTEN and anti-His Abs revealed that TAT-PTEN efficiently entered cells in a manner that depended on the length of incubation. In contrast, internalization of TAT-GFP reached a maximum at 15 s and plateaued for 30 min (Fig. 2*B*). To further confirm these results, we observed the intracellular distribution of the TAT fusion proteins in eosinophils. The confocal microscopic analysis of transduced TAT-PTEN (Fig. 3*A*) and TAT-GFP (Fig. 3*B*) demonstrated cytoplasmic localization accompanied with intense uptakes, possibly within endosomes. Our TAT-PTEN, unlike the dominant-negative form of TAT fusion proteins reported previously (17–19), is designed to function as an in situ phosphatase. PTEN is assumed to inhibit the PI3K pathway by dephosphorylating phosphatidylinositol 3,4,5-triphosphate, leading to the down-regulation of Akt, which lies downstream of PI3K. For this reason, we investigated the effect of TAT-PTEN on Akt activation in eosinophils to detect the in situ activity of the protein. After pretreatment of the eosinophils with 1 μM TAT-PTEN or TAT-GFP for 30 min, Akt phosphorylation induced by IL-5 or eotaxin was measured. As shown in Fig. 4, internalized TAT-PTEN significantly reduced Akt phosphorylation in eosinophils stimulated with IL-5 or eotaxin (Fig. 4)..

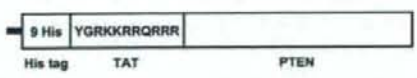

FIGURE 1. Structure of the TAT-PTEN fusion protein. Nine His residues and the 11-aa TAT peptide precede the N-terminal of the PTEN protein. The 11-aa of TAT comprise the protein transduction domain.

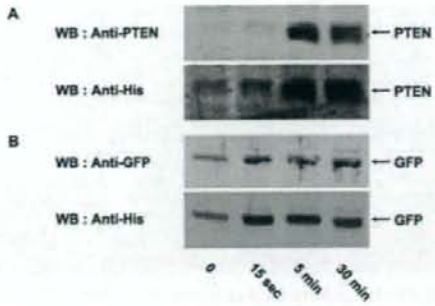

FIGURE 2. Kinetics of (*A*) TAT-PTEN and (*B*) TAT-GFP transduction into eosinophils. Eosinophils were incubated with 1 μM TAT-PTEN or TAT-GFP at 37°C for the indicated times. The TAT fusion protein in cell lysates was examined by Western blotting (WB) using anti-PTEN or anti-GFP Ab and then reprobed with anti-His Ab.

Effect of TAT-PTEN on eosinophil survival and chemotaxis

PTEN has been demonstrated to promote apoptosis in a number of tumor and immune cells (7–10, 24, 25). Thus, we investigated the role of PTEN in the maintenance of eosinophil survival. After incubating with TAT-PTEN or TAT-GFP for 30 min, eosinophils were stimulated with IL-5 for 24 h and viability was assessed by annexin V and propidium iodide staining. After 24 h, in the absence of IL-5, almost one-half of the eosinophils had undergone apoptosis. In contrast, eosinophil viability was >80% after stimulation with IL-5. Incremental increases in the concentration of TAT-PTEN, but not TAT-GFP, significantly abrogated the survival of eosinophils from atopic donors (Fig. 5). Interestingly, however, the effect of TAT-PTEN was not observed in the eosinophils from normal subjects (Fig. 5). We next studied the effect of TAT-PTEN on eotaxin-induced eosinophil chemotaxis since PTEN has been observed to be involved in the migration of *Dictyostelium* and mammalian cells (11–13). Eosinophils were incubated with and without the TAT fusion proteins and then applied to the upper Boyden chambers. The lower chambers contained 10 nM eotaxin. The cells that migrated and adhered to the lower surface of the membrane were counted under the light microscope. TAT-PTEN, but not TAT-GFP, dose-dependently reduced the chemotaxis of eosinophils, regardless of their atopic state (Fig. 6).

Effect of TAT-PTEN on allergic inflammation

Kwak et al. (26) have reported that the intratracheal administration of adenovirus-carrying PTEN cDNA significantly inhibits eosinophilic inflammation and bronchial hyperresponsiveness. We therefore investigated the in vivo effect of TAT-PTEN in a murine model of asthma. The OVA-sensitized mice were intranasally pretreated with and without TAT-PTEN or TAT-GFP, followed by intranasal OVA challenge. Airway inflammation was assessed by BALF cell analysis and histological examination. Following OVA challenge, the number of cells in the BALF increased due largely to an increase in eosinophils (Fig. 7). The increase in the numbers of BALF cells, particularly eosinophils, was significantly abrogated in mice pretreated with TAT-PTEN, but not in those treated with TAT-GFP (Fig. 7). A histological examination revealed that airway inflammation and the production of purple-stained mucus were augmented by OVA challenge (Fig. 8, *C* and *D*) compared with the PBS control (Fig. 8, *A* and *B*). The H&E staining revealed

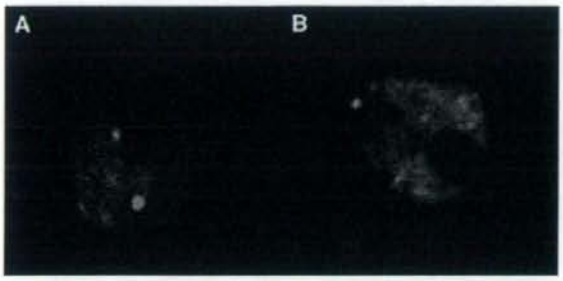

FIGURE 3. Intracellular localization of (*A*) TAT-PTEN and (*B*) TAT-GFP in eosinophils. Eosinophils were incubated with 1 μM FITC-conjugated TAT-PTEN or TAT-GFP for 30 min at 37°C. After preparation of a cytospin specimen, the cells were visualized under a confocal microscope.

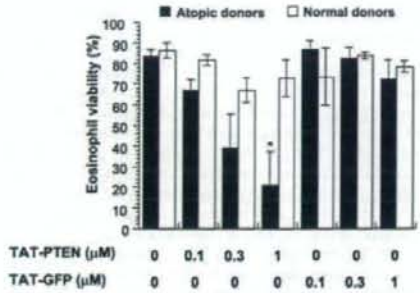

FIGURE 5. Effect of TAT-PTEN on eosinophil survival. Eosinophils were incubated with and without the TAT fusion proteins for 30 min. The cells were further cultured with IL-5 (1 ng/ml) for 24 h and then the viability of eosinophils was assessed by annexin V and propidium iodide staining. The data are expressed as means ± SEM (n = 3). *, $p < 0.05$ vs without the protein (ANOVA).

that airway inflammation was attenuated by the administration of TAT-PTEN (Fig. 8*E*). Pretreatment with TAT-PTEN markedly reduced the mucus-producing epithelial cells stained with AB/PAS (Fig. 8*F*). The effect elicited by TAT-PTEN was not observed in the case of TAT-GFP administration (Fig. 8,*G* and *H*). To study the effect of TAT-PTEN on Th1/Th2 balance, we measured the levels of cytokine/chemokine in BALF. The increase in the IL-5 level caused by OVA challenge was significantly inhibited by TAT-PTEN, but not by TAT-GFP (Fig. 9*A*). In contrast, the administration of TAT-PTEN significantly increased the RANTES level in BALF (Fig. 9*B*). Since the down-regulation of vascular endothelial growth factor (VEGF) expression in allergen-induced asthmatic lung by PTEN has recently been demonstrated (27), we also measured the VEGF concentration in BALF. Although TAT-PTEN tended to decrease the VEGF level, the difference was not significant (data not shown).

Discussion

In the present study, we developed a novel TAT fusion protein to investigate the role of PTEN in eosinophils and airway inflammation. TAT-PTEN, but not TAT-GFP, reduced the IL-5-induced survival of eosinophils obtained from allergic subjects. The eotaxin-induced eosinophil chemotaxis was inhibited by TAT-PTEN. The intranasal administration of TAT-PTEN, but not TAT-GFP, significantly inhibited the OVA-induced eosinophilic inflammation. This is the first report to clarify the role of PTEN in eosinophils. Moreover, we demonstrated the utility of TAT-PTEN as a therapeutic modality for the treatment of allergic diseases.

Eosinophils play a pivotal role in the pathogenesis of airway inflammation and remodeling in asthma. Therefore, it is of paramount importance to investigate eosinophil signaling and its functional relevance. Pharmacological inhibitors of intracellular signaling molecules have been widely used for this purpose. However, the data are often difficult to interpret because of the lack of inhibitor specificity. Although eosinophils are nonproliferating and terminally differentiated cells, transfection with plasmids or transduction with inhibitory nucleotides, such as antisense oligonucleotides and small interfering RNAs, may be unrealizable. To solve the above-mentioned problems, the TAT fusion protein system has become the focus of scientific attention. The first description of this system was made independently by Green and Loewenstein (28) and Frankel and Pabo (29), who demonstrated that the 86-aa HIV TAT protein could rapidly enter cells and subsequently transactivate the viral long terminal repeat promoter. Later, Fawell et al. (16) expanded on these findings by revealing that chemically cross-linking a 36-aa domain of TAT to heterologous proteins facilitated cell internalization. According to their results, the TAT fusion proteins were predominantly localized in the cell surface area following incubations of up to 20 min, with progressive accumulation in diffuse cytoplasmic, nuclear, and nucleolar regions with incubations of 30 min to 6 h. Several groups

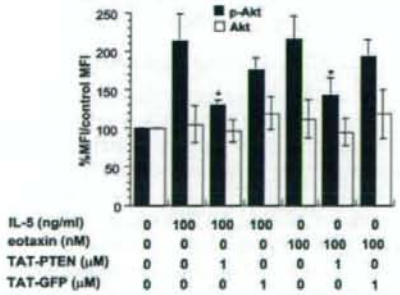

FIGURE 4. The in situ phosphatase activity of TAT-PTEN in eosinophils stimulated with IL-5 or eotaxin. Eosinophils were incubated with 1 μM TAT-PTEN or TAT-GFP for 30 min and then stimulated with 100 ng/ml IL-5 or 100 nM eotaxin for 3 min. Quantification of the phosphorylated and total Akt was performed using the Luminex System. The data are shown as a percentage of mean fluorescence intensity of unstimulated control and are expressed as means ± SEM (n = 4). *, $p < 0.05$ vs without the protein (ANOVA).

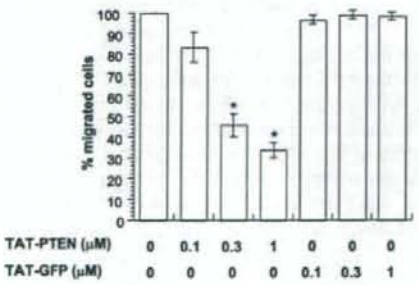

FIGURE 6. Effect of TAT-PTEN on eosinophil chemotaxis. Eosinophils were incubated with and without the TAT fusion proteins for 30 min. After incubation, the cells were subjected to the chemotaxis assay using Boyden microchambers. Eotaxin (10 nM) was used as the chemoattractant. The data are expressed as means ± SEM (n = 4). *, $p < 0.05$ vs without the protein (ANOVA).

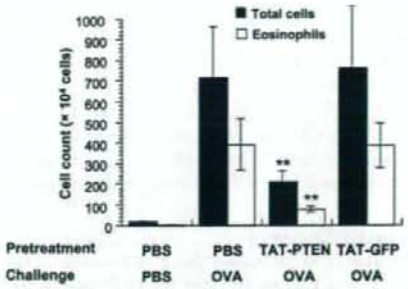

FIGURE 7. Effect of TAT-PTEN on BALF cells in a murine model of asthma. TAT-PTEN or TAT-GFP was intranasally administrated in OVA-sensitized A/J mice. The mice were intubated for lavage with a total of 5 ml of saline. Cells were pelleted from the lavage fluid and the cell number was counted under the light microscope following Diff-Quik staining. The data are expressed as means ± SEM ($n = 5$). **, $p < 0.05$ vs PBS-treated/OVA-challenged mice or TAT-GFP-treated/OVA-challenged mice (Mann-Whitney U test).

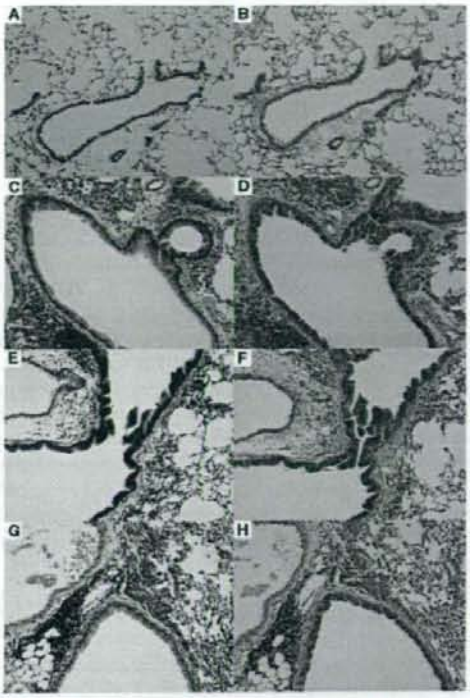

FIGURE 8. Effect of TAT-PTEN on the histological characteristics of lung tissue. Sections of lung were stained with H&E (*A*, *C*, *E*, and *G*) or AB/PAS (*B*, *D*, *F*, and *H*) stain. PBS-treated/PBS-challenged mouse (*A* and *B*), PBS-treated/OVA-challenged mouse (*C* and *D*), TAT-PTEN-treated/OVA-challenged mouse (*E* and *F*), and TAT-GFP-treated/OVA-challenged mouse (*G* and *H*). Original magnification, ×200.

have demonstrated that the TAT fusion proteins block specific signaling molecules such as Ras and PI3K in eosinophils (17–19). In the present study, we generated TAT-PTEN that was efficiently incorporated in eosinophils. It has been demonstrated that the expression of wild-type PTEN in PTEN-deficient tumor cell lines reduces phosphatidylinositol 3,4,5-triphosphate levels and inhibits Akt phosphorylation (30). We therefore examined the in situ phosphatase activity of TAT-PTEN in eosinophils and found that the Akt phosphorylation induced by IL-5 or eotaxin was inhibited by TAT-PTEN.

PTEN appears to play a particularly important role in regulating apoptosis in a variety of cell types. Somatic deletions or mutations of the *PTEN* gene are commonly detected in a large fraction of tumors (5) and overexpression of wild-type PTEN in $PTEN^{-/-}$ cell lines induces apoptosis (7–10). PTEN also inhibits Ag receptor signaling and cell survival in B cells and T cells (24, 25). Our results revealed that the transduction of PTEN inhibited the survival of eosinophils derived from atopic donors, but not those from normal subjects. Although mechanisms regarding the differential response of eosinophils based on atopic state are unclear, one possible explanation is as follows. We have previously found that Lyn, Jak2, and Raf-1, but not MAPK, are essential for the survival of eosinophils stimulated with IL-5 (31, 32). However, the role of PI3K in signaling between IL-5 and GM-CSF in eosinophil apoptosis remains controversial (33, 34). IL-5 and GM-CSF share a βc receptor that is critical for signal transduction. These results indicate that the Lyn (Jak2)-Ras-Raf-1 pathway may be common in survival signaling in the eosinophils from both atopic and normal subjects. In contrast, eosinophils from atopic donors, or primed in vivo in the allergic condition, possibly utilize the PI3K pathway as well as the Ras-Raf-1 pathway. In support of this finding, Pinho et al. (35) have established a model of allergic pleurisy in mice and found that treatment with the PI3K inhibitor abrogated the accumulation of eosinophils associated with an increased number of apoptotic events. Thus, it is reasonable that TAT-PTEN accelerates the apoptosis of atopic eosinophils by antagonizing the effect of PI3K.

The role of PTEN has recently been elucidated in *Dictyostelium* and mouse neutrophils (11–13). In the resting condition, PI3K is uniformly distributed in the cytoplasm, whereas PTEN is localized in the cell periphery. PI3K is translocated to the leading edge of the cell associated with delocalization of PTEN from the front in response to chemoattractants. Recently, Li et al. (36) have demonstrated that activated RhoA in the rear of leukocytes activates Rho-associated

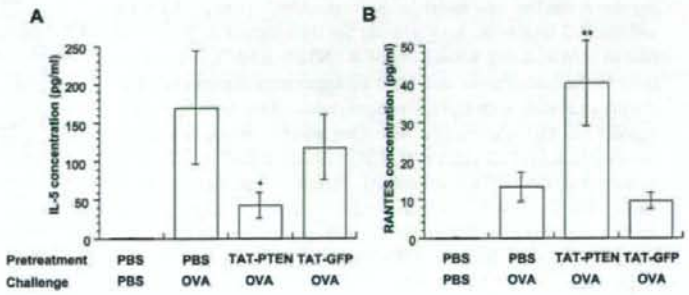

FIGURE 9. Effect of TAT-PTEN on cytokine/chemokine production. The concentration of IL-5 (*A*) and RANTES (*B*) in the BALF supernatant was measured using Bio-Plex Cytokine Panel and Luminex 200. The data are expressed as means ± SEM ($n = 5$). *, $p < 0.05$ vs PBS-treated/OVA-challenged mice; **, $p < 0.05$ vs PBS-treated/OVA-challenged mice or TAT-GFP-treated/OVA-challenged mice (Mann-Whitney U test).

coiled-coil-forming protein kinase (ROCK), subsequently forming a complex with and phosphorylating PTEN. Activated PTEN located at the back and lateral sides restricts phosphatidylinositol 3,4,5-triphosphate accumulation in the front of the cells, which is required for proper chemotaxis. Our previous report demonstrated that the Rho-ROCK pathway is activated by eotaxin and critical for eosinophil chemotaxis (37). Thus, the activation of Rho and ROCK may be responsible for endogenous PTEN activation in eosinophils. In some cells, however, overexpression of PTEN inhibits cell migration (38). Expression of the PTEN mutant G129E, which is deficient in lipid phosphatase activity but retains protein phosphatase activity, inhibits the migration of U87MG glioblastoma cells (39). This effect is likely to be mediated through the direct interaction of PTEN with FAK, followed by its dephosphorylation. In chemotactic PTEN-deficient Jurkat T cells, ectopically expressed PTEN was distributed homogenously in the cytoplasm (40). Nonetheless, in contrast to the results in *Dictyostelium* and murine neutrophils (11–13), PTEN attenuated actin polymerization and cell motility in Jurkat cells stimulated with stromal-derived factor 1 (40). In the present study, we observed that TAT-PTEN is largely distributed in the cytosol of eosinophils. Thus, the findings of Lacalle et al. (40) support our data demonstrating that TAT-PTEN blocks eotaxin-induced eosinophil chemotaxis. Taken together, it is possible that PTEN localization in migrating cells depends on cell type-specific factors.

Homozygosity for the null mutation of PTEN ($PTEN^{-/-}$ mice) results in early embryonic lethality (7, 40–42). $PTEN^{+/-}$ mice frequently develop a variety of cancers and autoimmune diseases (42–44). To overcome the lethality of $PTEN^{-/-}$, various cell-specific PTEN mutations have been generated using the Cre-loxP system. The T cell-specific PTEN-deficient ($Pten^{flox/-}$) T cells exhibit hyperproliferation, autoreactivity, secretion of increased levels of Th1/Th2 cytokines, and resistance to apoptosis (14). Similar phenomena, including hyperproliferation, resistance to apoptosis, and enhanced migration, are observed in PTEN-deficient ($bPten^{flox/flox}$) B cells (15). These results suggest inhibitory roles for PTEN in neoplasm formation and immune regulation. In a murine model of allergy, Kwak et al. (26) have established an elegant system for examining the role of PTEN. The intratracheal administration of adenovirus carrying PTEN cDNA significantly reduced airway eosinophil infiltration and bronchial hyperresponsiveness, indicating that the effect of PTEN is suppressive. The application of TAT fusion proteins, such as the dominant-negative forms of Ras and PI3K, has been reported in a murine model of allergy (45, 46). In the present study, we generated TAT-PTEN that efficiently blocks eosinophilic inflammation, mucus production, and IL-5 production in vivo. These results are consistent with those of Kwak et al. (26). We also observed less IL-5 production in the TAT-GFP-treated mice than in the mice not treated with TAT proteins. It has been shown that TAT transduction causes a phosphatidylserine flip from the inner to the outer cell membrane (47), which is commonly observed during cell apoptosis. Although the mechanism underlying this is unclear, the electrostatic interaction between TAT-GFP and the cell membrane may account for the reduced IL-5 production in BALF. Lung expression of RANTES mRNA is up-regulated in the Th1-skewed condition of a murine asthma model following treatment with CpG oligodeoxynucleotides or the adoptive transfer of Th1 clones (48, 49). Our results demonstrated the increase in RANTES level in BALF induced by TAT-PTEN, suggesting that TAT-PTEN modulates allergic inflammation by inducing the Th1 condition. Lee et al. (27) have recently demonstrated the down-regulation of VEGF expression in allergen-induced asthmatic lung by PTEN, which may indicate a further mechanism of TAT-PTEN action.

In conclusion, we generated TAT-PTEN that efficiently blocks eosinophil survival, chemotaxis, and airway inflammation. Targeting PTEN can be a therapeutic modality in the treatment of several cancers and autoimmune diseases. However, the molecular regulation of PTEN in eosinophilic inflammation remains to be clarified. Further studies are necessary to elucidate the detailed signaling complex around PTEN in allergy, the outcome of which may lead to the development of new molecular targeting therapy.

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Disclosures

The authors have no financial conflict of interest.

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Adherence to treatment by patients with asthma or COPD: Comparison between inhaled drugs and transdermal patch

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Summary
An Internet-based questionnaire study involving patients with asthma and chronic obstructive pulmonary disease (COPD) and parents of children with asthma was conducted to evaluate adherence to treatment, and convenience of inhalation and transdermal formulations. Valid responses were obtained from 1470 patients. Among asthmatic patients, the percentage of those who selected "taking as prescribed" was 52.7% for inhalant users and 83.2% for transdermal users. Among patients with COPD, the corresponding values were 54.7% and 86.6%. There was a significant difference ($p<0.01$) in treatment compliance between inhalation and transdermal formulations in both groups. The most common reason for poor adherence was "frequency of administration", and 83.2% of the patients preferred a once-daily administration. In addition, patients who had used both types of formulations preferred the transdermal ones. In conclusion, health care professionals should further educate their patients about the importance of treatment with inhalants, since poor adherence to treatment with inhalation formulations significantly hinders achievement of optimal efficacy. In addition, transdermal tulobuterol patch, which is administered once daily as a long-acting, β_2-agonist, appeared to be useful for long-term control of both asthma and COPD.

Introduction

Adherence to medical treatment is an essential factor in ensuring appropriate pharmacological efficacy. No drugs exert maximal effects unless patients take them as prescribed. Since bronchial asthma and COPD are chronic diseases, continuous treatment with inhalation agents is recommended for both of them, and numerous carefully controlled trials have confirmed that these agents are very effective for both pathological conditions. However, they are unlikely to exert full effects when treatment compliance is poor. In addition, some investigators have reported that

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adherence to treatment with inhalants is poor because of the complex procedures required to use them, as well as the tedious, frequent dosing.[1–4]

Japanese guidelines, such as the "Guidelines for the Prevention and Management of Asthma",[5] "EBM-Based Guidelines for Appropriate Use of Antiasthmatic Drugs",[6] and "Guidelines for the Diagnosis and Treatment of COPD",[7] recommend the use of tulobuterol patch (TP), a long-acting β_2-agonist (LABA). TP is the first long-acting transdermal patch preparation of the β_2-agonist tulobuterol, and is designed to exert sustained β_2-agonistic effects for 24 h when applied once daily. Tamura et al.[8] recently found in a randomized, double-blind, double-dummy, parallel-group multicenter trial that 4-week treatment with TP dose-dependently improved the morning and evening peak expiratory flow in patients with asthma who required treatment with inhaled corticosteroids (CS) and short-acting β_2-agonists (SABA). In a multicenter, randomized, open-label, parallel-group comparison study of COPD patients treated with either TP or inhaled salmeterol, Fukuchi et al.[9] demonstrated that TP improved St. George's Respiratory Questionnaire scores more effectively than inhaled salmeterol. These findings indicated that TP is useful for the management of stable COPD, with an efficacy equivalent or superior to that of inhaled salmeterol.

In addition, treatment compliance was significantly better with TP than with inhaled salmeterol in that study. However, adherence in inhalant and patch users in clinical practice, not in a clinical study based on a rigorous protocol, has not been reported. Thus, this study was conducted to clarify the adherence in clinical practice through an Internet survey.

Subjects and methods

Participants

The participants in this survey were 2000 patients with asthma who had been or were under treatment with dry-powder inhaler (DPI)-LABA, metered-dose inhaler (MDI)-SABA, DPI-CS, MDI-CS, or TP-LABA and 1000 patients with COPD who had been or were under treatment with DPI-LABA, MDI-SABA, MDI-anticholinergic, or TP-LABA. These patients were randomly selected from among 10,100 patients with asthma and 1523 patients with COPD who voluntarily accessed a health care communication website, "My Hospitals" (http://www.my-hospitals.net), and agreed to answer questionnaires about their treatment. In the present study, a questionnaire (Table 1) was sent to the participants via E-mail. When the number of effective responses reached the target number of 1500, which corresponded to half the number of selected patients, the survey was concluded. In this survey, the subjects voluntarily replied to the questionnaire.

Methods of administration and contents of the questionnaire

The questionnaire survey was carried out during February 15–26, 2005. The participants were asked to answer questions about self-management of asthma or COPD via the My Hospitals website. Protection of privacy of the data collected via the website was achieved by allowing access to the site only to the participants via a user ID along with a password-restricted log-in, and by encrypting the data they entered. The questionnaire included questions concerning gender, age, and underlying disease and its severity. The severity of asthma was evaluated according to the criteria for severity described in the "Guidelines for the Prevention and Management of Asthma 2003"[5] and of COPD, according to the British Medical Research Council (BMRC) Dyspnea Scale.[10]

Table 1 Questionnaire.

Q1. Are you taking drugs currently prescribed for treatment of asthma/COPD as directed by your physicians? Please provide an answer for each drug.
Asthma: DPI-LABA, MDI-SABA, DPI-CS, MDI-CS, TP-LABA
COPD: DPI-LABA, MDI-SABA, MDI-anticholinergic, TP-LABA
1. Taking as prescribed
2. Sometimes failing to take as prescribed
3. Often failing to take as prescribed
4. Always failing to take as prescribed

Q2. Which part of the treatment regimen is difficult to follow? Please provide an answer for your each drug (please select all that apply).
1. Method of administration
2. Frequency of administration
3. Timing of administration
4. Other ( )

Q3. Please indicate the most preferable frequency of administration for your asthma/COPD drugs.
1. Once a day, 2. Twice a day, 3. Three times a day or more.

Q4. Please rate the convenience of use of your drugs for asthma/COPD using the following rank scale.
1. Difficult, 2. Slightly difficult, 3. Easy, 4. Very easy.
The following questions are for patients who use or have used both inhaled drugs and transdermal drug.

Q5. Would you like to replace inhaled drugs with transdermal drug?
1. Definitely yes, 2. Probably yes, 3. Unsure, 4. Probably not, 5. Definitely not.

Q6. Would you like to replace transdermal drug with inhaled drugs?
1. Definitely yes, 2. Probably yes, 3. Unsure, 4. Probably not, 5. Definitely not.

Statistical analysis

The answers obtained were tabulated separately for each formulation, and the data were analyzed with stratification by disease.

Using SPSS software, answers regarding adherence to treatment, convenience, and willingness to replace drug formulation were examined by the Mann–Whitney *U*-test, and answers regarding reasons for poor adherence were examined by *Z*-test, for comparisons of inhaled and