G. 研究発表

1. 論文発表

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H. 知的財産

特になし

厚生労働科学研究費補助金(免疫アレルギー疾患予防・治療研究事業)
分担研究報告書

代替医療の客観的評価，あるいはアンケートを利用した評価，および新規治療法の開発

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研究協力者 ： 米倉 修二 千葉大学医学部附属病院 耳鼻咽喉・頭頸部外科医員
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研究要旨

アレルギー疾患に対して行われている様々な代替医療の科学的評価を行うことを目的に、本年度はメントール、鼻翼開大テープ、アロマセラピー、花粉対策マスク、花粉飛散情報について検討を開始した。メントール吸入、鼻翼開大テープにより鼻腔抵抗の低下がみられたが、メントール吸入は20～40分程度の一過性のものであり、鼻翼開大テープでは粘着テープを使用するため長期使用困難者もみられた。アロマセラピーについてはfunctional MRIを用いた検討を開始した。花粉飛散期のマスク着用の有効性について検討したが、着用に高い満足を感じると答えた割合は 12%にとどまった。詳細な花粉飛散情報の提供の有効性についてはその精度に評価は大きく依存した。局所リンパ節を Th1 優位に誘導出来る可能性がある細胞免疫治療は花粉症に対して有効性が期待され検討を進める価値があると考えられた。

A.研究目的

アレルギー疾患に対して様々な代替医療が行われているが，その有効性についての科学的評価はほとんど行われていない．今回，アレルギー性鼻炎に対して高い頻度で行われている代替医療のうち，アロマテラピー，ミント(メントール)，鼻翼開大テープ，さらにマスク，花粉飛散情報も含めてその有用性の評価を行う．

B.研究方法

1)メントール(ペパーミントオイル)を通年性アレルギー性鼻炎患者ボランティアに吸入させた後に 120 分まで継時的に鼻腔通気度計を用いて鼻腔抵抗を測定した．試験終了後に自覚症状について問診を行った．

2)鼻翼開大テープを通年性アレルギー性鼻炎患者ボランティアに装着し，その後の鼻腔抵抗の変化を 7 時間まで測定した．また，試験終了時に自覚症状について問診を行った．

3)アロマテラピーの影響についてボランティアにバラエキスを酸素と共に鼻カヌラで鼻腔に送り込み，functional- MRI (f-MRI)画像を撮影した．撮影範囲は OM line に平行で後交連に接するスライスを中心とした 5 スライスで 270 秒間撮影した．反応の特異性を高めるための吸入の on-off の変化を 30 秒間隔で繰り返し，同期する血流の変化がある場合を反応陽性と判断した．

4)スギ花粉症患者を対象に，花粉飛散期のマスク着用の有無，マスク着用の効果についての評価をアンケート調査により検討した．

5)携帯電話を介してメール配信により，毎朝その日の 1 時間毎の詳細な花粉飛散予報をスギ花粉飛散シーズンを通して希望するスギ花粉症患者さんに提供し，シーズン終了後に花粉飛散情報の有用性についてアンケート調査を行った．

6)海綿由来の糖脂質(ガラクトシルセラミド)でパルスした抗原呈示細胞の頭頸部癌患者の鼻粘膜下投与は所属リンパ節，血中に Th1 優位な反応を形成するが，投与を受けた患者の IgE 値の変化について検討した．また，鼻アレルギー感作マウスを用いてこの糖脂質パルス抗原呈示細胞の上気道投与を行い，IgE 抗体価，抗原誘発後の鼻症状について検討を進めた．

(倫理面への配慮)

本研究の遂行にあたっては，調査，試験対象者から十分な了解を得ることとし，文書による同意を得て行われた．研究の方法，必要性，有用性，さらに拒否しても不利益にならないことを十分説明した後に，本人から同意の得られた場合のみ行った．これらの検討は千葉大学医学部倫理委員会に申請し，許可を得て行われた．

C.研究結果

1) メントール吸入後，鼻腔通気抵抗は通年性アレルギー性鼻炎患者ボランティア10名で平均25%まで低下し，40から 100 分間持続した．その後は徐々に増加したが，rebound 現象は明らかではなかった．10 例中 1 例で刺激により鼻汁分泌を訴えたが，8 例で自覚的にも鼻閉の一時的改善を認めた．

2) 鼻翼開大テープによりアレルギー性鼻炎患者 5 名で平均30%の鼻腔抵抗の低下がみられ，試験終了まで改善は認められた．自覚的にも全例で鼻閉の改善がみられたが，1 例で装着部のかゆみ，1 例で痛みを訴えた．テープ除去後のかぶれを 1 例が訴えた．

3) f-MRI 検査にてバラエキス吸入後に脳血流の変化を確認することが 10 例中 5 例で可能であった．反応部位は側頭葉のみならず，後頭葉にも認められた．様々なアロマオイルを準備し，好みに応じたアロマの吸入による影響，臨床効果の評価を検討している．

4) 1240 名のスギ花粉症患者中常にマスクを着用している患者は男性 10%，女性 14%であり，効果については 80%の着用患者が認めたが，充分な効果と満足している割合はこの 12%にとどまった．

5) 携帯メール配信による詳細な花粉飛散情報を行い，アンケートに回答した 130 名の患者のうち，花粉予報の実感が比較的一致，良く一致したと回答した割合は 74%で，あまり一致していないが24%であった．予報が少し役立った，非常に役に立ったは 76%，あまり役に立たなかったは 20%，今後予報配信を使用したいは 68%と予報の実態が一致した患者で役に立った．今後の利用を希望する割合が高かった．一方，役立った理由としては花粉暴露を避けられた，洗濯や掃除に利用した，外出調整，薬の使用の工夫が高くみられた．

6) 海綿由来の糖脂質(ガラクトシルセラミド)パルス抗原呈示細胞の鼻粘膜下投与を受けた頭頸部癌患者 15 名中 1 名でスギ花粉 IgE，1 名でダニ IgE 抗体価が認められたが，いずれも抗原呈示細胞投与 5 週後には特異 IgE 抗体に低下がみられた．スギ花粉症患者の 1 例はスギ花粉飛散ピーク時であったが低下を示していた．

D.考察

メントール吸入，鼻翼開大テープにより鼻腔抵抗の低下がみられ，メントール吸入は一過性ではあるが 40-100 分継続していた．鼻翼開大テープの効果は全例で装着中認めたが，粘着テープの副作用から連日使用は困難例も少なくないであろう．今回の検討症例は通年性アレルギー性鼻炎で中等症以下の症例であり，大量の抗原暴露を受けるスギ花粉飛散ピーク時に再検討する必要がある．アロマテラピーについて脳機能への影響を f-MRI を利用して検討する目途がたてられた．検討を継続したい．花粉症に対するマスクの症状緩和作用は期待されるほど高くはなく，これはスギ花粉の鼻中への侵入が生じ得るためで，万全ではないことについて患者啓蒙が必要である．花粉飛散予報は，有効性が評価されており，当然のことながら実感と予報が一致した患者で評価が高く，予報精度の向上が望まれる．ガラクトシルセラミド抗原呈示細胞の鼻粘膜投与はスギ花粉症の飛散前の治療として有用性が期待され，既に臨床試験でヒトに投与され，かつ患者自身の細胞を用いて安全であることからハードルは低くオーダーメイド治療としても意義があり，動物実験で有効性を詳細に確認後，臨床試験に進みたい．

E.結論

様々な代替医療について客観的評価法，あるいは詳細なアンケート調査により科学的にその有用性，限度も明らかにするべく今後の評価の進捗が期待できる．

F.健康危険情報

なし

G.研究発表

1．論文発表

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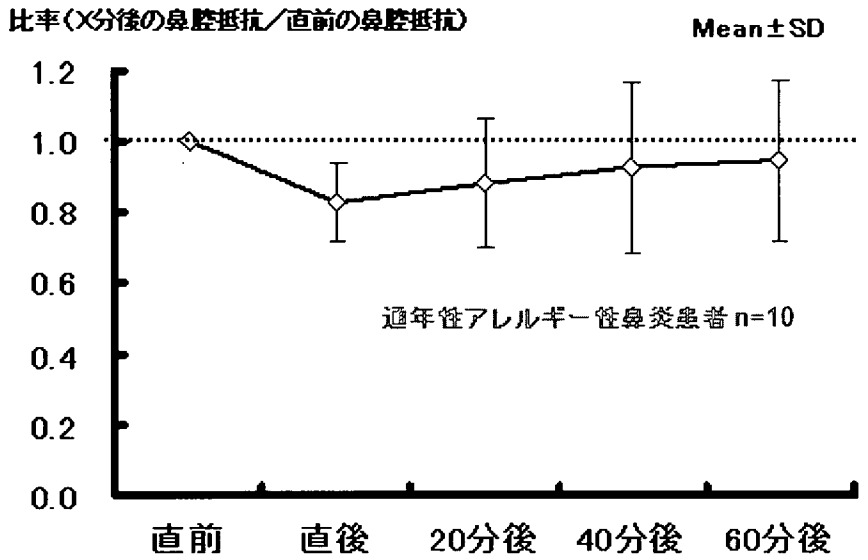
2．学会発表

なし

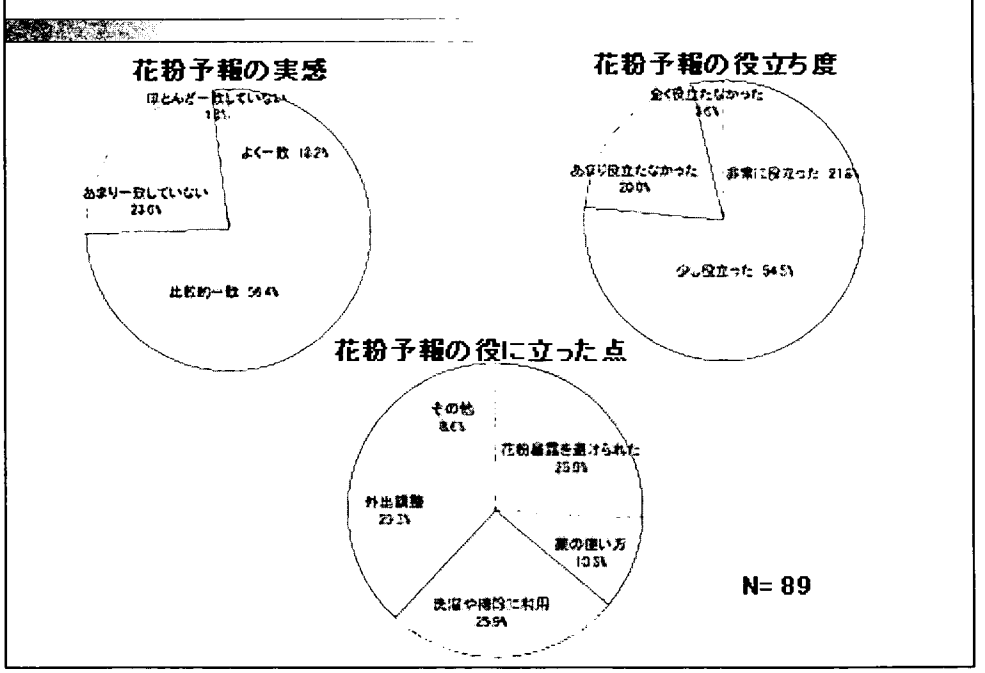
H.知的財産権の出願・登録状況

なし

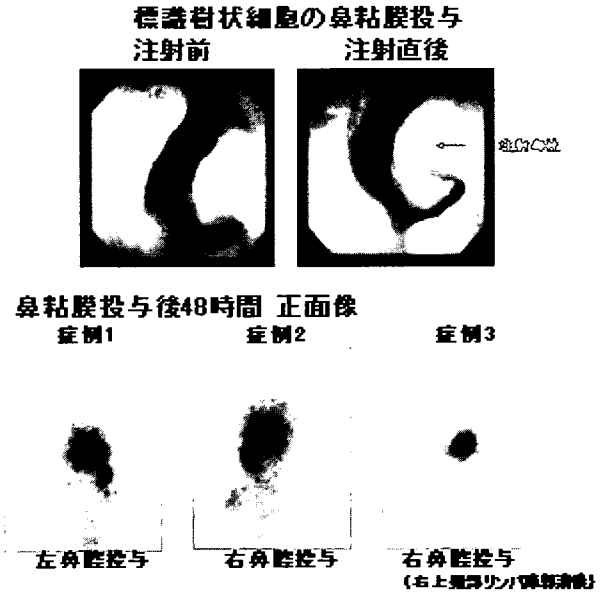
ペパーミント吸入後の鼻腔抵抗の変化



詳細な携帯メール花粉予報の評価: アンケート調査



樹状細胞の鼻粘膜下投与療法の検討



厚生労働科学研究費補助金(免疫アレルギー疾患予防・治療研究事業)
分担研究報告書

代替医療の実態と有効性の科学的評価
プロバイオティックスによる制御性T細胞・制御性サイトカインの誘導

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研究要旨
近年, 過剰な免疫応答の除去や免疫寛容の誘導・維持に中心的に働く細胞として, 制御性 T 細胞, 特に $CD4^+$ $CD25^+$ $Foxp3^+$ T 細胞 (Treg) が認知されている. 一般に Treg は cell-cell contact あるいは産生する IL-10 や TGF-β などの制御性サイトカインの作用によりその作用を示す. 今回我々は, プロバイオティックスによる免疫寛容の誘導を検証することを目的に, 代表的なプロバイオティックスである乳酸菌を取り上げ, 乳酸菌抗原がヒト末梢血単核細胞 (PBMC) からの制御性サイトカインや Treg を誘導しうるのか検討した. PBMC を 100 μg/ml の乳酸菌 (KW3110 株) 抗原にて刺激した. 培養 12 時間および 96 時間後に上清を回収し, 上清中の IL-10 および TGF-β を ELISA にて測定した. また培養 96 時間後に細胞を回収し, $CD4^+$ $CD25^+$ $Foxp3^+$ 細胞, すなわち Treg の頻度を FACS にて観察した. 乳酸菌抗原刺激に対してヒト PBMC は培養 12 時間後に有意な IL-10 産生を示した. IL-10 産生は培養 72 時間後においても有意に認められたが, 産生量は減弱した. 乳酸菌抗原刺激に対する TGF-β 産生は認めなかった. 乳酸菌抗原刺激 96 時間後の $CD4^+$ $CD25^+$ $Foxp3^+$ 細胞比率は非刺激との間に有意な差を認めなかった. 以上より, 乳酸菌抗原はヒト PBMC レベルにおいて, Treg 非依存性に IL-10 産生を誘導する可能性が示唆された.

A. 研究目的

近年, 過剰な免疫応答の除去や免疫寛容の誘導・維持に中心的に働く細胞として, 制御性 T 細胞, 特に $CD4^+CD25^+$ $Foxp3^+$ T 細胞 (Treg) が認知されている. 一般に Treg は cell-cell contact あるいは産生する IL-10 や TGF-β などの制御性サイトカインの作用によりその作用を示す.

衛生仮説を背景に, 乳酸菌などのプロバイオティックスがアレルギー疾患の予防や緩和に効果を示す可能性が報告されている. またその作用として上述の免疫制御作用を介する可能性が注目されているが, 検討は未だ十分ではない.

そこで今回我々は, プロバイオティックスによる免疫寛容の誘導を検証することを目的に, 代表的なプロバイオティックスである乳酸菌を取り上げ, 乳酸菌抗原がヒト末梢血単核細胞 (PBMC) からの制御性サイトカインや Treg を誘導しうるのか検討した.

B. 方法

乳酸菌として KW3110 株を入手した. KW3110 株は凍結融解を繰り返し, 粗抗原を抽出した. 花粉症患者を含むボランティアより採血を行い, 末梢血単核細胞(PBMC)を分離した. PBMC を 100 μg/ml の乳酸菌抗原にて刺激した. 培養 12 時間および 96 時間後に上清を回収し, 上清中の IL-10 および TGF-β を ELISA にて測定した. また培養 96 時間後に細胞を回収し, CD4, CD25 および Foxp3 の三重染色を行い, PBMC における $CD4^+$ $CD25^+$ $Foxp3^+$ 細胞, すなわち Treg の頻度を FACS にて観察した.

C. 結果

乳酸菌抗原刺激に対してヒト PBMC は培養 12 時間後に有意な IL-10 産生を示した (非刺激 0 ± 0 pg/ml, 乳酸菌抗原刺激 433.1 ± 81.2 pg/ml: $p<0.001$). IL-10 産生は培養 72 時間後においても有意に認められたが, 産生量は減弱した (22.7 ± 8.0 pg/ml: $p=0.004$). 一方, 乳酸菌抗原刺激に対する TGF-β 産生は認めなかった. 非刺激時の $CD4^+$ $CD25^+$ $Foxp3^+$ 細胞の PBMC における頻度は 3.09 ± 1.22 % であった. 乳酸菌抗原刺激 96 時間後の $CD4^+$ $CD25^+$ $Foxp3^+$ 細胞比率は 5.58 ± 2.64 % であり, 非刺激との間に有意な差を認めなかった ($p=0.179$).

D. 考察

以上の結果より, 乳酸菌抗原は本邦においてもヒト PBMC からの IL-10 産生を誘導することが明らかとなった. Niers

らはヒト PBMC においてさまざまな乳酸菌が IL-10 産生を誘導することを報告しており (Niers LEM, et al. Clinical Exp Allergy 35: 1481, 2005), 今回の結果はこれに矛盾しないと思われた. ただし, 乳酸菌による IL-10 産生は刺激早期にみられることが示され, 持続した IL-10 産生を得るためには反復した刺激が必要である可能性が示唆された.

一方, 乳酸菌による Treg の誘導に関しては, 肯定的な見解 (Baroja ML, et al. Clinical. Exp. Immunology 149: 470, 2007) がみられる一方で, 否定的な報告 (Taylor AL, et al. Pediatr. Allergy Immunol. 18: 10, 2007) もあり, 結論は出ていない. 今回の結果は後者を支持するものである. プロバイオティックスの制御性サイトカインの誘導機構についてより詳細な解析を進めているところである.

E. 結論

乳酸菌抗原は, ヒト PBMC レベルにおいて Treg 非依存性に IL-10 産生を誘導する可能性が示唆された.

F 健康危険情報

なし

G 研究発表

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H 知的財産権の出願・登録状況

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   なし

厚生労働科学研究費補助金(免疫アレルギー疾患予防・治療研究事業)
分担研究報告書

アレルギー治療の作用機序の解析

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研究要旨

アレルギー疾患に対する代替医療について,科学的評価が可能なものについては作用機序の検討を行い,代替医療の問題点を明らかにすると同時に有用である可能性を明らかにする.実際には,基礎免疫学の立場から,アレルギーに対する代替医療(乳酸菌等プロバイオティクス投与など)の作用機序の解明に向けた基礎研究を行う.I 型アレルギーの発症は Th1/Th2 のバランスによって制御されていることがわかっており,まずは Th1/Th2 のバランスの制御に注目し,これらに作用するかどうか等の検討を行う.また,Th2 免疫反応に関与する樹状細胞,マスト細胞,レギュラトリーT 細胞,NKT 細胞などの関与に関して検討を行う.これによって,作用機序の解明を目ざす.また,アレルギー発症の要の細胞であるメモリーTh2 細胞の成立,生存,機能維持に関する分子レベルでの検討,ならびに CD69 分子をターゲットにしたアレルギー性炎症の制御に関する基礎研究をおこなう.総合的な細胞レベル及び分子レベルの研究により,乳酸菌等プロバイオティクス等の作用機序を明らかにしたい.

A. 研究目的

基礎免疫学の立場から,アレルギーに対する代替医療(乳酸菌等プロバイオティクス投与など)の作用機序の解明に向けた基礎研究を行う.本年度は Th2 免疫反応に関与する細胞の機能に与える影響を調べる.アレルギー発症の要の細胞であるメモリーTh2 細胞の成立,生存,機能維持に関する分子レベルでの検討,ならびに CD69 分子をターゲットにしたアレルギー性炎症の制御に関する基礎研究をおこなうことを目的とした.

B. 研究方法

抗原(アレルゲン)特異的な TCR を持つエフェクター Th2 細胞から生体内で大量のメモリーT 細胞を作る系を樹立した.この系では,$5x10^6$程度のメモリーTh1/Th2 細胞を得ることができる.それを使ってクロマチンレベルの解析(転写活性と相関するヒストン H3-K9 のアセチル化,ヒストン H3-K4 のメチル化を指標に)を行った.ポリコーム分子やトライソラックス分子のノックアウトマウスを用いて,メモリーTh2 細胞の形成と機能維持に関する研究を行った.また,CD69 ノックアウトマウスを用いてアレルギー性気道炎症発症と持続の制御に関する研究を行った.

(倫理面への配慮)
千葉大学の動物実験指針にしたがって動物実験を行った.

C. 研究結果

次の3点の結果が得られた.1.ポリコーム分子の一つである bmi-1 のノックアウトマウスでは,Th1/Th2 ともにメモリー細胞が形成されないことが分かった.2.その機序として細胞の生存に関わる分子 Noxa の発現調節ができなくてアポトーシスが亢進しているためであることが分かった.3. CD69 ノックアウトマウスでは気道炎症反応が低いこと,Th2 細胞上の CD69 分子が気道炎症の発症に重要であることが分かった.

D. 考察

アレルギー患者では,アレルゲン特異的なメモリー Th2 細胞が症状の発症に深く関わっている.今回,ポリコーム分子群の様な細胞の生存維持に関与する分子に関して解析を行ったところ,メモリーTh1/Th2 細胞形成に関わる重要な機能が明らかになった.このことは,この分子の活性を制御することで,一方では強力な持続機能を持つワクチンの開発が考えられ,他方ではアレルゲン特異的なメモリーTh2 細胞の抑制を通じて Th2 細胞に焦点を当てたアレルギーワクチンの治療法開発の可能性が示唆される.今後,正常のヒトやアレルギー患者,また代替治療(乳酸菌等プロバイオティクス投与など)を行った患者等においてのメモリーTh2 細胞での bmi-1 の発現量と機能相関に関して解析したい.また,CD69 分子に関しては,炎症巣への活性化免疫細胞の遊走に関わる分子であるということが示唆されているが,アレルギー性気道炎症の発症に Th2 細胞上の CD69 分子が重要な働きをしていることがわかり,この分子をターゲットにした治療法の開発と,代替治療を行った患者等において CD69 の発現について解析を行いたい.

E. 結論

メモリーTh2 細胞の形成に重要な分子として Bmi-1 の同定を行った(Yamashita et al. J. Exp. Med. 2008).また,CD69 分子がアレルギー性気道炎症の発症に重要な分子であることがわかった.この分子をターゲットにした治療法の開発の可能性が提示された.代替治療(乳酸菌

等プロバイオティクス投与など)を行った患者において，ポリコームや CD69 に焦点を当てたアレルゲン特異的なヒト Th2 細胞の解析の有用性が示唆された.

F. 健康危険情報
特になし

G. 研究発表
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H. 知的財産権の出願・登録状況(予定を含む)

1. 特許取得

1) 出願中

出願番号:特願 2005-210606 号
発明の名称:アレルギー性喘息の治療薬
発明者:中山俊憲, 長谷川明洋
出願日:平成 17 年 7 月 20 日

2. 実用新案登録

なし

3. その他

なし

Ⅲ. 研究成果の刊行に関する一覧表

1.Delaunay,J., Sasajima,H., Yokota,M., Okamoto,Y. Side-by-side comparison of automatic pollen counters for use in pollen information systems. *Annals of Allergy,, Asthma and Immunology* 98,553-558,2007.

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Side-by-side comparison of automatic pollen counters for use in pollen information systems

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Background: Recent effort to build an unmanned pollen monitoring network in Japan has led to new developments in automatic pollen counters. In-the-field performance tests of these automatic counters have not been reported.

Objective: To characterize recently developed automatic pollen counters, with a view of using their data in pollen information systems.

Methods: We performed side-by-side comparisons between 2 recently developed automatic pollen counters and 2 reference samplers at 2 sites during the 2005 pollen season.

Results: Both automatic counters were found to have similar overall performance in terms of their correlations with the reference samplers. The linear correlation coefficient between the hourly values of the counters and one of the reference samplers was larger than 0.8 at both sites for both counters. Although these results are encouraging, our analysis also points to weaknesses of the investigated automatic counters in the areas of pollen discrimination, minimum measurable concentration, and calibration. Both counters were found to be affected by large concentrations of particulate matter, although the conditions and extent to which the particulate matter disrupted the measurements differ for the 2 sensors. The effect of particulate matter is particularly noticeable at the start and end of the pollen season, that is, when pollen concentration is low relative to particulate matter concentration. Further, it was found that one of the automatic counters could not differentiate snow particles from pollen grains.

Conclusions: The tested automatic pollen counters had good overall performances, but weaknesses in the areas of pollen discrimination, minimum measurable concentration, and calibration still have to be addressed for these counters to find widespread use in the allergy community.

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INTRODUCTION

Monitoring of airborne pollen has recently attracted much attention because of its potential contribution to both allergen avoidance measures by providing individuals with allergy with pollen alerts[1] and the evaluation of cross-pollination between genetically modified crops and their wild relatives.[2,3] Automatic pollen monitoring has only been introduced recently in Japan in an attempt to provide the general public with alerts for Japanese cedar (*Cryptomeria japonica*) and hinoki cypress (*Chamaecyparis obtusa*) pollen. The cedar and cypress tree species are major sources of airborne pollen, carrying potent allergens that have been reported to be the main cause of pollinosis in Japan.[4] Today, it is estimated that more than 1 in 10 Japanese citizens has pollinosis. Further, the cedar and cypress pollen share a common antigen (70% of Japanese patients with cedar pollen allergy also developed cypress pollinosis) and have their season shifted in time but overlapping so that the pollen season of the combined cedar and cypress pollen is unusually long. The cedar pollen season starts in February and ends in the beginning of April, whereas the cypress pollen season starts in March and can last until the beginning of May, thus making a pollen season of approximately 12 weeks, twice as long as the typical 6-week ragweed pollen season. Thus, cedar and cypress allergy patients are exposed to pollen for a long time, making the development of a pollen alert system desirable. Current pollen alerts are generated by information systems that use the data collected by a network of automatic pollen counters as one of their model inputs to compute pollen forecasts.[5–7] Besides the application in allergy prevention, the spread of transgenes through pollen of genetically modified crops needs to be monitored to evaluate the impact of genetically modified crops, which could lead to disruption of natural habitats.[2,3] The environment evaluation of genetically modified crops requires detailed data on pollen dispersal that can only be collected with an automatic network of pollen counters.

Automatic pollen counters that are widely used in Japan include the KH3000 (Yamato, Yokosuka, Japan),[8] Kowa (Hamamatsu, Japan),[9] and NTT (Tokyo, Japan)[10] counters. Recently, a new pollen counter developed by Shinyei Corporation (Kobe, Japan) was introduced on the market and has triggered much interest because of its new pollen discrimination principle and its low cost. The design of the new automatic counter is based on the design of the standard particle counter in which a defined volume of air is circulated through a fine pipe that is intersected by a laser beam. When

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a particle passes through the laser beam, a scattered signal is detected, the intensity of which is related to particle size and optical index. The measured intensity of the scattered light can be related to particle size. In addition to the scattered intensity, the Shinyei counter includes a measure of the change in the polarization state of scattered light, which is known to be related to the particle shape and its internal structure. Pollen grains generate intensity and polarization signals that are different from those of nonpollen particles, so that pollen can be recognized from these 2 measures. The application of this pollen recognition principle may lead to some errors, because it was recognized that under certain circumstances (types of particulate matter) some overlapping between the scattered intensity and polarization values of pollen and particulate matter takes place. The KH3000 counter uses the spherical shape of pollen grains to discriminate them from other particles (the nearly perfect spherical shape of pollen is not found in particulate matter that lacks a biological origin such as sand and soot). To discriminate spherical particles from others, 2 laser beams and their respective detectors are used to measure the intensity of the scattered light from the same particle but measured at 2 different incident angles. If the particle is spherical and homogeneous, the 2 intensities will be the same. The existing automatic counters together with their main characteristics are listed in Table 1. Extensive comparisons against reference samplers, such as the gravimetric Durham sampler and the volumetric Burkard sampler (Burkard Manufacturing Co, Rickmansworth, England), remain scarce, resulting in poor characterization of actual counter performance in the field and, therefore, in ambiguity as to how the data should be used in a pollen information system. The new counter introduced by Shinyei and the lack of data on actual performance of the available counters prompted us to conduct our own side-by-side comparison.

MATERIALS AND METHODS

During the cedar and hinoki pollen season of 2005, we conducted extensive side-by-side comparisons between the KH3000 and Shinyei automatic pollen counters and the Durham and Burkard reference pollen samplers. Our measurement campaign was performed at 2 different sites, namely, on the roof of a 4-story building in the Chiba University Campus (140.133° east and 35.602° north) and on a 5-story building of the Akita Health Institute (140.898° east and 39.717° north). The measured items together with the measurement periods are given in Table 2. Although the Burkard samplers used at both sites were of a different type, they were of a similar design except for the vacuum source, which uses a fan in the old version (7-day spore trap) and a mechanical pump in the new version (SporeWatch trap). The pollen counts on the Melinex tape used in the Burkard samplers and on glass slides used in the Durham samplers were performed under a microscope at a magnification of ×400 by trained staff. Both cedar and hinoki pollen grains were counted, because the automatic counters do not distinguish these pollen species, which are similar in size and shape. Durham daily counts were only available at the Chiba site and were determined by observing an area of 1 cm^2 (in this case, cedar and hinoki were counted separately). For the Burkard counts, the observed areas of the Chiba data and the Akita data were 0.5 × 5 mm^2 and 2 × 14 mm^2, respectively. The KH3000 counters were operated with a sand gravimetric trap, which is thought to filter out yellow sand particles originating from deserts in China and Mongolia and sporadically blowing across Japan. The actual performance of the sand trap with regard to discrimination between pollen and sand has not been reported to our knowledge. The recently developed Shinyei pollen counter[11] exists in 2 versions: the original version, referred to as Shinyei, which has to be placed in a weather instrument shelter, and a modified version, referred to as NTT-Shinyei, which has a higher flow rate and is protected by an all-weather casing. The measurement results obtained with the original version of the NTT counter[10] are not shown because the NTT counter was updated to the NTT-Shinyei counter. The KP1000 Kowa counter[9] was found to be difficult to operate during a long period (we have had experience during 2 cedar pollen seasons) because of high running cost and repeated failures, so we decided not to include data from this counter in our study.

RESULTS

Figure 1 shows the variation in time of the daily deposition count and the average daily concentration for the Chiba site. The deposition count was determined by using a Durham sampler and counting separately cedar and hinoki pollen grains. The average daily concentration was obtained by averaging hourly concentrations measured by the automatic pollen counters. These counters cannot distinguish cedar from hinoki pollen grains and thus should be compared with observations of the total count of cedar and hinoki pollen grains. As shown in Figure 1, the hinoki pollen contribution to deposition counts was significant from the middle of March and predominant at the end of March. For the Akita

Table 1. Main Characteristics of the Most Widely Used Automatic Counters and the Burkard Reference Sampler

Counter	Measurement principle	Pollen discrimination	Flow rate, L/min
Burkard	Impactor and microscopy	Microscope observation	10
KH3000	Scattering from 2 beams	Spherical shape	4.1
Kowa	Scattering and fluorescence	Size and fluorescence	4.0
Shinyei	Scattering and polarization	Size and shape	0.9 (original), 2.2 (NTT-Shinyei)
NTT	Scattering	Size	30

Table 2. List of Instruments and Their Period of Operation at Both Measurement Sites

Site (period of operation)	Durham	Burkard	KH3000	Shinyei
Chiba (1/24/05–4/24/05)	C	3/25–3/30 (SporeWatch, 1-hour sampling)	C (with sand filter)	C (NTT-Shinyei)
Akita (3/28/05–4/25/05)	NA	C (7-day sampler, 2-hour sampling)	C (with sand filter)	C (Shinyei original)

Abbreviations: C, complete dataset; NA, dataset not available.

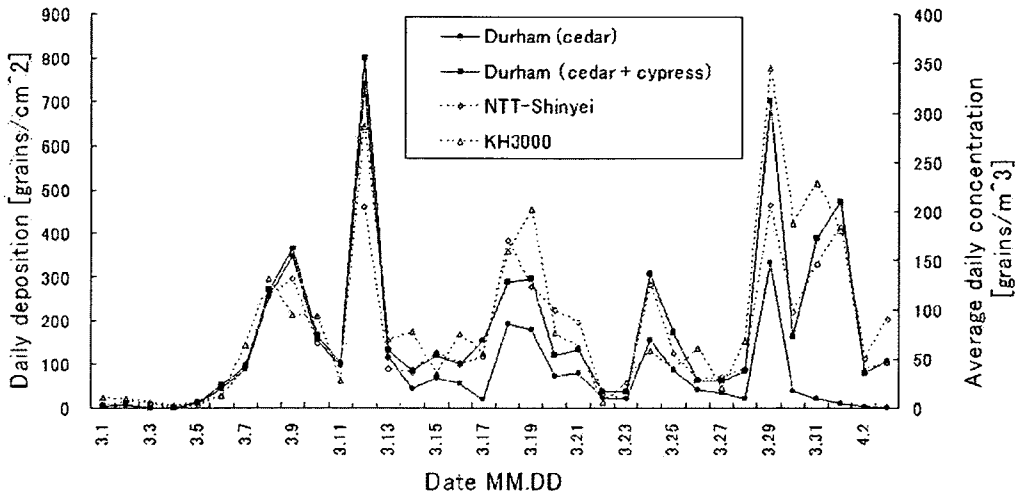

Figure 1. Comparison between daily deposition and daily average concentration of pollen at the Chiba site for the complete pollen season. The daily deposition counts for the cedar and hinoki total and for the cedar only are shown to illustrate the contribution of hinoki to the total count of pollen. Average daily concentrations were computed from hourly concentration values measured by automatic counters.

site, hinoki pollen contribution is known to be negligible during the entire pollen season because of the quasi-absence of the hinoki species in this area. From Figure 1 it is clear that the variations in daily deposition counts determined by the Durham reference sampler are well reproduced by the variations in the daily average concentrations of the automatic counters. Note that the relation between deposition and concentration depends on local factors, such as irregular topography and micrometeorological conditions, and is therefore varying over space and time. In our comparison, we assume the average over a day of these factors to be constant. The linear correlation coefficients between the Durham counts and the concentrations of both automatic counters were higher than 0.9, strongly suggesting that the automatic counters correctly approximate daily pollen variations.

Figure 2 shows the pollen concentration time series as measured with the automatic counters and the reference sampler at both sites. Timing of the pollen bursts are well reproduced by the 2 automatic counters. This is further evidenced by the high values of the linear correlation coefficients between the counters and the reference sampler. For the Chiba data, both a Shinyei and a NTT-Shinyei counter were used and the correlations were computed on hourly concentrations. For the Akita data, a Shinyei module was used and the correlations were computed on 2-hour average concentrations. At the Chiba site, the linear correlation coefficients were 0.89, 0.92, and 0.90 for the KH3000, Shinyei, and NTT-Shinyei counters, respectively. At the Akita site, the linear correlation coefficients were 0.83 and 0.81 for the KH3000 and Shinyei counters, respectively. It also appears from Figure 2 that the intensity of the burst is not always well determined by the automatic counters. All counters underestimated pollen concentrations, pointing to calibration problems in the automatic counters. Small variations in pollen concentrations (<100 grains/m^3) as measured by automatic counters did not compare well with those determined with the reference sampler. In the Akita time series of Figure 2b, we found a high correlation between a sleet episode recorded by the Japan Meteorology Agency on March 29 to 30 and high counts of the KH3000. During this sleet episode, no pollen was observed in the Burkard reference concentration series. This strongly suggests that the KH3000 counted snow particles as pollen grains. This phenomenon was not observed with the Shinyei counter.

In Figure 3 and Figure 4, we examine in more detailed measurement errors in pollen concentration determined by the automatic counters for the Akita series. The concentrations measured by the automatic counters were first corrected for bias introduced by calibration errors using a linear calibration curve between the reference and the automatic counters, and then the residuals were computed and used to derive the absolute errors between the automatic counters and

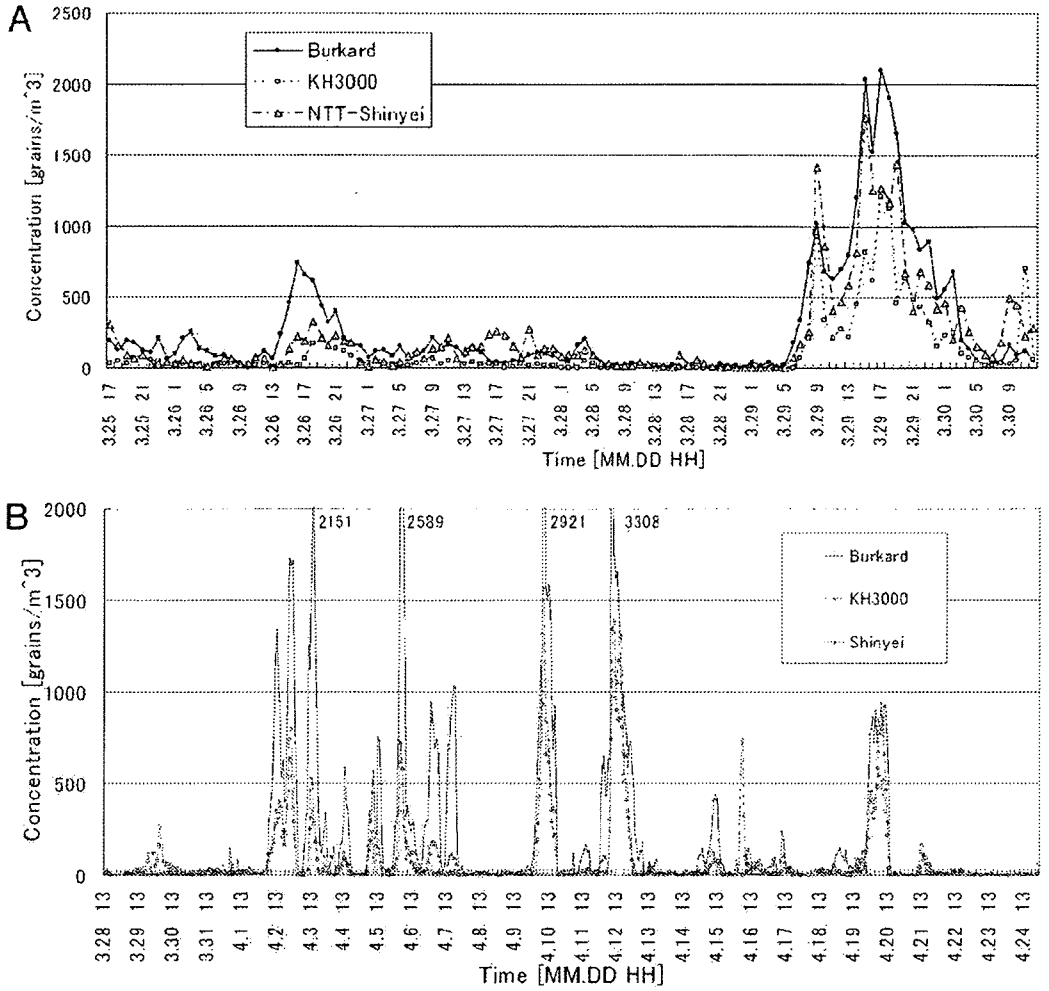

Figure 2. Comparison between pollen concentrations measured with a Burkard, KH3000, and Shinyei counter at the Chiba site (A) and the Akita site (B). In section B, the maximum value of the concentration axis was set to 2,000 grains/m^3 to make visible the variations for all counters (actual values for the Burkard counts that fall outside the plotted range are indicated on the right side of each peak). The 1-hour average and 2-hour average of pollen concentration are plotted for the Chiba site and the Akita site, respectively.

the reference sampler. Figure 3a-b shows detailed time variations in the corrected concentrations together with the reference concentration for a low-concentration region and a high-concentration region of the Akita series, evidencing poor (good) correlation between the automatic counters and the reference sampler for the low (high) concentration region. Figure 4 shows the absolute errors in concentration measurement between the automatic counters and the reference sampler as a function of the averaged concentration. The curves indicated as "observed" refer to errors estimated from differences between the reference sampler and the automatic counters, whereas the curves indicated as "statistical" refer to errors estimated from the theory of statistical fluctuations. When counting airborne pollen grains, unavoidable statistical fluctuations in the observations result from the random nature of the observed process. These fluctuations are not related to any instrument error; that is, an estimate of the statistical fluctuations gives the lower limit to the measurement error for an ideal instrument. An estimate of the fluctuation error can be obtained from the SD of the observation distribution, which is known to follow a Poisson distribution in a counting experiment. The SD of a Poisson distribution is the square root of the mean of the counted events, which was used to compute the statistical errors of Figure 4. The difference in the statistical errors between the 2 automatic counters is explained by a difference in the sampled volumes (see the flow rate column of Table 1), that is, a difference in the number of counted pollen grains for the same concentration. For both automatic counters, the observed errors are found to be much larger than the statistical errors, pointing to the

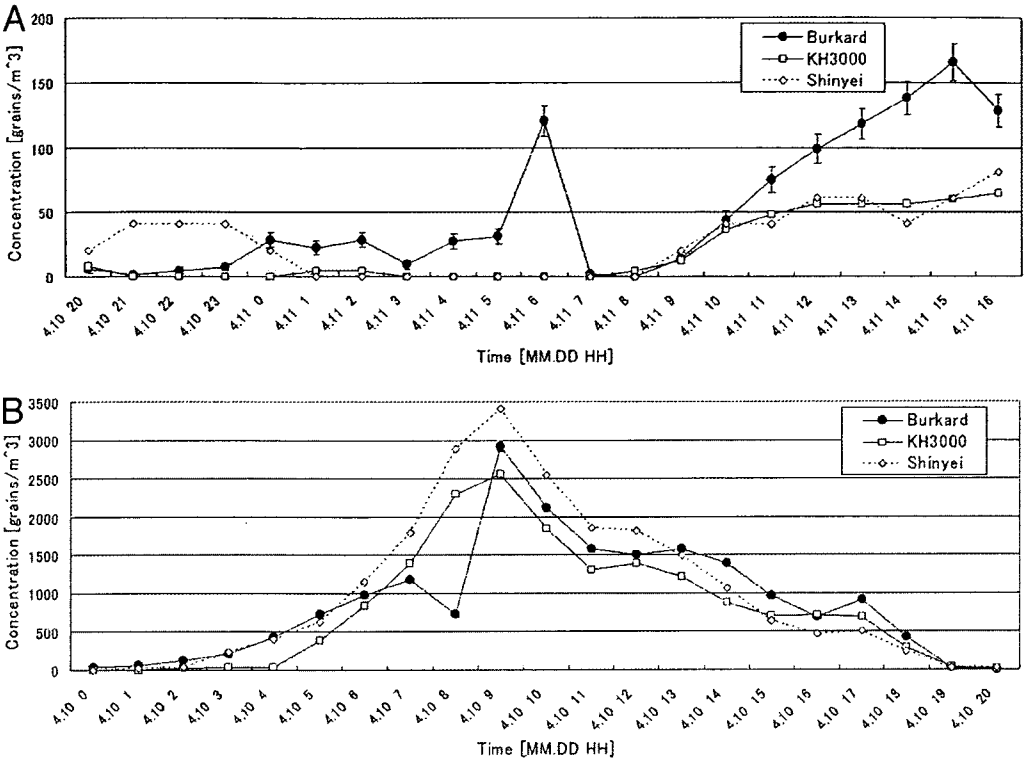

Figure 3. Detailed variations in pollen concentration with time at Akita as measured with a Burkard sampler and a KH3000 and Shinyei counter for a low-concentration region (A) and a high-concentration (B) region. The error bars of the Burkard series represent statistical uncertainty, which arises from statistical fluctuations in the pollen counts and were estimated as the square root of the counted pollen grains. (Note that the values of the error bars in section B are too small compared with the extent of the y-axis to be represented.)

existence of instrumental errors. For concentration in the 0 to 50 grains/m^3, the error of the Shinyei counter is as large as the measured concentration, indicating that data collected with this counter should be used for concentrations larger than 50 grains/m^3.

DISCUSSION

A side-by-side comparison conducted at 2 different sites between reference samplers and automatic pollen counters revealed some weaknesses of the investigated counters in the areas of pollen discrimination, minimum measurable concentration, and calibration. These imperfections rooted in the design of the investigated automatic counters directly affect the accuracy of the measured pollen concentration and limit their applications. Apart from changes in counter design, which fall outside the scope of this report, we would like to point out a possible area of improvement. Because the 2 investigated automatic counters have been found to exhibit different characteristics in their ability to discriminate pollen from other particles, combining observations of both counters to filter out false peaks should lead to some improvements in the accuracy of these pollen counters when used simultaneously. Also some recommendations when setting up pollen counters in weather shelters (commonly used to protect instruments from sunshine and precipitation) may be useful. When operating a counter in weather shelter, the air should be sampled outside the shelter through a channel like the ones of the KH3000 or the NTT-Shinyei counters. Sampling inside the shelter could result in measurement errors, because pollen grains that are inevitably deposited inside the shelter are likely to be reemitted at a later time.

Pollen discrimination problems and calibration inaccuracies greatly hamper the use of automatic pollen counter data in a pollen forecasting system and should be addressed. The minimum measurable concentration of the investigated counters was found to be approximately 50 grains/m^3, a concentration that may be too high to provide useful information to patients with allergy. The value of the minimum concentration to be monitored in a pollen information system has to be debated and agreed on by the allergy community. For the automatic pollen counters to be successfully integrated in a pollen monitoring network, further developments in the pointed areas have to be made.

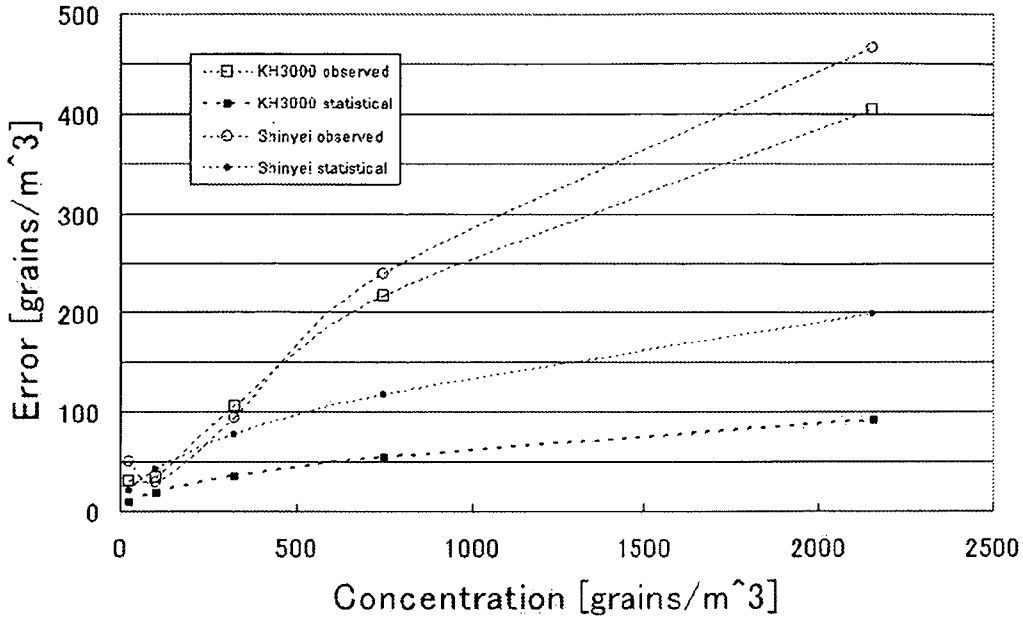

Figure 4. Error analysis of automatic pollen counters performed on the Akita dataset. Absolute errors are computed between the concentrations measured with the Burkard reference sampler and the automatic counters. The 2-hour average concentrations were used and errors computed in the following bins: 0 to 50, 50 to 150, 150 to 500, 500 to 1,000, and more than 1,000 grains/m^3.

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Migration of Tumor Antigen-Pulsed Dendritic Cells After Mucosal Administration in the Human Upper Respiratory Tract

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Abstract Tumor-specific peptide-pulsed dendritic cells (DC) were administered via different routes to a group of patients with head and neck cancers. The migration and homing patterns of such antigen-stimulated cells was carefully studied employing single photon emission computed tomography (SPECT). The DC administered directly into the nasal submucosa quickly migrated very rapidly to the regional neck lymph nodes in the neck. However, after inoculation of the cells into the palatine tonsils, the DCs remained close to the site of administration and did not migrate to the regional lymph nodes or to other mucosal regions. After nasal submucosal administration of the DC, tumor-antigen-specific cytotoxic T cells were detected in the ipsilaterals but not in the contra lateral lymph nodes. These results suggest that after antigen processing, the regional lymph nodes serve as inductive sites for development of mucosal immune responses and for induction of memory cells during the local immunological responses in the nasopharyngeal-associated lymphoid tissue in man.

Keywords Dendritic cell · migration · mucosal vaccines · human studies · cancer vaccines

Abbreviations

DC	dendritic cell(s)
NALT	nasopharyngeal-associated lymphoid tissue
SPECT	single photon emission computed tomography
ROI	region of interest
HLA	human leukocyte antigen
CTL	cytotoxic T lymphocyte

Introduction

A distinct nasal-associated lymphoid tissue (NALT) located in the anterior nasal cavity is an important inductive site of mucosal immune responses in the rodents and functions in a similar manner to the Peyer's patches in the gut and other organized lymphoid tissues [1–4]. The nasopharyngeal tonsils and the salivary glands and other tissues, collectively referred to as Waldeyer's Ring, appear to represent the equivalent of NALT in man. It includes isolated lymphoid follicles having an overlying lymphoepithelium with M cells [5–8]. Recently, it has been proposed that the upper respiratory tract could be an attractive vaccine administration route because of its ability to mount effective mucosal as well as systemic immune responses [9–13]. However, the role of such immunization in cancer immunotherapy remains to be elucidated.

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Recently, dendritic cells (DC) have been shown to play a crucial role in the induction of the primary T-cell-dependent immune responses. DC-based cancer immunotherapy has been shown to induce significant immunological responses and some clinical improvement in patients with different malignant disorders, including gastric carcinoma [14], prostate cancer [15], and the adenocarcinoma of colon [16]. The most effective route of administration of the tumor antigen-pulsed DC to induce effective anti-tumor responses remains to be determined. The migration and selective homing of DC, after the uptake of antigen from peripheral tissues and its delivery to the regional draining lymph nodes, is considered to be a distinct immunological property. In human studies, peripheral blood DCs administered in the upper thigh have been observed to migrate to the regional inguinal lymph nodes [17]. However, the migration patterns of DC from the upper respiratory tract have not been studied extensively in humans.

Most head and neck tumors arise from upper respiratory or gastrointestinal mucosa, and the lymph nodes in the neck region are the principal draining sites for these tissues. Thus, intramucosally administered DC may be expected to migrate to the regional lymph nodes and effectively induce mucosal anti-tumor responses locally.

In the present studies, we examined the migration of autologous, tumor antigen-pulsed DC, after administration into the nasal submucosa, direct inculcation into the nasopharyngeal tonsils, or after intravenous injection in a small number of patients with different carcinomas of head and neck region.

Materials and Methods

Eleven patients with squamous cell carcinomas of head and neck region were enrolled in this study, as shown in Table I. All patients were positive for human leukocyte antigen (HLA) A2402 and included four subjects with maxillary cancer and seven subjects with pharyngeal carcinoma. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from each patient.

SART-1 peptides

SART-1 is a carcinoma rejection antigen identified by using a cytotoxic T lymphocyte (CTL) clone developed from a patient with esophageal cancer. The clinical grade SART-1 peptides (SART-1 690–698) recognized by HLA-A2402 restricted tumor-specific cytolytic T lymphocytes [18] were prepared by NeoMPS. Inc., San Diego, CA. HLA-matched HIV-specific peptides (HIV Env 584–592) were used as controls [19].

Preparation of DCs

Peripheral blood mononuclear cells (PBMC) were prepared from 100 ml of peripheral blood from each patient. The cells were incubated for 2 h in a six-well plate under 5% CO_2 at 37°C. After the removal of non-adherent cells, the adherent cells were cultured in AIM V medium (Gibco, Rockville, MD) containing granulocyte macrophage colony stimulating factor (GM-CSF; 1,000 U/ml) and IL-4 (600 U/ml; Primmune K.K, Osaka, Japan) for 7 days to generate a DC-rich cell population. The cultured cells were harvested by vigorous washing. The enriched DCs were then cultured overnight in a medium containing 2 μg/ml squamous cell carcinoma specific SART-1 peptide. The DCs were then washed and resuspended in normal saline containing 5% autologous serum from a given patient. The phenotypic markers of the DC were determined by flow cytometry using monoclonal antibodies to HLA-DR, CD83, CD80, and CD86 (BD Pharmingen Fujisawa, Tokyo, Japan).

Table I Patients Profiles

Patient	Age/gender	Tumor lesion	Stage	Labeled DCs	Un-labeled DCs
1	68/M	maxilla	T4N1M0	ns	
2	72/M	maxilla	T4N0M0	ns	
3	67/M	maxilla	T4N0M0	ns	
4	47/M	pharynx	T3N1M1	ton	
5	56/M	pharynx	T2N1M0	ton	
6	70/F	maxilla	T3N0M0	iv	
7	56/M	pharynx	T3N1M0	iv	
8	73/M	pharynx	T2N3M0		ns
9	56/M	pharynx	T4N2M0		ns
10	71/F	pharynx	T4N2M0		ns
11	66/M	pharynx	T4N0M0		ns

DCs were administered into *ns* nasal submucosa, *ton* intratonsil, *iv* intravenous.

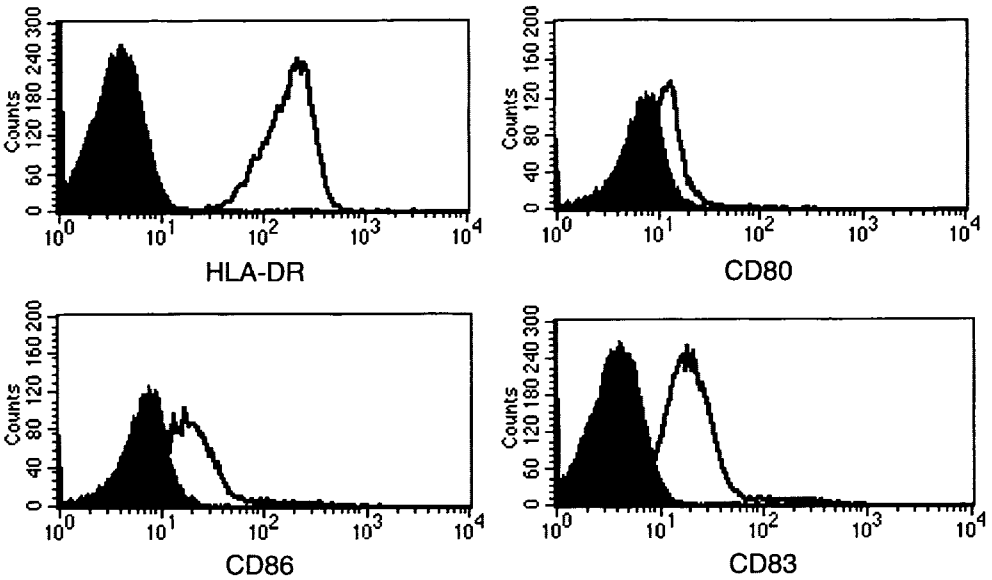

Fig. 1 The SART-1 pulsed DC possess an activated phenotype, including an upregulation of HLR-DR, CD80, CD86, and CD83, shown as *white*. The isotype match control is shown as *black*.

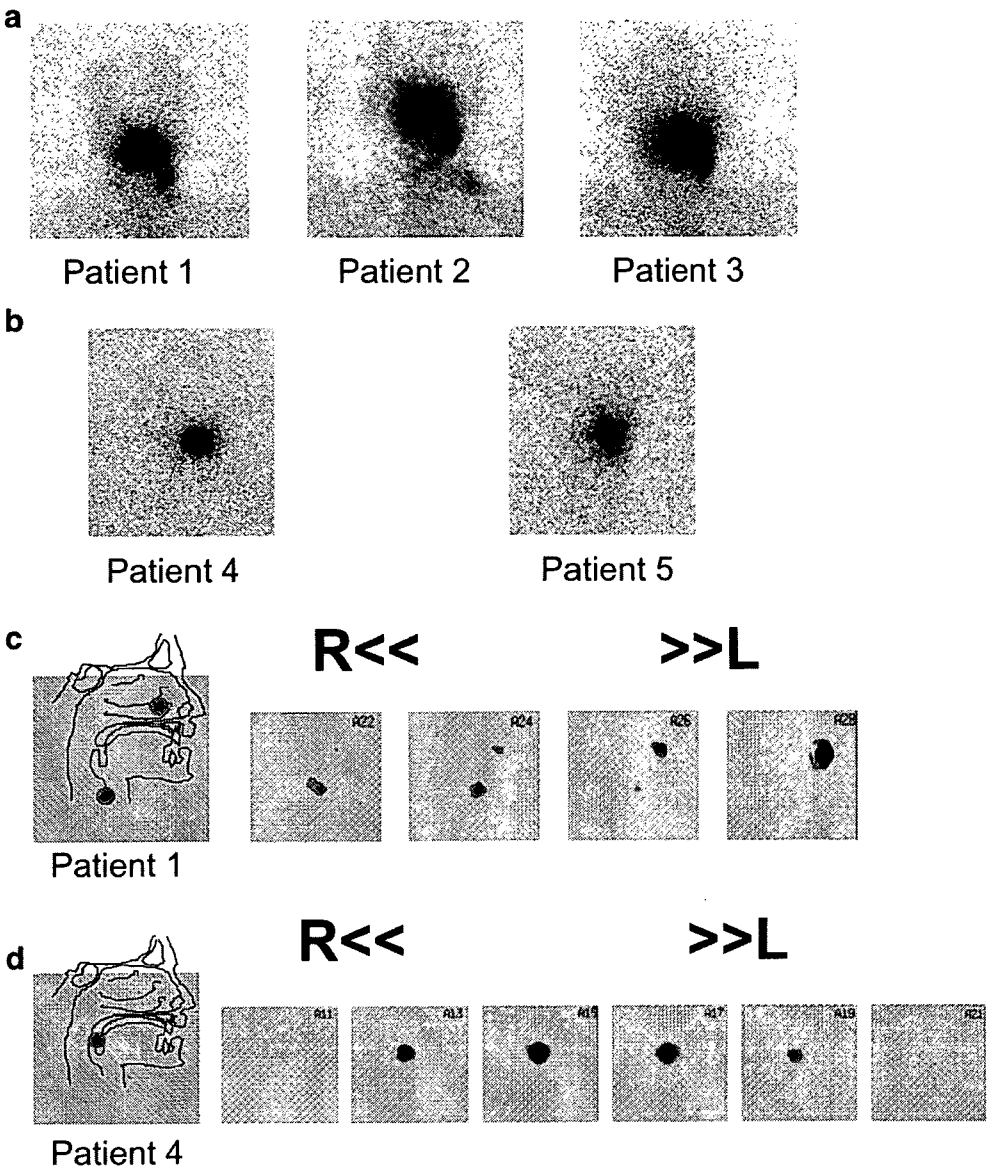

Fig. 2 An anterior image of the head and neck region recorded 48 h after injection. These patients were administered with DC into the left nasal inferior turbinate or tonsil. The DCs were intense in the neck as in the nose (**a**) or into the left palatine tonsil (**b**). A sagittal SPECT image of the head and neck region 48 h after injection. A series images were made at 2-cm intervals. The intense spots were observed in the injection site of nose and the regional lymph nodes (**c**). In contrast, when DCs were injected into the left palatine tonsil, the intense spots were only observed in the injected tonsil (**d**).