慢性副鼻腔炎とアレルギー

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●慢性副鼻腔炎
●鼻アレルギー
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はじめに

近年，先進諸国におけるアレルギー疾患の有病率は，著しく増加していると考えられている。われわれが行った耳鼻科専門医とその家族を対象としたアンケート調査によれば，季節性のスギ花粉症の有病率は16.2%，通年性のアレルギー性鼻炎の有病率は18.7%であった[1]。アレルギー疾患の発症には，環境因子や遺伝的因子が複雑に絡みあっていると考えられるが，特に環境因子の割合が多いと考えられている。アレルギー疾患の発症に関与していると考えられている環境因子としては，花粉やダニ，ハウスダストなどのアレルゲンの増加，ディーゼルエンジンなどによる大気汚染，食生活・栄養状態の変化などがあげられる。最近注目を浴びている環境因子として衛生環境の変化，つまり過剰な清潔志向による抗菌グッズの普及，予防接種の普及や抗生薬の乱用による細菌曝露の減少である。

これらの環境衛生の変化が，アレルギー性疾患の増加に関与しているのではないかとするHygiene Hypothesis(衛生仮説)が，近年注目を集めている。本稿では，慢性副鼻腔炎と鼻アレルギーとの関連について，疫学的事実を踏まえ述べてみたい。

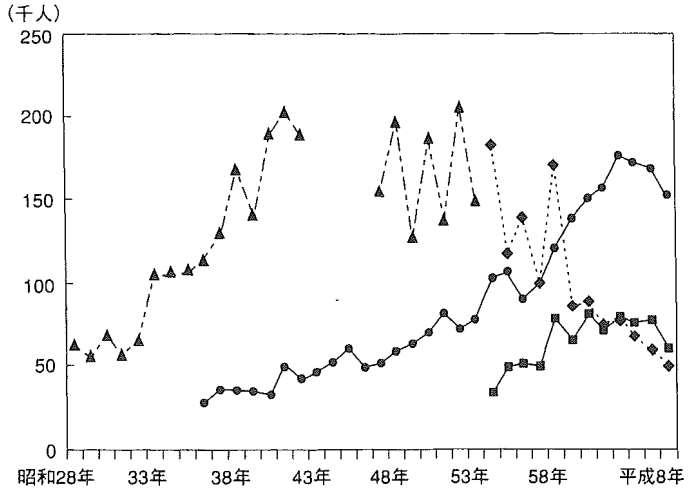
Ⅰ．衛生仮説とは

Strachanは，1989年にアレルギー疾患の発症やアトピー性素因を抑制する環境因子について疫学調査を行い，報告した。その報告のなかで，1958年の3月に生まれた17,414名について23年間追跡調査を行い，11歳，23歳の時点で，hay feverや慢性湿疹の保有率は同胞数に反比例していること，さらにそれは年長者の同胞数に依存すると述べている[2]。Strachanは，この結果の解釈として，衛生環境の向上による幼少時の感染症曝露の減少が，アレル

ギー疾患の増加の原因ではないかとする衛生仮説の概念を提唱した。その後，この衛生仮説を支持するいくつかの報告がなされた[3)-6)]。そして，現在では衛生仮説の根幹をなす理論として，Th1 (Helper T cell 1) /Th2 (Helper T cell 2)バランス説が広く支持されている。つまり，新生児の免疫応答は，Th2型の免疫応答が優勢であるが，幼少期にさまざまな感染性微生物への曝露によりTh1型の免疫反応が発達し，Th1とTh2のバランスがとれたものとなる。したがって，近年の衛生環境の整備により幼少期における感染の機会が減少することにより，結果としてTh2優位の免疫状態が生じ，アレルギーを発症しやすくなると考えられている[7)]。

Ⅱ．慢性副鼻腔炎とⅠ型アレルギー

本邦における慢性副鼻腔炎の疫学調査については，いくつかの報告がある。集団検診による検討では，鎌田ら[8)]は，1万人を対象とした集団検診を1964～1966年の間に行い，慢性副鼻腔炎の有病率を10.4％と報告している。同様に後藤ら[9)]は，2,357人の小学生を対象とした集団検診を1964～1965年の間に行い，慢性副鼻腔炎の有病率を12.5～17.2％と報告している。入院患者統計における検討では，高坂ら[10)]は，1940～1977年に耳鼻咽喉科の入院患者において鼻副鼻腔疾患の割合は34％で，そのうち慢性副鼻腔炎の占める割合は，1940年代には58％であったが1970年代には20％まで低下したと報告している。このように，慢性副鼻腔炎の有病率については，調査手法が確立されておらず，報告によってばらつきがある。また，鼻アレルギーについても同様に，その有病率についての疫学調査の方法がいまだ確立されておらず，報告によってばらつきが多い。先にも述べたように，われわれが1998年に行った日本全国の耳鼻咽喉科医およびその家族を対象にしたアンケート調査では，アレルギー性鼻炎の有病率は，10～40歳代にピークをもつ山型の年齢分布を示し，全体の有病率は季節性のスギ花粉症で16.2％，通年性のアレルギー性鼻炎で18.7％であった[1)]。鼻アレルギーの有病率は，近年増加していることは間違いないが，その正確な数値は不明である。そこで，われわれは，厚生労働省大臣官房統計情報部より経年的に発表されている全国推計患者数を調べた(図)。この全国推計患者数は，必ずしも実際の患者数と一致するとは限らないが，ある程度患者数の推移を知ることは可能である。この調査結果からは，昭和40年代より喘息患者数は徐々に増加しはじめ，昭和50年代後半からは増加が著しくなっている。同様に鼻アレルギー患者数も昭和50年代後半より増加しはじめ，平成5 (1993)年には慢性副鼻腔炎患者数と逆転している。それとは逆に，慢性鼻，副鼻腔炎患者数は昭和50年代はじめより減少しはじめ，昭和60年代前半には鼻アレルギー患者数よりも少なくなっている。平成14(2002)年度の報告では，喘息，鼻アレルギーの患者数は，鼻副鼻腔炎患者数のそれぞれ約3倍と1.3倍である。この結果からは，鼻アレルギーの増加の1つの因子として，慢性副鼻腔炎の減少があり，鼻アレルギーと慢性副鼻腔炎との間に，衛生仮説が成立するようにも思える。一方で，夜陣ら[11)]は，小中学生約1万人を対象にした1980年度および2000年度の学校検診における



図．全国推計患者数

‥◆‥：慢性副鼻腔炎，—●—：喘息，－▲-：鼻副鼻腔炎，—■—：鼻アレルギー

昭和28年から平成14年までの患者調査表(厚生労働大臣官房統計情報部)より抜粋した。

なお，昭和44年から47年の間の鼻副鼻腔炎患者数については記載されていなかった。

検討で，慢性副鼻腔炎では，両年度で有病率に顕著な差はなかったが，鼻アレルギーの有病率は，2000年度は1980年度に比べ有意に上昇していたと報告している。これら疫学的事実からは，喘息・鼻アレルギー患者数が増加していることは間違いないと思われるが，これを，慢性副鼻腔炎患者が減少したためと短絡的に結びつけることは困難と思われる。これまで行われてきた慢性副鼻腔炎および鼻アレルギーに対する疫学的調査では，調査方法や対象群が統一されておらず，今後は調査方法を標準化することが正確な有病率の把握には必要と思われる。

強力なTh1型の免疫反応を惹起する細菌としては，結核菌が知られているが，化膿性副鼻腔炎の起炎菌は通常，黄色ブドウ球菌や肺炎球菌である。これらのグラム陽性菌が結核菌と同様に強力なTh1型の免疫反応を惹起するほどの強い炎症を引き起こすとは考えにくい。しかしながら，主としてグラム陰性桿菌から産生されるエンドトキシンの濃度を測定した検討では，室内塵のエンドトキシン濃度が，非アレルギー児よりもアレルギー児のほうが低いと報告されている[12]。また，最近ではマクロファージや樹状細胞などの抗原提示細胞上に表出しているTLR(Toll-like receptor)を介するシグナルにより活性化された樹状細胞は，主としてTh1型の免疫反応を誘導することが知られており，細菌感染とアレルギーの関連について解明されつつある[13]。

ま と め

衛生仮説の概念が疫学的調査から提案され，さまざまな環境因子とアレルギー疾患との関連が，これまで報告されてきた。衛生仮説は，アレルギーの発症や病態を考えるうえで，大変興味深い理論ではある。しかしながら，こと慢性副鼻腔炎に関しては，鼻アレルギーの増加の因子として副鼻腔炎が関与していると断定するのは，現時点では困難のように思える。今後，標準化された調査方法を用いた大規模な疫学調査が行われることを期待したい。

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研究報告書
リアルタイムモニター飛散数と現状の治療によるQOLの関連性の評価と花粉症根治療法の開発
自然免疫系を介したスギ花粉症治療の基礎的研究

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研究要旨
微生物感染に対し生体は、Toll-like receptor (TLR)を介して pathogen-associated molecular patterns (PAMPs)を認識し、自然免疫系を活性化するとともにその後の獲得免疫の成立を促し、病原体から身を守っている。この機序にはアレルギー抑制に働く Th1 細胞の誘導が必須である。そこで二本鎖 RNA(dsRNA)および CpG-DNA を用いてアレルギー治療に使用できないか検討した。1μg/ml までの dsRNA は、鼻粘膜由来線維芽細胞において JNK の経路が働き、好酸球の浸潤を促進させる。さらに dsRNA は、抗原特異的な免疫グロブリン産生に関与する BLyS の産生を誘導する。IgE 産生・好酸球浸潤の点からは dsRNA は使用できないが、vaccination においては治療に使える可能性があった。CpG-DNA は、形質細胞様樹状細胞の関与のもと IgE 産生を抑制する。これは扁桃細胞においても同様であり、舌下免疫療法のアジュバンドとして vaccination 亢進と IgE 産生抑制の二面性を持って使用できる可能性を見出した。

A 研究目的

ヒトは生下時、Th2 (type 2 helper T cells) 優勢であり、その後微生物 (細菌・ウイルス) の感染や曝露によって、Th1 細胞の誘導がなされる。しかし Th1 への誘導がなされず、Th2 優勢がそのまま持続するとアレルギー疾患が発症すると言われている。これが衛生仮説であり、アレルギー性鼻炎増加の原因の一つと考えられている。鼻腔は気道の入り口であり、最もウイルスや細菌に曝露される器官と考えられている。鼻腔内組織はそれらの感染対していろいろな反応を起こし、生体を防御する。本研究では、ウイルス感染や細菌感染を模倣するような方法で生体内に反応を起こさせ、花粉症に対する新しい治療法になりえないか検討した。

B 方法

花粉症患者の下鼻甲介手術時に採取した下鼻甲介粘膜から、線維芽細胞を分離し用いた。すべての実験は、文書で患者の同意を得たのち行った。合成 doule stranded RNA (dsRNA)である polyI:C を RNA ウイルス感染の模倣する物質をして用い、線維芽細胞のケモカイン産生を調べた。細胞内シグナルの同定は、シグナル特異的阻害薬やリン酸化を検討して行った。細菌感染や DNA ウイルス感染の模倣として CpG-DNA を用いた。CpG-DNA はいくつかの型に分類されているが、樹状細胞の活性化を主作用とするAタイプ CpG-DNA と主に B 細胞に働き IgE 産生を抑制する B タイプ CpG-DNA を今回の実験では用いた。ヒト末梢血単核球及び口蓋扁桃より分離した細胞における、両タイプの CpG-DNA による免疫寛容の誘導および IgE 産生の抑制を検討した。形質細胞様樹状細胞はポジティブセレクション法にて行った。

C 結果

鼻由来線維芽細胞における10種のTLRの発現量を定量的に解析した結果、TLR3、4、9の発現量が特に多く認められた。鼻由来線維芽細胞を poly(I:C)で刺激すると、poly(I:C)の濃度依存的に増強する IL-8、RANTES の著明な産生亢進を認めた。Eotaxin、IL-1β、TNF-α、IFNα、IFNγ、IL-12 の産生亢進は認められなかった。poly(I:C)刺激による RANTES 産生は JNK と PI3 キナーゼの関与が、IL-8 産生は JNK、p38MAP キナーゼ、PI3 キナーゼの関与が考えられた IL-4 存在下では鼻由来線維芽細胞から Eotaxin が産生され、ERK の経路が関与することが判明した。さらに poly(I:C)の刺激を加えると JNK の経路も働き、相乗効果を認めることが判明した。すなわち poly(I:C)は JNK の経路を中心に活性化する。しかし高濃度(10μg/ml 以上)の poly(I:C)では、Eotaxin の産生は低下した。

鼻由来線維芽細胞を poly(I:C)で刺激すると多くの BLyS (B cell stimulator)を発現した。BLyS は IL-4 存在下でクラススイッチを誘導し、抗原特

異的な免疫グロブリン産生に関与している。すなわち鼻粘膜でのクラススイッチに関与していると考えられる。

CpG-DNA を用いて、鼻粘膜や扁桃にも存在が認められている形質細胞様樹状細胞(PDC)の活性化機序を検討した。PDC を末梢血より精製し、各種遺伝子・蛋白の発現と活性化を検討すると、PDC ではNF-κB p65/p50 が構成的に活性化されていた。CpG-DNA 刺激により、IFN-α と CXCL10/CCL3 が IFNαR 非依存性に誘導された。IFN-αは有意に IgE 産生を抑制した。p38MAPK と NF-κB の活性化および TLR9 認識経路の検討により、CpG-DNA は TLR9 下流で p38MAPK/NF-κB の相互活性化を介して STAT1 リン酸化を起こし、ISGF3 の核移行と IRF7 の発現を亢進して IFN-α/CXCL10/CCL3 を誘導することが示された。CpG-DNA 刺激 PDC では NF-κB と p38MAPK の協調的活性化により、IFN 誘導性遺伝子が IFNαR をバイパスして発現すると考えられる。扁桃細胞では A タイプ CpG-DNA、B タイプ CpG-DNA ともに有意な IgE 産生の低下を誘導し、IFNα、IFNγ の産生の亢進を認めた。それらの作用は形質細胞様樹状細胞によるものであった。しかしいずれの CpG-DNA も $CD4^+CD25^+$ 細胞の増加はフローサイトメトリーにて確認できなかった。

D 考察

poly(I:C)は好酸球浸潤の点からは、治療において大量に用いないと使用できない。臨床的にも重症のウイルス感染後鼻症状が著しく改善することを認めることもあり、この結果はうなずける。poly(I:C)が線維芽細胞に働き、抗原特異的免疫グロブリン産生に関与する BLyS を誘導することは、減感作療法などのブロッキング抗体の誘導には効果があるのではないかと推測する。しかし poly(I:C)刺激が JNK シグナルを活性化することが判明したので、JNK 阻害薬などを使用するとその用途は広がるのではないかと思われる。

CpG-DNA は、我々が以前からヒト末梢血 IgE 産生を抑制することを報告してきた。今回この現象に形質細胞様樹状細胞が深く関与していることが判明した。また扁桃細胞においても IFNα、IFNγ の産生亢進とともに IgE 産生を抑制した。さらに CpG-DNA は vaccination のアジュバンドとしてかなり有望であるとの報告もなされているので、本研究班の大きなテーマであるスギ舌下免疫療法と併用すると治療効果が増す可能性が高いと思えわれる。この点に関しては、BLys を誘導する poly(I:C)も使用可能である。

E 結論

CpG-DNA は、炎症反応の副反応をうまく押さえ込むと IgE 産生抑制、vaccination の観点から新しい治療法のなりうると考えられた。今後、唾液による CpG-DNA や poly(I:C)の分解の程度などを検討する必要性がある。抗原と結合させるとどのようになるのかなどの検討も必要である。

F 健康危険情報

なし

G 研究発表

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Roles of protein tyrosine kinase Syk in nasal polyps

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Summary

The non-receptor protein tyrosine kinase Syk is widely expressed and plays an important role in intracellular signal transduction in haematopoietic cells including B cells, mast cells, eosinophils, platelets, macrophages, neutrophils and T cells. We found that Syk is expressed in human nasal polyp tissue-derived fibroblasts and plays a critical role in chemokine production and activation of c-Jun N-terminal kinase 1 stimulated with lipopolysaccharide or IL-1. In mast cells, cross-linking FcεRI via IgE bound to multivalent antigen induces tyrosine phosphorylation of immunoreceptor tyrosine-based activation motifs, and binds and modifies the activity of Syk, thereby initiating downstream signalling. In eosinophils, Syk is essential for activating the antiapoptotic pathway and generating reactive oxygen intermediates in response to Fcγ receptor engagement. In nasal polyps, Syk inhibition might influence the levels and function of specific IgE to *Staphylococcus aureus* enterotoxins that are thought to drive local eosinophilic inflammation therein. The regulation of Syk expression may prove to be a useful strategy in the treatment of airway diseases.

Keywords chemokine, eosinophils, fibroblast, IgE, mast cells, nasal polyp, Syk

Introduction

Pathogenetic findings in nasal polyps show infiltrating cells including mast cells, lymphocytes, eosinophils and neutrophils that can release cytotoxic and neurotoxic products that give rise to vascular denervation, exudation and oedema [1, 2]. Nasal polyps and middle turbinate bones have been found to contain more macrophages, lymphocytes, plasma cells, HLA-DR-positive cells and eosinophils than inferior turbinates [3]. Furthermore, median levels of histamine, tryptase and eosinophil cationic protein (ECP) are significantly higher in nasal lavage of patients with nasal polyps than in samples from subjects with normal nasal mucosa. Because tryptase and ECP in nasal fluids are correlated with symptom scores, eosinophils and mast cells are believed to play key roles in the pathogenesis of nasal polyposis [4].

Recent studies have demonstrated strong local up-regulation of IgE synthesis in nasal polyps with the formation of specific IgE to *Staphylococcus aureus* enterotoxins, suggesting a possible role of superantigens in these pathologic processes [5, 6]. The concentrations of IL-5, ECP, total IgE and specific IgE to *S. aureus* enterotoxins were significantly increased in aspirin-sensitive patients compared with aspirin-tolerant patients with nasal polyps as well as in normal controls [7]. Hence, staphylococcal superantigens may drive local eosinophilic inflammation in nasal polyp tissue [8]. Figure 1 shows a proposed mechanism of nasal polyp formation. Numerous epithelial and inflammatory cells participate in this process under a variety of conditions including hypoxia, oxidant exposure and bacterial, fungal and viral infection with or without allergy. Nasal fibroblasts also play an important role in both nasal polyposis and allergic rhinitis through the release of biologically active factors [9, 10].

We have found that the non-receptor protein tyrosine kinase Syk is expressed in numerous primary human nasal polyp tissue-derived fibroblast lines [11]. Syk is a widely expressed tyrosine kinase that plays an important role in intracellular signal transduction in haematopoietic cells including B cells, mast cells, eosinophils, platelets, macrophages, neutrophils and T cells [12–19]. Here, we focus on the roles of Syk in nasal polyps formation and discuss the implications for therapy based on our results using human nasal polyp tissue-derived fibroblasts.

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Human mast cells and B cells

Human mast cells and basophils expressing the high-affinity IgE receptor FcεRI play a key role in allergic diseases. FcεRI cross-linking stimulates the release of allergic mediators [20]. FcεRI aggregation induces release of preformed mediators and synthesis of later-acting leukotrienes, chemokines and cytokines [21]. The FcεRI is a heterotetramer consisting of a single IgE-binding α-subunit, a β-subunit and two disulfide-linked γ-subunits. The β- and γ-subunit cytoplasmic tails each contain a conserved immunoreceptor tyrosine-based activation motif (ITAM). Cross-linking FcεRI via IgE bound to multivalent antigen including staphylococcal superantigens induces tyrosine phosphorylation of ITAMs and binds and modifies the activity of Syk, which plays a critical role in initiating downstream signalling [22, 23] (Fig. 2a).

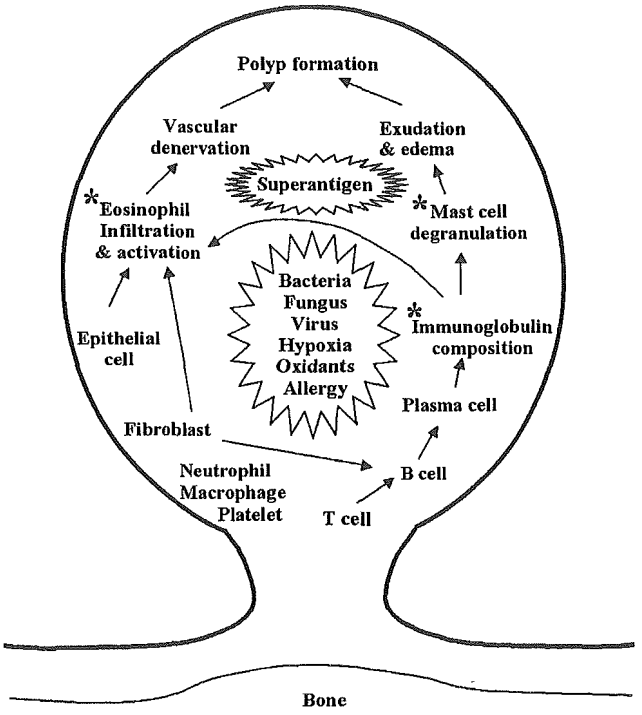

Fig. 1. Mechanism of nasal polyp formation. *Some commonly observed pathological findings in nasal polyps.

We demonstrated an association between B cell antigen receptor (BCR) and Syk and activation of Syk by cross-linking [12]. The BCR is a complex between membrane Ig and the Ig-α and Ig-β heterodimer. The cytoplasmic domains of Ig-α and Ig-β each contain an ITAM. Cross-linking activates Syk through ITAMs and thereby induces vigorous signalling reactions [24] (Fig. 2b). Specific IgE to *S. aureus* enterotoxins is produced in nasal polyps through this signalling because foreign antigens are recognized by BCR as an obligatory early step in B cell activation.

Nasal fibroblasts

Fibroblasts, a rich source of chemokines, interact with eosinophils and thereby play a key role in the pathogenesis of airway disease. Human nasal fibroblasts cultured from nasal polyp tissue express a variety of cytokines that induce differentiation of human haemopoietic progenitor cells [25]. Figure 3a shows Syk expression in the cytosol of nasal polyp-derived fibroblasts by immunohistochemical staining using the traditional ABC technique [26].

High concentrations of regulated on activation, normal T cell expressed and secreted (RANTES) have been demonstrated in nasal polyp specimens [27], and cultured nasal polyps have been shown to release RANTES spontaneously [28]. Stimulation with lipopolysaccharide (LPS) induces expression of RANTES mRNA in cultured nasal fibroblasts and secretion of RANTES protein [29]. The level of Syk expression is associated with RANTES production induced by LPS stimulation in nasal fibroblasts [11]. Overexpression of wild-type Syk increases RANTES production from human nasal fibroblasts. However, fibroblasts transfected with inactive Syk vector fail to produce high levels of RANTES.

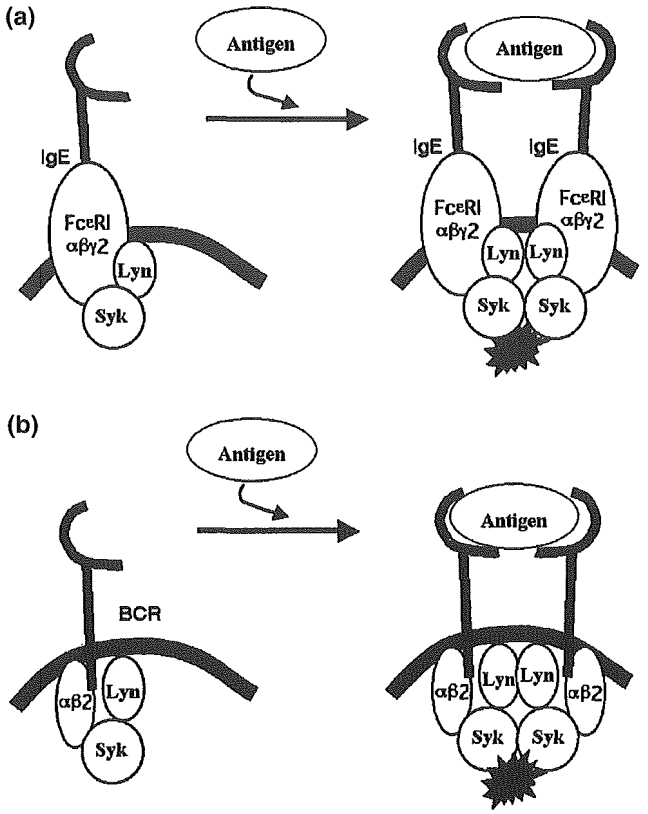

Fig. 2. Syk plays critical roles in intracellular signal transduction in human mast cells (a) and B cells (b).

Pre-treatment of antisense oligodeoxynucleotides to Syk inhibits RANTES production and activation of c-Jun N-terminal kinase 1 (JNK1) stimulated with LPS.

IL-1 induces interaction of TNF receptor-associated factor 6 (TRAF6) with IL-1 receptor-associated kinase that is rapidly recruited to the IL-1 receptor after IL-1 induction. We found that Syk plays an important role in IL-1-induced chemokine production through a signalling complex involving Syk and TRAF6. Overexpression of wild-type Syk by gene transfer enhanced RANTES production from nasal fibroblasts stimulated with IL-1. Decrease of Syk expression by administration of Syk-antisense inhibited RANTES production in response to IL-1. Syk is required for the IL-1-induced chemokine production with TRAF6 in fibroblasts of nasal polyps through JNK and p38 phosphorylation [30] (Fig. 3b).

Roles of Syk in various other cells

Table 1 shows some recently reported roles of Syk in other cells located in nasal polyp tissues. In human eosinophils, Syk is essential for the activation of the antiapoptotic pathway(s) induced through the IL-3/IL-5/granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor β-subunit [15]. Furthermore, eosinophils derived from Syk (−/−), but not wild-type mice, were incapable of generating reactive oxygen intermediates in response to Fcγ receptor engagement, although eosinophil differentiation and survival were not affected [31].

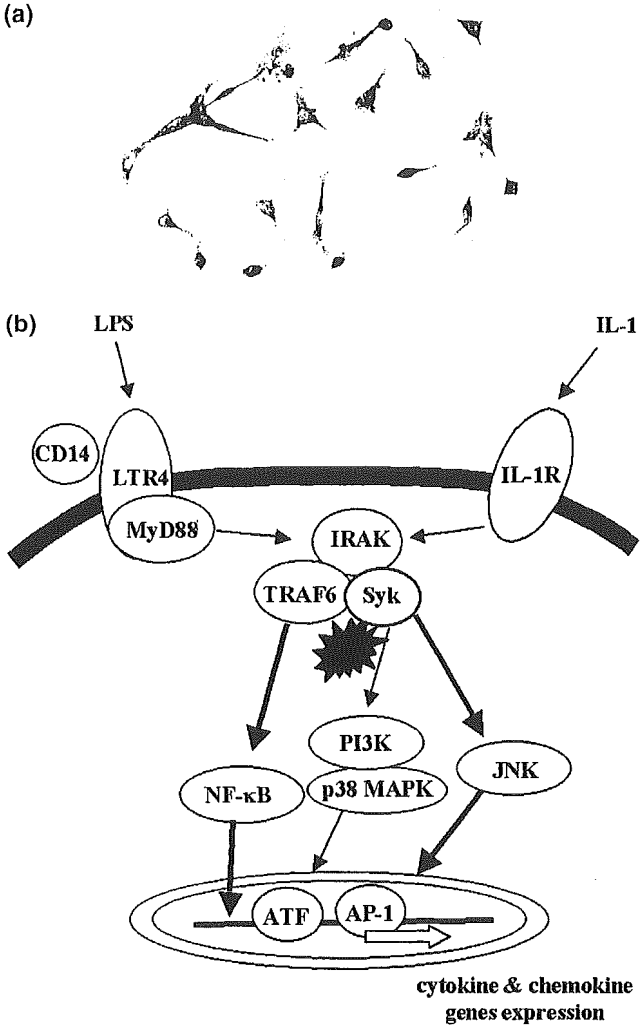

Fig. 3. Syk expressed in human nasal fibroblasts (a) and its signal transduction (b).

We found that Syk plays an important role in LPS-induced chemokine production from nasal fibroblasts. In neutrophils, Syk associates with Toll-like receptor 4 (TLR4) following LPS stimulation, and plays a pivotal role in the LPS-induced signalling pathway and monocyte chemoattractant protein (MCP)-1 expression [32]. Syk (−/−) neutrophils fail to undergo respiratory burst, degranulation or spreading in response to pro-inflammatory stimuli while adherent to immobilized integrin ligands or when stimulated by direct cross-linking of integrins [33]. In response to Fcγ receptor engagement, Syk (−/−) neutrophils were incapable of generating reactive oxygen intermediates [34]. Syk (−/−) macrophages were also defective in phagocytosis induced by the Fcγ receptor [34]. In monocytes, Syk is essential for β_2 integrin signalling and cell spreading [35]. Collagen induces tyrosine phosphorylation of Syk in platelet aggregation [36], and Syk phosphorylation is required for dendritic cell maturation induced by Fc receptor-mediated antigen presentation [37].

While we have demonstrated the roles of Syk in nasal fibroblasts, it has also been reported that Syk proteins are expressed in other non-haematopoietic cells such as endothelial and epithelial cells. The proliferation and migration of human umbilical vein endothelial cells are severely impaired by adenovirus-mediated expression of Syk dominant-negative mutants [38]. In Jurkat T cells, TNF activates Syk protein tyrosine kinase, leading to TNF-induced mitogen-activated protein kinase (MAPK) activation, nuclear factor-κB (NF-κB) activation and apoptosis [39].

Table 1. Roles of Syk in various cells

Cell	Cellular function or signal transduction	References
Eosinophils	Antiapoptotic pathway(s) through the IL-3/IL-5/GM-CSF-Rβ	[15]
	Generating reactive oxygen mediators in response to FcγR engagement	[31]
Neutrophils	Association with TLR4 (LPS stimulation), MCP-1-expression	[32]
	Degranulation or spreading (proinflammatory stimuli)	[33]
	Generating reactive oxygen mediators in response to FcγR engagement	[34]
Platelets	Collagen-induced signal transduction	[36]
Macrophages	Phagocytosis induced by FcγR engagement	[34]
Monocytes	β2 integrin signalling and cell spreading	[35]
Dendritic cells	FcR-mediated antigen presentation and cell maturation	[37]
Endothelial cells	Proliferation and migration	[38]
Epithelial cells	TNF-induced NF-κB	[39]

GM-CSF, granulocyte-macrophage colony-stimulating factor; TLR, Toll-like receptor; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; NF-κB, nuclear factor-κB.

Conclusion

Fibroblasts are a rich source of chemokines, cytokines and other inflammatory mediators, and as such are known to play a major role in the pathogenesis of airway diseases including bronchial asthma, cystic fibrosis and rhinosinusitis with polyps. Nasal fibroblasts produce RANTES [9, 10], eotaxin [40], MCP-1 and GM-CSF [41]. On LPS stimulation, RANTES expression leads to eosinophilic recruitment and activation [28, 42, 43]. Syk is required for this process [11]. LPS also increases IL-4-induced production of eotaxin, which is a potent mediator in the development of tissue eosinophilia [44], and significantly induces gene expression and production of GM-CSF and IL-8 in nasal tissue-derived fibroblasts [45]. We have demonstrated that IL-8 may be an important aspect of the effect of treatment on nasal polyps [46].

LPS induces tyrosine phosphorylation of Syk and activates JNK1 in nasal fibroblast lines. The Syk-generated signal cooperates to enhance JNK activation in T lymphocytes [47], and MAPK activation has been shown to be compromised in the macrophages of Syk (−/−) mice after Fcγ receptor stimulation [35]. Syk is an important component leading to activation of NF-κB in human monocytic cell lines [48]. Decreased Syk expression has been shown to attenuate JNK1 activation in nasal fibroblast lines in the same way that oxidative stress-induced JNK activation is significantly

decreased in B cells that do not express Syk [49]. Experiments on the roles of src homology 2 (SH2) domains of Syk have revealed that the C-terminal SH2 domain of Syk is required for induction of JNK activation in oxidative stress [50]. Recently, TLR has been implicated in the recognition of various bacterial cell wall components including LPS [51]. Syk associates with TLR4 upon LPS stimulation [32]. TRAF6 mediates both IL-1- and LPS-induced signalling. We found a signalling complex involving Syk and TRAF6 after IL-1 induction, leading to chemokine production and subsequent eosinophil infiltration [30].

An Syk-negative variant of rat basophilic leukemia-2H3 cells failed to release histamine by FcεRI aggregation, whereas reconstituted cells with stable expression of Syk could release histamine [52]. Syk-deficient mast cells failed to degranulate, synthesize leukotrienes and secrete cytokines [53]. Furthermore, Syk may be critical in cell survival after damage in inflammatory diseases, as antiapoptotic pathways involve Syk-dependent signalling [15, 49].

Syk expression affects chemokine production in airway diseases. Syk antisense oligodeoxynucleotides delivered by aerosol to the lungs *in vivo* depressed Syk expression and pulmonary inflammation [54]. Syk is associated with Fc receptors and the B cell receptor involved in allergic diseases, antibody-mediated autoimmune diseases and nasal polyps. Syk inhibition might control the levels and function of specific IgE to *S. aureus* enterotoxins in nasal polyps. Because the role of Syk in regulating vascular homeostasis and other housekeeping functions is minimal or masked by redundant Syk-independent pathways, targeting Syk may be an optimal approach to the effective treatment of a multitude of chronic inflammatory diseases without undue toxicity [55]. In conclusion, manipulation of Syk expression may prove to be a useful strategy in the treatment of airway diseases such as asthma and nasal polyposis.

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Double-stranded RNA induces production of RANTES and IL-8 by human nasal fibroblasts

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Abstract

Double-stranded RNA (dsRNA) and the viral RNA mimic, polyinosine–polycytidylic acid (poly(I:C)), are recognized by toll-like receptor 3 (TLR3) that mediates the innate immune response to viral infections. In this study, we investigated the effects of poly(I:C) on the production of chemokines (IL-8, RANTES, and eotaxin), Type I IFNs (IFNα and IFNβ), Th1-cytokines (IL-12 and IFNγ), and pro-inflammatory cytokines (TNF-α and IL-1β) by human nasal mucosa-derived fibroblasts. Human nasal fibroblasts were treated with poly(I:C), and levels of cytokines and chemokines were measured by ELISA. Incubation with poly(I:C) significantly enhanced the secretion of RANTES and IL-8. However, eotaxin, IL-1β, TNF-α, IFNα, IFNγ, and IL-12 were not secreted from nasal fibroblasts stimulated with poly(I:C). The JNK inhibitor SP600125 and the PI3-kinase inhibitor LY294002 significantly blocked the poly(I:C)-induced release of RANTES and IL-8, whereas the p38 MAP kinase inhibitor SB203580 suppressed poly(I:C)-induced secretion of IL-8, but not RANTES. Nasal fibroblasts play an important role in initiating antiviral responses and inflammation of the nasal cavity by producing chemokines leading to enhanced inflammatory cell recruitment.

Keywords: dsRNA; poly(I:C); Chemokine; IL-8; RANTES; Nasal fibroblast

Introduction

Viral infections of mammalian cells result in the activation of innate immune responses mediated by Type I IFNs, IFNα and IFNβ, and other cytokines [1,2]. Most of the viruses causing upper respiratory infections including rhinoviruses, coxsackievirus, echovirus, influenza viruses, and RSvirus are RNA viruses. RNA viruses synthesize double-stranded RNA (dsRNA) during replication [3], and this is a potent stimulus for innate anti-viral responses through the secretion of cytokines [4]. It is known that dsRNA binds only intracellular targets, including the dsRNA-dependent protein kinase (PKR) [5]. However, cells derived from PKR KO mice still responded to the synthetic dsRNA analogue, polyinosine–polycytidylic acid (poly(I:C)), suggesting the existence of another receptor expressed on the cell surface, which recognizes dsRNA [6,7]. Recently, toll-like receptor (TLR) 3-deficient mice have been shown to have reduced responses to dsRNA and poly(I:C), suggesting that TLR3 is involved in the recognition of dsRNA [8]. Toll-like receptors play a key role in innate immunity by recognizing conserved microbial pathogen-associated molecular patterns (PAMPs) [9–11]. Recognition of the invading pathogen then triggers production of cytokines and chemokines and up-regulation of co-stimulatory molecules in phagocytes and antigen presenting cells, leading to the activation of T cells [8,12–14].

The nasal mucosa is often affected by viral infection. Thus, it is suspected that dsRNA might also be an important stimulus for the synthesis of cytokines and chemokines. Recently, it was proposed that fibroblasts are not passive players in the immune system. Fibroblasts have been considered mainly a physical barrier, but several studies have shown that they may be important modulators of local inflammation due to their capacity to release a variety of pro-inflammatory mediators, including IL-8, RANTES, eotaxin, and GM-CSF [15–17]. Eotaxin, RANTES, and GM-CSF are implicated in the recruitment and enhanced survival of eosinophils [18–20], and IL-8 is a potent chemoattractant for neutrophils [21].

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Infiltration by these inflammatory cells causes acute inflammation of the nasal mucosa and helps to exacerbate chronic inflammation including allergic rhinitis and nasal polyps. Thus, fibroblasts are important sentinel cells in the immune systems.

In this study, we investigated the effects of poly(I:C) on the production of chemokines (IL-8, RANTES, and eotaxin), Type I IFNs (IFNα and IFNβ), Th1-cytokines (IL-12 and IFNγ), and pro-inflammatory cytokines (TNF-α, IL-1β) in human nasal mucosa-derived fibroblasts. Signaling pathways for the activation by poly(I:C) in nasal fibroblasts were also examined by using inhibitors of MAP kinase (U0126, SB203580, SP600125) and LY294002.

Materials and methods

Reagents

The following reagents were used: SP600125 as the specific inhibitor for JNK (BIOMOL, Plymouth Meeting, PA), SB203580 as the specific inhibitor for p38 MAP kinase (Promega, Madison, WI), U0126 as the specific inhibitor for MEK-1 (Promega), LY294002 as the specific inhibitor for PI3 kinase (Promega), poly(I:C) (Amersham Bioscience, Piscataway, NJ), IL-1β (PeproTech EC, England), TNFα (PeproTech EC), IL-4 (PeproTech EC), IFNγ (PeproTech EC), LPS (MERCK bioscience, Germany), CpG, a synthetic oligodeoxynucleotide that contains CpG motifs mimicking bacterial DNA (5′-ACCGATCGTTCGGCCGGTGACGGCACCA-3′) [22], p44/42 MAP Kinase rabbit polyclonal antibody (Ab) (Cell Signaling, Beverly, MA), SAPK/JNK rabbit polyclonal Ab (Cell Signaling), phospho-p44/42 MAPK (E10) mouse monoclonal Ab (Cell Signaling), phospho-SAPK/JNK (G9) mouse monoclonal Ab (Cell Signaling), phospho-p38 MAPK (28B10) mouse monoclonal Ab (Cell Signaling), phospho-AKT (587F11) mouse monoclonal Ab (Cell Signaling), AKT Rabbit polyclonal Ab (Cell Signaling), and p38 (A12) mouse monoclonal Ab (Santa Cruz Biotechnology, Santa Cruz, CA).

Human nasal mucosa-derived fibroblast cell culture and stimulation

Nasal mucosa of the inferior turbinate was obtained from patients with chronic sinusitis or allergic rhinitis when they underwent nasal surgery. Nasal specimens were cultured in 10-cm dishes containing RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated FCS (Gibco, Grand Island, NY), 0.29 mg/ml glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37°C in 5% CO_2 and humidified air. Nasal fragments were removed and the first passage was performed. After a period of 3–4 weeks, nasal mucosa-derived fibroblast cell lines were established. The cells were used at passage numbers 3–5. Epithelial cells were confirmed not to be contaminated by immunohistochemical examination using cytokeratin and vimentin markers. The cells were then placed in a 24-well flat-bottomed tissue culture plate (Corning, Corning, NY) at an initial density of 1×10^5 cells/well for cytokine production or 10-cm dish for Western blotting and RT-PCR. When the cells were growing in sub-confluent conditions, the culture medium was replaced with serum-free RPMI 1640 medium, and then the cells were stimulated by poly(I:C). Where indicated, cells were also pretreated for 1 h with pharmacological inhibitors. Inhibitors were dissolved in dimethyl sulfoxide (DMSO) at 10 mM and further dilutions were made in cell culture medium. DMSO vehicle controls were included in each experiment.

Cytokine and chemokine assay

The cells were cultured in the presence of poly(I:C) for appropriate periods, then culture supernatants were harvested and stored at −80°C. Amounts of cytokines and chemokines in the cell culture supernatant were measured with commercially available ELISA kits. All the kits were purchased from Biosource International (Camarillo, CA) except for the IFNβ ELISA kit, which was purchased from TFB (Tokyo, Japan). Measurements were performed according to the manufacturer's directions. All samples were assayed in duplicate.

Immunoblot analysis

The cells were washed twice with ice-cold PBS and collected by scraping, then centrifuged and pelleted at 4°C. The cells were homogenized in lysis buffer [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM PMSF, 0.5 mM EDTA, 0.6 μM leupeptin, 2 μM pepstatin A, and 1 mM PMSF] by pipetting and sonication. Protein concentrations were measured using the BioRad Protein Assay Kit (BIORAD, Hercules, CA) in all experiments. Lysates were centrifuged at 10000 rpm for 10 min at 4°C and the supernatants were used for immunoblotting. The supernatants were added to a twofold volume of sample buffer [95% laemmli sample buffer (BIORAD) and 5% 2-mercaptoethanol]. After heating at 95°C for 5 min, the samples were electrophoresed. Proteins were transferred electrophoretically onto polyvinylamidedifluoride (PVDF) membranes (Amersham Bioscience). The blotted membranes were rinsed with 5% non-fat dry milk diluted in PBS containing 0.1% Tween 20 for 60 min at room temperature, then incubated with the appropriate antibodies for 16 h at 4°C. After being washed, the membranes were treated with HRP-conjugated anti-mouse immunoglobulin (Ig) Ab or HRP anti-rabbit Ig Ab (DAKO, Carpinteria, CA) for 60 min at room temperature. Peroxidase color visualization was achieved with TMB membrane Peroxidase Substrate (KPL, Gaithersburg, MD).

Real time PCR

Total RNA was extracted using a total RNA isolation NucleoSpin™ RNA II Kit (MACHERY-NAGEL, Düren Germany). The reverse transcription reaction was performed with TaqMan® RT Reagents (Applied Biosystems Japan, Tokyo, Japan) using random hexamer primers. The amplification of TLRs and β2microglobulin-cDNA was performed in a MicroAmp optical 96-well reaction plate (Applied Biosys-

tems). All TaqMan® probe/primer combinations used in this study were TaqMan® Gene Expression Assay products purchased from Applied Biosystems. β2-Microglobulin was chosen as the reference housekeeping gene because it is convenient to assay and highly expressed. Furthermore, in order to select the housekeeping gene, we evaluated it using a TaqMan® Human Endogenous Control Plate, which was most suitable. TaqMan® PCR was performed in a 20-μl volume using TaqMan® Universal PCR master mix (Applied Biosystems). The reaction was performed in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Reaction mixtures were pre-incubated for 2 min at 50°C. The PCR program was 10 min of Taq Gold activation at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C (maximum ramping speed between temperatures). Human cDNA equivalent to 50 ng of total RNA from each sample was assayed in each tube.

The threshold cycle number (Ct) was determined with sequence Detector Software (version 1.1: Applied Biosystems) and transformed using comparative Ct methods as described by the manufacturer with β2microglobulin as the calibrater gene.

Data and statistical analysis

Data in the text and figure legends are expressed as the mean ± SEM of observations obtained from human nasal fibroblasts cultured from 8 donors, if not otherwise specified. Statistical analysis was performed using the Wilcoxon signed-ranks test to assess the difference in cytokine production levels. Macintosh computers (Apple computer, Cupertino, CA) with Statview software (Abacus Concepts, Berkeley, CA) were used for all statistical analyses.

Results

Human nasal fibroblasts express TLRs

We first set out to determine the expression of TLRs on human nasal fibroblast cells. The expression of mRNA for TLRs on fibroblasts was confirmed by real time RT-PCR. To assess the relative expression levels of TLR mRNAs on the cells, a logarithmic scale was used in Fig. 1a. TLR3, 4, and 9 were highly expressed. TLR1, 2, 5, and 6 were also detected,

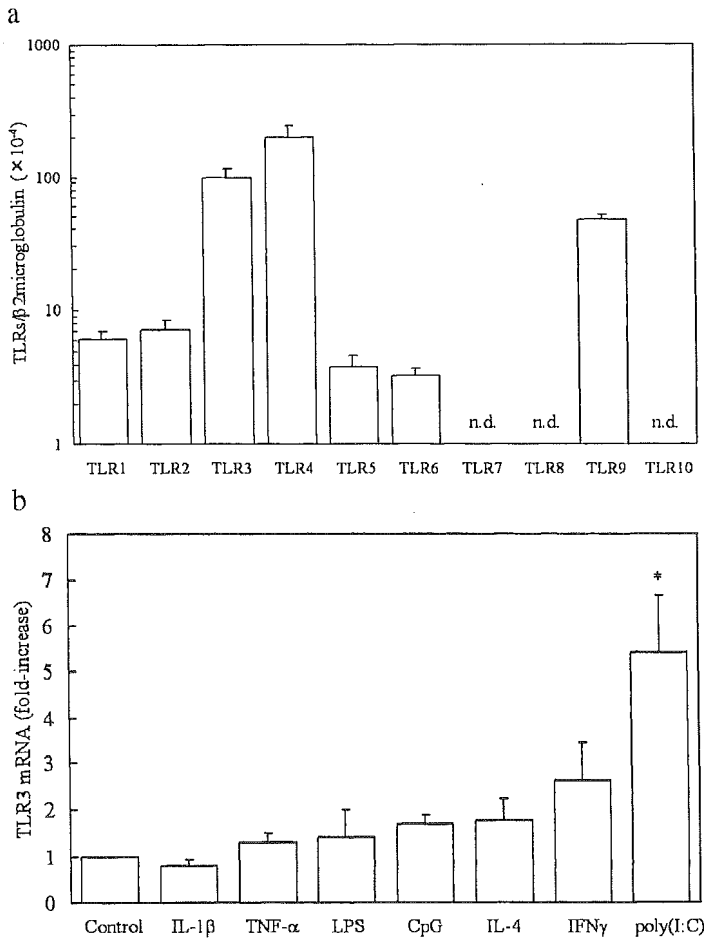

Fig. 1. (a) TLR1-10 mRNA expression levels were assayed by real time RT-PCR using a cDNA template derived by reverse transcriptase from 50 ng of RNA from human nasal fibroblast cells. Reactions were performed in three wells, and results are expressed relative to expression levels of β2microglobulin. Data are presented as the mean ± SEM (n = 12). (b) Analysis of expression of TLR3 mRNA induced by cytokines and PAMPs using real time PCR. Fibroblasts were treated with IL-1β (2 ng/ml), TNF-α (10 ng/ml), LPS (5 μg/ml), CpG (100 μg/ml), IL-4 (10 ng/ml), IFNγ (10 ng/ml), or poly(I:C) (10 μg/ml) for 6 h. Total RNA was isolated and reverse transcribed to cDNA. The cDNAs were used for real time PCR as described in Materials and methods. Data are expressed as the mean ± SEM of the fold increase relative to the control (n = 6). *$P < 0.05$ compared with control using Wilcoxon's signed-ranks test.

but their expression levels were lower than those of TLR3, 4, and 9. Human nasal fibroblast cells did not express TLR 7, 8, or 10 (Fig. 1a).

We examined the effect on TLR3 mRNA expression of the TLR ligands LPS, CpG, and poly(I:C), pro-inflammatory cytokines IL-1β, and TNFα, Th1 cytokine IFNγ, and Th2 cytokine IL-4. Fibroblasts were treated with the agonists for 6 h, and TLR3 mRNA expression was assessed by real time PCR. The expression of TLR3 mRNA was increased 5.4-fold ($P < 0.05$) in nasal fibroblasts by poly(I:C). However, other PAMPs and cytokines had no significant effect on the level of mRNA for TLR3.

Human nasal fibroblasts produce IL-8 and RANTES when stimulated with poly(I:C)

Production of IL-8, RANTES, eotaxin, IL-1β, TNF-α, IFNα, IFNβ, IFNγ, and IL-12 in poly(I:C)-stimulated nasal fibroblasts was determined in the supernatants by ELISA. Culture supernatants from the fibroblast cells stimulated with various concentrations of poly(I:C) were collected 48 h after the stimulation. A significant release of IL-8 and RANTES was observed with 0.1 μg/ml poly(I:C) (Fig. 2a), and a release of IFNβ was observed with 10 μg/ml poly(I:C), but the amount of IFNβ was very small (Fig. 2b). The rank order for maximal release stimulated by poly(I:C) from human nasal fibroblast cells was IL-8 > RANTES >> IFN-β. The release of eotaxin, IL-1β, TNF-α, IFNα, IFNγ, and IL-12 could not be detected by ELISA (<1–10 pg/ml) at 8, 24, 48, 72, and 148 h after treatment with poly(I:C). Since IL-8 production reached a plateau on stimulation with 10 μg/ml of poly(I:C), 10 μg/ml of poly(I:C) was used in all subsequent experiments. The time course of IL-8 and RANTES production was determined. Concentrations of IL-8 and RANTES in the supernatants changed with time with significant increases detected at 8, 24, 48, and 72 h (Fig. 2c). Optimal harvest time was 48 h after stimulation with 10 μg/ml of poly(I:C).

Poly(I:C) induces phosphorylation of p38 MAP kinase, JNK, and AKT

To determine poly(I:C)-induced intracellular signaling, p38 MAP kinase, JNK, and ERK in nasal fibroblast cells were immunoblotted after stimulation with 10 μg/ml of poly(I:C). The exposure of the cells to poly(I:C) triggered a slow phosphorylation of p38 MAP kinase and JNK. Amounts of phosphorylated threonine and tyrosine of p38 MAP kinase and JNK in poly(I:C)-stimulated cells increased at 30 min (Fig. 3a) and 60 min (Fig. 3b), respectively. The activation of ERK in poly(I:C)-stimulated cells was unchanged during 2 h (Fig. 3c). In addition to MAP kinase, AKT, regulated by the phosphoinositide products of PI3-kinase, was examined. Amounts of phosphorylated serine of AKT in poly(I:C)-stimulated cells increased at 120 min (Fig. 3d). The upper panels of the figure show that equal amounts of p38 MAP kinase, JNK, ERK, and AKT were immuno-

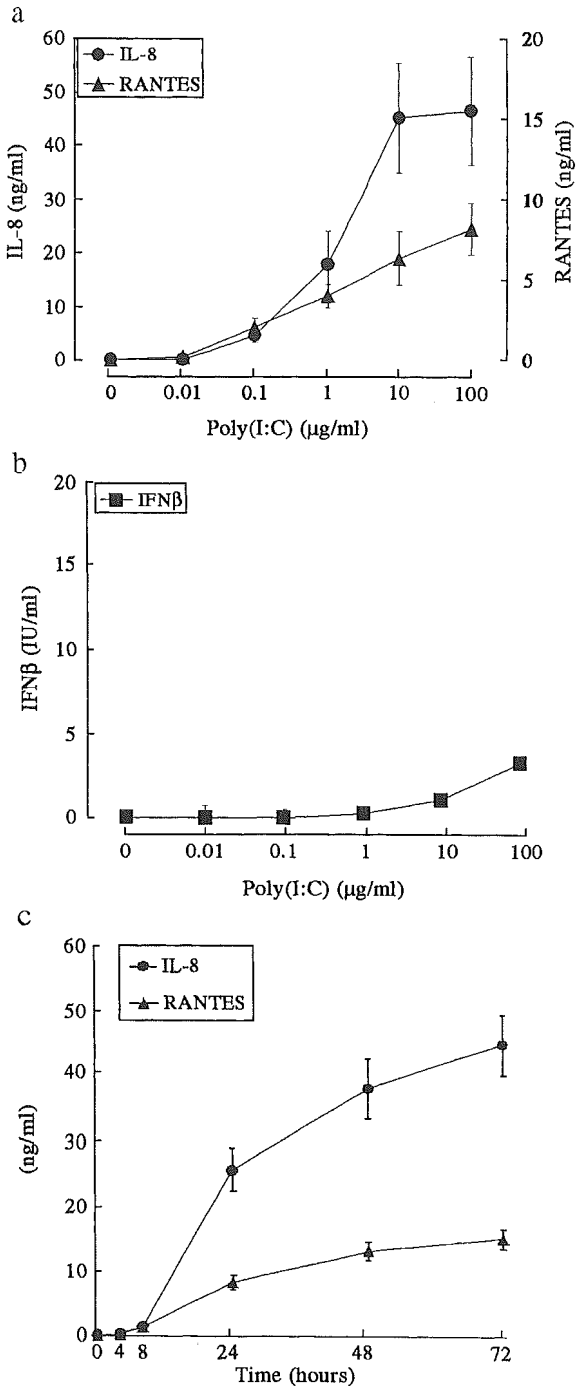

Fig. 2. Poly(I:C) induces production of IL-8, RANTES, and IFNβ by human nasal fibroblasts. Human nasal fibroblast cells were cultured either with medium or with various concentrations of poly(I:C) (0.01, 0.1, 1, 10, and 100 μg/ml) for 48 h. The concentrations of IL-8 (circles), RANTES (triangles) (a), and IFNβ (b) in the culture supernatants were determined by ELISA. Human nasal fibroblast cells were cultured with either medium or poly(I:C) (10 μg/ml) and the concentrations of IL-8 (circles) and RANTES (triangles) in the culture supernatants were determined at 4, 8, 24, 48, and 72 h after stimulation (c). The results are expressed as the mean ± SEM.

blotted with phosphorylation-independent specific antibodies to p38 MAP kinase, JNK, ERK, and AKT regardless of the time course, indicating that poly(I:C)-induced p38 MAP kinase, JNK, ERK, and AKT phosphorylation occurred in

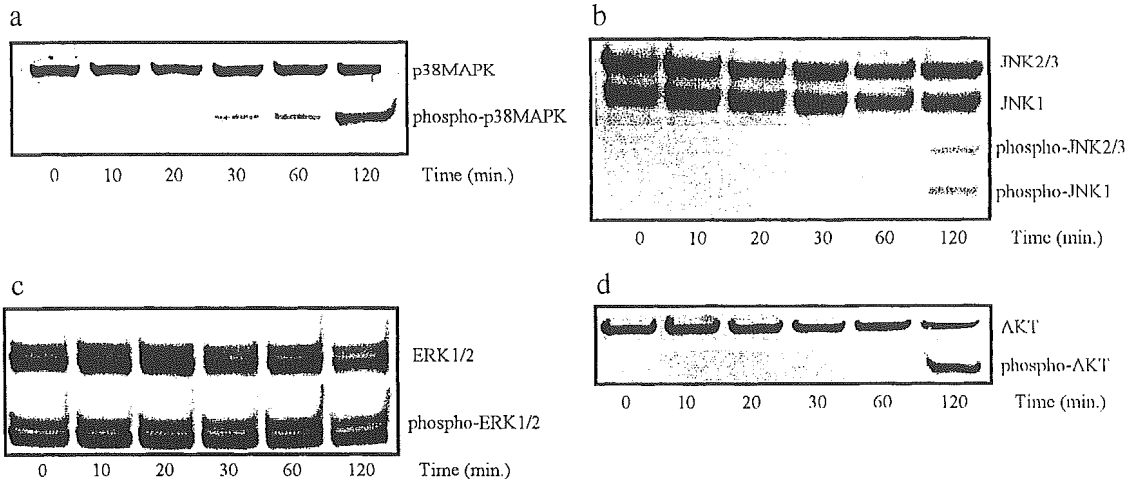

Fig. 3. Poly(I:C) induced the threonine and tyrosine phosphorylation of p38 MAP kinase and JNK but had no effect on the phosphorylation of ERK. Poly(I:C) also induced the serine phosphorylation of AKT. Human nasal fibroblast cells were separated by 10–20% SDS–PAGE, transferred to membranes and blotted with a specific antibody to phosphorylated p38 MAP kinase (phospho-p38) (a, bottom), p38 MAP kinase (a, top), phospho-JNK (b, bottom), JNK (b, top), phospho-ERK (c, bottom), ERK (c, top), phospho-AKT (d, top), or AKT (d, bottom).

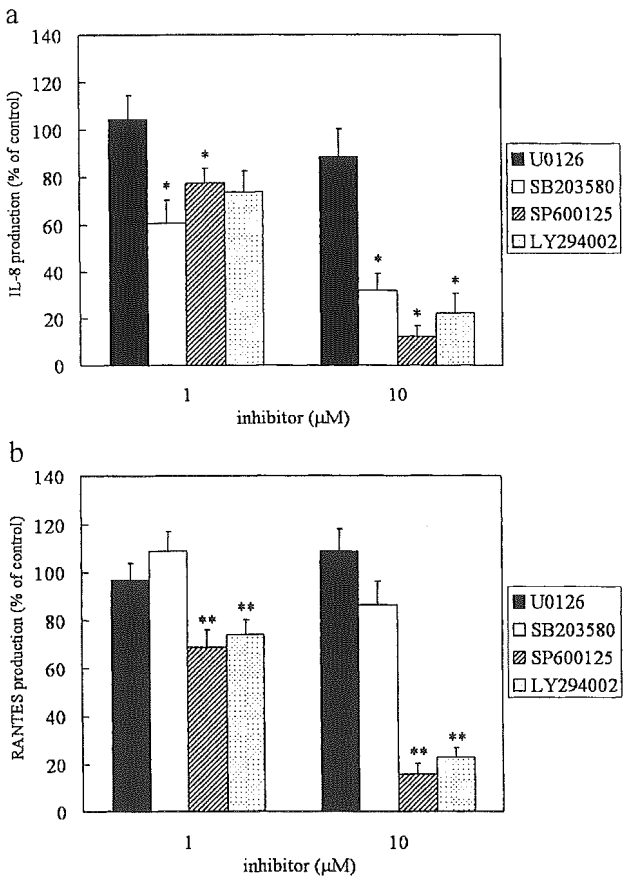

Fig. 4. Human nasal fibroblasts were pre-incubated with SB203580, an inhibitor of p38 MAP kinase; U0126, an inhibitor of MEK; SP600125, an inhibitor of JNK; or LY294002, an inhibitor of PI3 kinase. Data in the graphs show the effects of U0126, SB203580, SP600125, and LY264002 on IL-8 (a) and RANTES (b) production in cells stimulated with poly(I:C) (10 μg/ml) for 48 h. Data are presented as mean values ± SEM (IL-8 $n = 7$, RANTES $n = 10$). *$P < 0.05$, **$P < 0.01$ compared with the levels without inhibitor using Wilcoxon's signed-ranks test.

the absence of changes in ERK, p38 MAP kinase, JNK, and AKT protein levels (Figs. 3a–d).

Effect of MAP kinase inhibitors and PI3 kinase inhibitor on IL-8 production in nasal fibroblast cells stimulated with poly(I:C)

The p38 MAP kinase inhibitor SB203580, the JNK inhibitor SP600125, the ERK inhibitor U1026, and the PI3-kinase inhibitor LY294002 were examined to determine whether they inhibit production of IL-8 in nasal fibroblasts stimulated with poly(I:C) (Fig. 4a). Pre-incubation with SB203580, SP600125, and LY294002 suppressed the poly(I:C)-induced production of IL-8 in a dose-dependent manner. The JNK inhibitor SP600125 had the greatest effect. SP600125 decreased the production of IL-8 in poly(I:C)-stimulated nasal fibroblasts by 87.9% compared to the control level ($P < 0.05$). The ERK inhibitor U0126 had no effect on the levels of IL-8 present in the supernatants of cells stimulated with poly(I:C). There were no differences in cell shape and viability among the four inhibitors (data not shown).

Effect of MAP kinase inhibitors and PI3 kinase inhibitor on RANTES production in nasal fibroblast cells stimulated with poly(I:C)

The four inhibitors were also examined to determine whether they affect the production of RANTES by poly(I:C)-stimulated nasal fibroblasts (Fig. 4b). The same samples used to measure IL-8 levels were examined for RANTES. SP600125 and LY294002 inhibited the production of RANTES by nasal fibroblast cells stimulated with poly(I:C) in a dose-dependent manner. SP600125 had the greatest effect inhibiting production in poly(I:C)-stimulated nasal fibroblasts by 84.3% relative to the control ($P < 0.01$). LY294002 had a similar effect to SP600125 on the production of RANTES by nasal fibroblasts ($P < 0.01$). SB203580 and U0126 had no effect on the level of

RANTES present in the supernatants of cells that were stimulated with poly(I:C).

Discussion

In this study, we demonstrated that nasal mucosa-derived fibroblasts express a large amount of TLR3 mRNA. Stimulation with poly(I:C) directly induced production of IL-8 and RANTES in nasal fibroblasts. The signal pathway for IL-8 and RANTES production was via JNK and PI3 kinase. Additionally, p38 MAP kinase was also important for the production of IL-8.

The nose is the target of a substantial number of infectious agents that produce agonists for TLRs, including gram-negative and positive bacteria, mycobacteria, fungi, viruses, and numerous helminthes. Despite this fact, relatively little is known about the expression of toll-like receptors on nasal fibroblast cells. The presence of TLRs on primary bronchial epithelial cells and BEAS-2B airway epithelial cells [23], as well as alveolar and bronchial epithelial cells [24], has been demonstrated. TLR3 was highly expressed on nasal fibroblast cells as it was on primary bronchial epithelial cells. The expression of TLR3 in primary bronchial epithelial cells was positively regulated by the influenza A virus and by dsRNA [24], and expression of TLR3 in the MRC-5 human lung fibroblast cell line and A549 human lung epithelial cell line was positively regulated by respiratory syncytial virus [25]. We confirmed that poly(I:C) also up-regulated TLR3 expression in human nasal fibroblasts.

Poly(I:C) is a potent and selective stimulus for the secretion of IL-8 and RANTES, but not pro-inflammatory cytokines (IL-1β and TNF-α), Th1 cytokines (IFNγ and IL-12), or eotaxin. We previously showed that pro-inflammatory cytokines and IFNγ induced production of RANTES by nasal fibroblasts [17,26,27]. However, these cytokines were not expressed in nasal fibroblast cells stimulated with poly(I:C). Thus, RANTES was produced as a direct effect of poly(I:C), not in an autocrine manner. Type I IFNs, IFNα and IFNβ, are key effectors in the innate immune responses to viral infections. However, in this study, significant production of IFNα and IFNβ by nasal fibroblasts was not induced by a viral mimic, poly(I:C). We also confirmed that IFNα and IFNβ were not detected in the culture supernatants even if the incubation was extended to 7 days after poly(I:C) stimulation. Recently, several studies showed that plasmacytoid dendritic cells (pDCs) play a pivotal part in antiviral immune responses because of their extraordinary capacity to produce Type I IFNs against viral infection [28,29]. They also sense viral ssRNA or its degradation product via TLR7/8, leading to the production of Type I IFNs [30,31]. In contrast, nasal fibroblasts may not recognize the viral genome of ssRNA viruses because TLR7/8 mRNA was not detected in nasal fibroblasts. Since RNA viruses produce dsRNA during RNA replication, it is considered that in the proliferative phase of the infection, nasal fibroblasts have an antiviral effect.

Poly(I:C), a synthetic dsRNA polymer, was shown to act as an adjuvant [32], and has been used to mimic RNA viral infections. All TLRs share a common cytoplasmic signaling domain, the toll-interleukin 1 (IL-1) receptor domain (TIR domain). This domain mediates the association between TLRs and adaptors, such as MyD88 and TIRAP [11,33]. MyD88 is used by most TLRs. However, only TLR3 was associated with another adaptor, TRIF (also called TICAM-1) [34–36]. Signal transduction pathways downstream of TRIF lead to NF-κB, IRF-3, and MAP kinases [37–39]. NF-κB is most commonly the transcription factor associated with IL-8 and RANTES production [40,41]. AP-1, which can be activated by p38 MAP kinase and JNK, is also an important positive regulator of IL-8 and RANTES promotor activity [42,43]. Although it is not clear which pathway mediates the signaling of poly(I:C) to MAP kinases, it is suggested that poly(I:C) activates the TLR3-TRIF-MAP kinase pathway in nasal fibroblast cells.

The observation that the p38 MAP kinase inhibitor SB203580 blocks production of IL-8 but not RANTES suggested that there are different signaling pathways involved in the up-regulation of these two chemokines by poly(I:C). There are conflicting reports about the involvement of p38 MAPK and RANTES. In bronchial epithelial cells, Gern et al. reported that SB203580 did not inhibit the production of RANTES induced by dsRNA [44]. Kujime et al. reported that SB203580 did inhibit RANTES production induced by Influenza in vitro [45]. In airway smooth muscle cells, Hallsworth et al. reported that SB203580 did not inhibit the production of RANTES induced by IL-1β [46]. Maruoka et al. reported that SB203580 did inhibit RANTES production induced by PAF [47]. In nasal fibroblasts, the result might change if the stimulation is different. Gern et al. also reported that SB203580 inhibited IL-8 production induced by dsRNA in bronchial epithelial cells [44]. However, the mechanisms activating p38 MAP kinase and producing IL-8 in epithelial cells and fibroblast cells also remain unclear. Further study is necessary.

Our study added the result to Gern's work that JNK and PI3-kinase are also important to the production of IL-8 and RANTES stimulated by poly(I:C). Recently, Sarkar reported that the PI3 kinase-AKT pathway plays an essential role in the TLR3-mediated TBK-1 to IRF-3 pathway [48]. IRF-3 binds to the promotor region of RANTES and plays a crucial role in gene expression [49,50]. There is no report that IRF3 binds to the promotor region of IL-8. It is thought that the pathway to IRF-3 does not lead to IL-8 production though it may lead to RANTES production. NF-κB plays an important role in the gene expression of IL-8 [51]. Xianwu Li reported that PI3-kinase is an important mediator of LPS signaling leading to the activation NF-kB through AKT in human microvascular endothelial cells [52]. The signal transduction pathway downstream of TLR3-TRIF-PI3K may associate with not only IRF3 but also NF-κB.

RANTES is a highly effective chemoattractant for T lymphocytes, monocytes, eosinophils, and basophils [20,53]. Infections with some viruses have been shown to induce RANTES expression in a wide variety of cells [54–59]. Thus, virus-induced RANTES expression could be a major element in the pathogenesis of viral infection. IL-8 is an important

mediator of the inflammatory response to many stimuli, including viruses [60–62]. Most importantly, IL-8 is a major neutrophil chemoattractant and activator [21]. It was confirmed in our laboratory that IL-1β, TNFα, and LPS also induced production of both IL-8 and RANTES by nasal fibroblasts (data not shown). But less RANTES was produced in response to IL-1β, TNFα, and LPS than to poly(I:C). However, the rank order for maximal capacity to stimulate production of IL-8 by human nasal fibroblast cells was IL-1β > TNF-α > LPS > poly(I:C). This difference may contribute to the specific role of nasal fibroblasts in protection against viral infections. T cells and granulocytes recruited to the airways are likely to contribute to antiviral activity. On the other hand, increased cellular inflammation could also add to airway obstruction and dysfunction, leading to symptoms in the upper and lower airway. If the function of chemokines secreted during viral infections was defined further, specific inhibitors of the signal pathway associated with chemokine production might be used to regulate cellular inflammation in the pathogenesis of virus-induced inflammation of the nasal cavity, rhinosinusitis and exacerbations of allergic rhinitis.

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The efficacy of short-term administration of 3 antihistamines vs placebo under natural exposure to Japanese cedar pollen

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Background: Japanese cedar pollinosis, a common disease with morbidity of approximately 20% in the Japanese population, is characterized by subjectively irritating symptoms during an annual 3-month period.

Objective: To investigate the effectiveness of cetirizine hydrochloride, loratadine, and fexofenadine hydrochloride in reducing pollinosis symptoms induced while walking in a park during the pollen season.

Methods: A randomized, double-masked, placebo-controlled trial was conducted in 113 individuals with Japanese cedar pollinosis during 2 days in March 2003 in Osaka Expo Park, Osaka, Japan. Participants (aged 20–57 years) were divided into 4 groups according to treatment assignment: cetirizine hydrochloride, 10 mg/d; fexofenadine hydrochloride, 120 mg/d; loratadine, 10 mg/d; and placebo (lactose), twice daily. Symptoms were recorded hourly during the study. Furthermore, all the patients completed the Japanese version of the Rhinoconjunctivitis Quality of Life Questionnaire before and after the trial.

Results: Self-evaluated symptom scores in all 3 active treatment groups showed significant improvements compared with the placebo group. Furthermore, the cetirizine group showed significant improvement in the domains of frequency of nose blowing and nasal obstruction compared with placebo. In addition, improvement in Japanese Rhinoconjunctivitis Quality of Life Questionnaire scores was higher in the cetirizine group than in the loratadine and placebo groups.

Conclusion: Cetirizine seems to be more effective than fexofenadine and loratadine at reducing subjective symptoms in this study population.

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INTRODUCTION

Japanese cedar pollinosis (JCP) is a relatively common disorder in Japan, presenting serious social problems between February and April each year. The age-adjusted prevalence of JCP in Japan in 2001 was 19.4% using the cross-sectional random sampling method, and the estimated prevalence after correlation of possible biases was 13.1%.[1] Because the beneficial effects of H_1-specific antagonists on improving symptoms in patients with seasonal allergic rhinitis (SAR) are well established, antihistamine drugs are administered as the mainstay of treatment in most of these patients. However, owing to the availability of many varieties of antihistamines, including second-generation H_1-receptor antagonists, it is overwhelming for Japanese physicians to decide which drug provides the greatest clinical benefit and the fewest adverse effects in patients with JCP. Hence, the rating of antihistamines principally from the standpoint of efficacy by using placebo-controlled, randomized, double-masked clinical trials has considerable merit for patients and physicians alike.

Quality of life (QOL) is a subtle concept that includes many physical and psychological factors. Although the symptoms of JCP, such as sneezing, rhinorrhea, nasal congestion, and itchy and watery eyes, are not life threatening, they unarguably reduce QOL in patients with the disease. Hence, to evaluate impairment in QOL caused by SAR and to evaluate the clinical benefits of anti-SAR medications, not only total symptom scores but also QOL scores, as evaluated by the 36-Item Short-Form Health Survey[2] or the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ), have been popularly used worldwide in recent years.[3–5] Several such studies have reported that general QOL score is sufficiently sensitive for use in antiallergy drug trials in Western countries.[6] However, Asian people, including the Japanese, have different cultures and lifestyles than their Western counterparts; thus, it remains unclear whether QOL score is correlated with total symptom score in patients who take antihistamines to control their JCP.

In this context, we conducted a randomized, double-masked, placebo-controlled study of the effects of 3 second-generation antihistamines (cetirizine hydrochloride, 10 mg; fexofenadine hydrochloride, 120 mg; and loratadine, 10 mg) against naturally induced symptoms caused by walking in a park during the Japanese cedar pollen season. Study drugs

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