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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の関連性の評価研究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と網羅的解析

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研究要旨

自然免疫を活性化させることによってスギ花粉症の治療ができないかどうか、ウイルスを模倣した doule stranded RNA (dsRNA)と細菌感染を想定した CpG-DNA を用いて基礎的な検討を行った。その結果、鼻由来線維芽細胞においては、dsRNA 刺激によって IL-8、RANTES の著明な産生亢進と IL-4 存在下での Eotaxin 産生亢進が認められた。また dsRNA と LPS 刺激により鼻由来線維芽細胞から BLyS が発現した。BLyS は IgE クラススイッチを促進させた。以上から dsRNA の治療薬の可能性は低いが、抗 BLyS 抗体によって末梢リンパ球からの IgE 産生を抑制できた。一方、B タイプ CpG-DNA はヒト形質細胞様樹状細胞に働き IFN-αと CXCL10/CCL3 を IFNαR 非依存性に誘導し、IFN-αは有意に IgE 産生を抑制した。

福井県在住 1590 名の抗原特異的 IgE と遺伝子のデータベースを作成した。候補遺伝子アプローチによる遺伝子多型解析では、インターフェロンガンマーリセプター1 (IFNGR1) において有意な相関が認められ、自然免疫の関与が示唆された。一方全ゲノムアプローチでの解析では、一次ならびに二次解析で、36 遺伝子までに絞り込めた。また花粉曝露による網羅的蛋白解析では、曝露後に 19 因子の変動を見いだした。そのうち alpha-1-antitrypsin と Apolipoprotein A-1 を同定し、花粉曝露の炎症反応惹起が確認でき、抗炎症作用が治療効果に影響を及ぼす可能を見出した。

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に対して反応し、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反応を起こし、花粉症に対する新しい治療法になりえないか検討するとともに自然免疫系に注目した候補遺伝子アプローチを行った。

花粉症は、IgE-dependentな疾患である。そのためIgE産生 (クラススイッチ) を抑制できる手段は、ほとんどがアレルギー性鼻炎の治療手段になりえると考えられている。我々は、鼻粘膜においてIgEクラススイッチが誘導されていること、鼻粘膜における線維芽細胞がアレルギー性鼻炎の発症、病態に大きく関与していることを報告してきた。そこでBLySと代表的肥満細胞内シグナルのSykについて検討した。

網羅的蛋白解析では、全蛋白の変化を検討することができる。このような解析法は、予想もしえない遺伝子や蛋白が機能していることを見出すことができる画期的な方法である。スギ花粉を曝露した場合どのような蛋白が変動するのか網羅的に調べ、スギ花粉症治療での新しい戦略を考案したいと考えた。

B. 研究方法

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として用い、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。

また鼻粘膜由来線維芽細胞における BLyS の発現を real-time PCR で測定し，刺激後の発現の変化を検討した。刺激 48 時間後に培養上清を採取し、ウエスタンブロット法にて BLyS の蛋白産生の変化を観察した。IgE クラススイッチに関しては、ヒトB細胞株 Ramos2G6 に GFP 発現ベクターを遺伝子導入し、IL-4 及び抗 CD40 抗体または BLyS で刺激後に，フローサイトメトリーにて GFP 陽性細胞の数で定量比較した。一方でヒト B 細胞を IL-4 及び BLyS で刺激し，２日後の RNA を回収し，RT-PCR で Activation-induced cytidine deaminase (AID) の発現を検討した。ヒト B 細胞を IL-4 及び抗 CD40 抗体で刺激し，14 日後の上清を採取し，IgE を ELISA にて測定し，BlyS 中和抗体前処理の場合と比較した。回収したタンパク質リン酸化アレーとウエスタンブロットにより細胞内情報伝達を解明した。

手術にて切除されたアレルギー性鼻炎患者と非アレルギー性鼻炎患者の鼻粘膜と鼻茸組織からホルマリン標本を作製し、免疫組織化学を行った。顕微鏡下で Syk を発現している細胞をカウントし、比較検討した。また免疫二重染色を行い、Syk 陽性細胞の同定を行った。

福井県在住の 1590 名から本人の承諾を文書で得て、採血を行った。年齢は 20 歳から 50 歳までとした。得られた採血から DNA サンプルと抗原特異的 IgE (7 種)、症状、アレルギー疾患合併率、ペット、インフルエンザ予防接種からなるデータベースを構築した。10 万の遺伝子多型は、イルミナ社製全ゲノム用 SNP ジェノタイピングアレイを用いて検討した。候補遺伝子は、自然免疫系遺伝子 30 遺伝子 100 遺伝子多型を調べた。スギ花粉暴露による蛋白の変動は、大阪医科大学花粉曝露センターでの花粉曝露室での花粉曝露前後で採血後、血漿を回収し、網羅的蛋白を行った。解析法は、Ettan DIGE システムにて二次元電気泳動を行い、Decyder により各スポットの発現強度を GeneSpring により解析した。統計的有意な変化を示しているスポットについて質量分析 (mass spectrometry) を行った。ゲルを銀染色し、目的のスポットを Ettan Spot Picker により切り出した後，MALDI-TOFMS : AXIMA-CFR plus を用いて解析、得られた Peptide mass fingerprint を Mascot データベースにより解析した。Mascot で得られた結果と Swiss-2DPAGE のデーターが一致した、または複数回の MALDI-TOFMS→Mascot 解析により結果が再現された場合に同定したと判定した。網羅的蛋白解析、遺伝子解析は、福井大学医学部倫理委員会の承認を得た。サンプルは、番号で取り扱い個人の同定はできなくした。

C.　研究結果

鼻由来線維芽細胞では、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 経路の関与が判明した。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。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。

鼻由来線維芽細胞では Poly IC と LPS 刺激により BLyS が発現した。刺激 48 時間後に、BLyS 蛋白産生も確認された。BLyS 発現誘導は、Poly IC の作用が最も強く濃度依存性に誘導し、扁桃由来線維芽細胞でもみられたが、下甲介粘膜由来線維芽細胞で特に強くその１０倍であった。皮膚由来線維芽細胞では BLyS 発現みられず、組織特異性が認められた。Poly IC によよ BLyS 発現は、PI3K 阻害剤により抑制された。細胞内情報伝達をタンパク質リン酸化アレイで検討すると，Rho, Syk, Vav, c-Src, TRAF6, p-Selectin の関与が証明された 。

ヒトB細胞を IL-4 及び BLyS で刺激すると AID の発現が誘導された。同様に IL-4 と抗 CD40 抗体で刺激し，IgE を ELISA にて測定すると，BLyS

中和抗体前処理により IgE 産生が減少した。Ig クラススイッチベクターを用い共刺激における実験では，抗 CD40 抗体が低濃度では BLyS は IgE クラススイッチを有意に増強したが，高濃度の存在下では BLyS の影響はなかった。

アレルギー性鼻炎患者の鼻茸には、非アレルギー患者の鼻茸に比べ有意に多くの Syk 陽性細胞が認められた。二重染色の結果、Syk 陽性細胞は多くが好酸球であった。しかしアレルギー性鼻炎患者鼻粘膜の粘膜下層と鼻腺細胞においては、非アレルギー性鼻炎患者のそれと Syk 陽性細胞数に違いはなかった。

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 では NF-κB p65/p50 が常時活性化されていた。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、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。

福井県在住 1590 名のデータベースを作成した。その結果、20 歳代、30 歳代、40 歳代での血清中スギ特異的 IgE 抗体陽性率は、それぞれ 58%、48%、49%であったが、症状発現率は 37%、35%、38%であった。すなわちこの 10%～20%は、感作成立後何らかの免疫学的機序で症状発現が抑えられていることを意味していた。ペットの有無では、スギ感作陰性症状陰性群でもっとも飼っている率が高く、ついでスギ感作陽性花粉症症状陰性群、感作陽性症状陽性群が有意に低かった。インフルエンザの予防接種を行っている群は、行っていない群よりも有意に血清中総 IgE 値が低値であった。

候補遺伝子アプローチによる遺伝子多型解析では、インターフェロンガンマーリセプター1（IFNGR1）において有意な相関が認められた。全ゲノムアプローチでの解析では、一次ならびに二次解析で、36 遺伝子までに絞り込めた。

花粉曝露による網羅的蛋白解析では、曝露後に 19 因子の変動を見いだした。そのうち alpha-1-antitrypsin と Apolipoprotein A-1 の 2 因子を同定し得た。

D. 考察

感染を模倣した Poly IC 刺激によって、BLyS が発現され、BLyS 自身弱い CD40 刺激下では、IgE クラススイッチを促進する働きがあることが証明された。このことは、経験的に風邪などのウイルス感染が起こると、アレルギー症状は悪化し、鼻閉などが増強する現象と一致する。弱い CD40 刺激下とは、T 細胞や肥満細胞が少ないもしくは弱い条件でも、鼻粘膜において線維芽細胞からの BLyS によって IgE が産生される可能性が高くなることを示している。これまで線維芽細胞は好酸球遊走因子や好酸球を生存させるサイトカイン・ケモカイン産生が主として検討されていたが、IgE クラススイッチにも関与している可能性が高いことは、大変興味深い。これまで抗 BLyS 抗体は、シェーグレン症候群・SLE・リウマチ性関節炎などの自己免疫疾患に臨床応用されている。今後 BLyS のアレルギー性鼻炎臨床病態との関連、影響について検討する。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。

アレルギー性鼻炎患者の鼻粘膜においては、上皮層での Syk 陽性である好酸球が重要な役割を担っている可能性が高い。粘膜下層での好酸球はあまり重要でない可能性がある。Syk に対する阻害薬が開発され、臨床治験も欧米で行われ、効果が報告された。その投与法として点鼻法がとられていたが、今回の鼻粘膜上皮での Syk 陽性細胞数の差はまさしく点鼻法の正しさを示しているのかもしれない。

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 IFN ・、IFN ・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。

スギ花粉症発症においてペットの有無、インフ

ルエンザ予防接種の有無が関連していた。遺伝子多型においても感染に重要なリセプターが関与していた。これらのことは細菌感染の重要性とウイルス感染による発症・増悪化に関与することが示唆された。また蛋白分析では、炎症に関与する蛋白の変動が認められ、花粉症がアレルギー炎症に深く結びついていることが判明した。

E. 結論

鼻粘膜においては、BLyS は IgE クラススイッチ促進因子として働いていた。BLyS 中和抗体（抗 BLyS 抗体）が IgE 産生を減少させたことから、アレルギー性鼻炎の治療薬としての期待が高まってきた。また BLyS が関与するシグナル Rho, Vav, c-Src, TRAF6, p-Selectin と Syk を標的として分子治療もアレルギー性鼻炎の治療候補であると思われる。この後は、RNAi を利用した抑制系の開発や特定細胞を標的にした抗体との組み合わせによって効率的なシグナル抑制系を樹立すべきであると考えている。

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。そして舌下免疫療法に CpG-DNA を併用させいきたい。

スギ花粉飛散期におけるウイルス感染の防御は、発症予防に重要な因子であると思われた。臨床的には、ワクチンによるウイルス感染防御からのアレルギー発症予防の可能性が高い。

蛋白の網羅的解析から、スギ花粉症において炎症性蛋白の発現が認めら、炎症治療も重要な治療要因になることが証明でき、新しい治療標的の探索と新薬作成を検討したいと考えた。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

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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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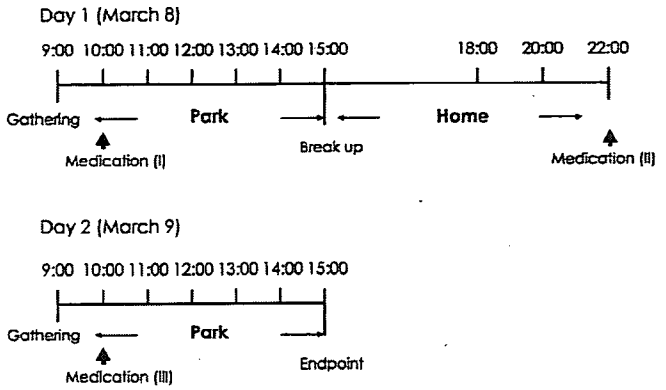

Figure 1. Study sequence. Nasal symptom scores and Japanese version of the Rhinoconjunctivitis Quality of Life Questionnaire scores were recorded before administration of the study agents at 10 AM on day 1.

and placebo were administered for 2 days to clarify the early effects on suppression of nasal symptoms, as evaluated by total symptom scores, and on improvement in scores on the Japanese version of the RQLQ (JRQLQ).

PARTICIPANTS AND METHODS

Participants

Eighty-five men and 92 women were recruited to the study from the general public through the Clinical Research Center of Osaka Medical College. For inclusion, individuals had to experience moderate or worse nasal symptoms of SAR between February and April 2002 and had to exhibit Japanese cedar specific IgE. Individuals with upper respiratory tract infections and sinusitis were excluded. Finally, 52 men and 61 women aged 20 to 57 years (mean, 34 years) were selected. All the participants resided in Osaka. Informed consent was obtained from each patient before entry into the study, which was approved by the ethics committee of Osaka Medical College.

Study Agents

Patients were randomly assigned to receive cetirizine hydrochloride, 10 mg daily; fexofenadine hydrochloride, 120 mg daily (administered as two 60-mg doses); loratadine, 10 mg daily; or placebo (lactose), twice daily. The medication doses used in this study are the standard daily doses used in Japan. All test agents, including placebo, were prepared at the Department of Pharmacologic Research Graduate School, Nihon University (Funabashi, Japan). Patients were not allowed to take any antiallergic agents beginning 3 days before the start of the study. However, the use of clemastine fumarate as a rescue drug was permitted on an as-needed basis between 3 PM and midnight on the first day of the study. Patients were instructed not to use antiasthma medications, antibiotics, H_1-antagonists, or decongestants for the duration of the study.

Methods

This was a 2-day, randomized, double-masked, parallel-group study conducted during the peak of the Japanese cedar pollen season (March 8 and 9, 2003) at Osaka Expo Park. The study sequence is outlined in Figure 1. All eligible patients visited the study site at 9 AM on day 1 and were questioned concerning their nasal symptoms and QOL during the baseline period. Individuals were given the first dose of study agent at 10 AM, and then they were asked to walk around the park without wearing a hat or cap, accompanied by a guide. Patients recorded their symptoms in diaries hourly between 11 AM and 3 PM while in the park and at 6, 8, and 10 PM at home. Individuals in the fexofenadine group took their second dose of fexofenadine and the others took placebo at 10 PM.

The next morning, all the patients returned to the park and took their third dose of study medication, including placebo, at 10 AM. Patients were then asked to complete their nasal symptom diaries hourly between 10 AM and 3 PM in the park. At the end of the study, patients completed their second JRQLQ.

Recording and Assessing Symptoms and QOL

The numbers of paroxysmal sneezes and occasions when patients blew their noses were recorded on nasal symptom forms. Nasal congestion, nasal itching, eye itching, and watering of the eyes were recorded on an analog scale from 0 (none) to 10 (very severe). In addition, QOL was surveyed in accordance with the JRQLQ.[7] This questionnaire includes 17 questions in 6 domains designed to measure the effects of rhinoconjunctivitis symptoms on disease-specific QOL. The JRQLQ was developed from the original 28-item RQLQ with permission from the Japan Academic Association for Copyright Clearance (Tokyo, Japan) and the Copyright Clearance Center Inc (Danvers, MA). An overall JRQLQ score was computed by taking the mean scores of the 17 items in the instrument; scores, therefore, ranged from 0 to 4, with higher scores indicating poorer QOL, which is different from the original RQLQ (7-point scale from 0 to 6).[7]

Airborne Japanese Cedar Pollen Count

A Durham pollen collector[8] was set up in the park to measure the amount of pollen during the study. In addition, the amount of airborne pollen in Osaka was measured at 8 locations on the study days.

Statistical Methods

Nasal symptom scores at 10 AM on day 1 in the 4 treatment groups were compared using the Tukey test. Baseline comparability of the 4 groups was analyzed using longitudinal analysis of variance. Evaluation of change in QOL status between baseline and the end of the study in each group was compared using the Wilcoxon signed rank test. Comparisons among the 4 groups were made using nonparametric methods (Mann-Whitney *U* test).

RESULTS

Participants

Seven individuals did not participate because of sickness on the study days. Background characteristics of the study groups are given in Table 1. No significant differences were observed among the 4 groups in terms of the number of

Table 1. Patient Background Characteristics

	Treatment group			
	Cetirizine (n = 30)	Fexofenadine (n = 28)	Loratadine (n = 28)	Placebo (n = 27)
Sex, No.				
Male	14	13	14	11
Female	16	15	14	16
Age, mean, y	34.1	34.1	32.5	34.6
Total symptom score, mean	12.1	11.7	10.0	12.3
Overall QOL score, mean	1.11	1.12	0.94	1.18

Abbreviation: QOL, quality of life.

paroxysmal sneezes, the number of times patients blew their noses, nasal congestion, nasal itching, eye itching, and watering of the eyes before administration of the study agents at 10 AM on day 1 (Table 2).

Airborne Japanese Cedar Pollen Count

The mean pollen counts at 8 facilities located in different areas of Osaka were 43.6 grains/cm^2 on day 1 and 40.9 grains/cm^2 on day 2. At Osaka Expo Park, the mean pollen counts were 34 grains/cm^2 between 9 AM and 3 PM on day 1 and 18 grains/cm^2 during the morning of day 2.

Changes in Symptom Scores

Reduction rates of mean values of total symptoms recorded by patients at all times by drug group are shown in Figure 2. Cetirizine use produced a 45% to 48% mean reduction in total

Table 2. Changes in Rhinoconjunctivitis Symptom Scores During the Study

Date and time	Change in symptom score						
	Sneeze	Nose blow	Stuffiness	Nasal itch	Eye itch	Eye watering	Total
			Cetirizine Group				
March 8							
10:00	0.50	1.63	2.79	2.15	2.75	2.26	**12.08**
12:00	0.13	0.63	1.49	1.16	1.85	0.99*	**6.25**
15:00	0.66*	1.20	1.78*	1.37	1.74*	1.32*	**8.07**
March 9							
12:00	0.03	0.80	2.21*	1.03	1.51*	1.14*	**6.72**
15:00	0.26*	0.83	1.93*	0.98	2.27*	1.19*	**7.46**
			Fexofenadine Group				
March 8							
10:00	0.61	1.18	2.66	2.79	2.88	1.60	**11.72**
12:00	0.07	0.36	1.49	1.37	1.79	0.90*	**5.98**
15:00	0.25*	1.43	2.11	1.69	2.12*	1.39*	**8.99**
March 9							
12:00	0.21	0.71	2.68	1.70	2.25	1.12*	**8.67**
15:00	0.25*	1.14	2.91	1.88	1.65*	0.82*	**8.65**
			Loratadine Group				
March 8							
10:00	0.82	1.21	1.88	1.91	2.37	1.82	**10.01**
12:00	0.36	0.64	1.80	1.80	1.70	0.92*	**7.22**
15:00	1.43	1.43	2.41	2.75	2.58	1.60*	**12.20**
March 9							
12:00	0.29	0.61	2.01*	1.94	1.33*	0.74*	**6.92**
15:00	0.75	0.82	2.09	1.73	1.56*	0.96*	**7.91**
			Placebo Group				
March 8							
10:00	0.67	1.29	2.77	2.19	2.87	2.52	**12.31**
12:00	0.15	0.74	2.41	1.81	2.56	2.34	**10.01**
15:00	2.14	2.14	3.48	2.66	4.03	3.70	**18.15**
March 9							
12:00	0.48	1.04	3.98	2.02	3.33	2.59	**13.44**
15:00	1.00	1.78	4.11	2.61	4.05	2.58	**16.13**

* $P < .05$ compared with baseline.

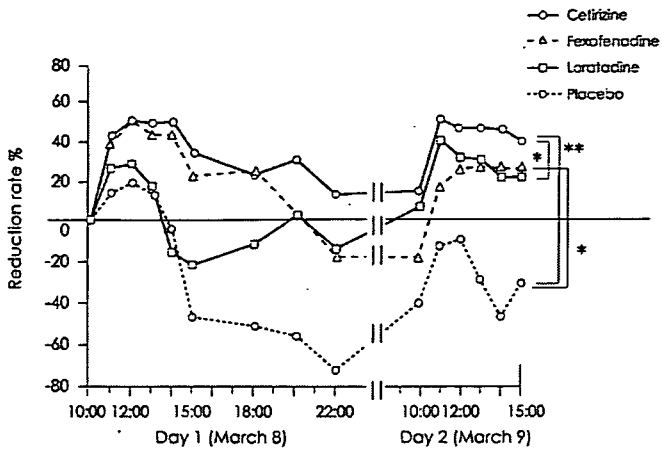

Figure 2. Mean hourly reductions in total symptom scores vs baseline. Asterisk indicates $P < .05$ (cetirizine vs loratadine and fexofenadine vs placebo); double asterisk, $P < .01$ (cetirizine vs placebo).

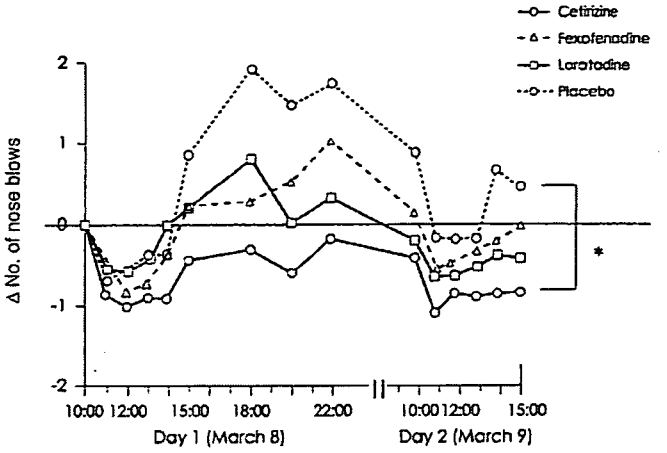

Figure 3. Change in the number of nose blows from baseline. Asterisk indicates $P < .05$ (cetirizine vs placebo).

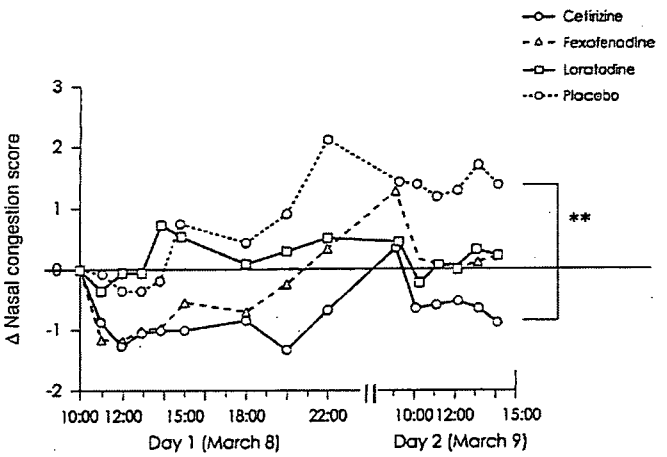

Figure 4. Change in visual analog scale scores for nasal congestion. Double asterisk indicates $P < .01$ (cetirizine vs placebo).

symptom scores compared with baseline 1 to 3 hours after administration on both days (Table 2). Mean percentage reductions with cetirizine use were consistently larger than those with fexofenadine, loratadine ($P = .04$), and placebo ($P = .006$) use.

Fexofenadine use produced a 42% to 48% mean reduction in total symptom scores within 1 to 3 hours of administration on day 1, but the reduction rate on day 2 was much lower than that observed on day 1 (Table 2). However, fexofenadine therapy significantly reduced total symptom scores compared with placebo use ($P = .04$). Loratadine use produced no significant reductions in total symptom scores overall compared with placebo use, although a 30% to 40% mean reduction was observed in this group on day 2 (Table 2). The effect of the first dose of loratadine on nasal symptoms disappeared within 4 hours on day 1. However, the second administration of loratadine continued to suppress nasal symptom through 3 PM on day 2.

The checkpoint analysis obtained at 3 PM on day 1 showed greater reductions in total symptom scores with cetirizine (34.0%; $P = .001$ vs baseline) and fexofenadine (22.8%; $P = .03$ vs baseline), whereas loratadine (−21.9%) and placebo (−47.5%; $P = .008$) showed significant increases compared with baseline. Similarly, the end point analysis obtained at the end of the study revealed the greatest reductions in total symptom scores in the cetirizine group (38.9%; $P = .005$ vs baseline), followed by the fexofenadine (25.9%) and loratadine (20.9%) groups. Aggravation of symptoms was noted in the placebo group. Total symptom scores in the 4 groups at 4 checkpoints are given in Table 2.

Group Comparisons by Symptom

Each symptom was compared at all times in the 4 groups. Cetirizine therapy significantly reduced the number of times the nose was blown (Fig 3) and nasal congestion (Fig 4) relative to placebo use and suppressed nasal itching more than loratadine and placebo use (Fig 5). Fexofenadine therapy was significantly more effective at reducing nasal itching than was placebo use. The 3 active treatments were better than placebo use in terms of reducing sneezing, eye itching, and eye watering. No differences were observed among the 3 active treatment groups in reducing these symptoms.

The onset of action of cetirizine, fexofenadine, and loratadine was observed as a reduction in eye watering within 2 hours of administration. Sneezing and eye itching in the cetirizine and fexofenadine groups and nasal congestion in the cetirizine group were significantly reduced compared with in the placebo group at 3 PM on days 1 and 2. Loratadine therapy significantly reduced nasal congestion and eye itching compared with placebo use at midnight on day 2 (Table 2).

Use of Rescue Drug

The use of rescue drug in the cetirizine, fexofenadine, loratadine, and placebo groups was calculated to be 7% (2/30),

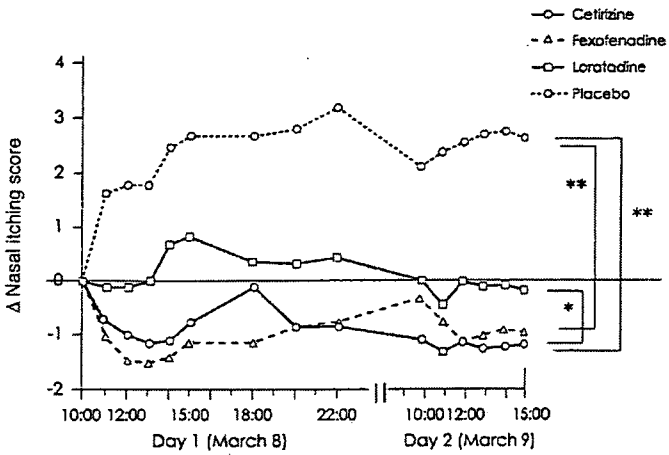

Figure 5. Changes of visual analog scale scores for nasal itching. Asterisk indicates $P < .05$ (cetirizine vs loratadine); double asterisk, $P < .01$ (cetirizine vs placebo and fexofenadine vs placebo).

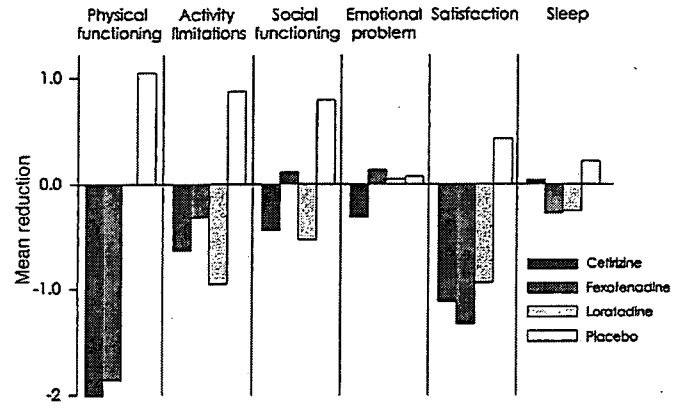

Figure 6. Mean reductions in 6 item scores in the Japanese version of the Rhinoconjunctivitis Quality of Life Questionnaire during the double-masked study with the administration of cetirizine, fexofenadine, loratadine, or placebo.

18% (5/28), 21% (6/28), and 22% (6/27), respectively. Although use of rescue medication was not statistically different among the 4 groups, use in the cetirizine group was markedly lower than that in the other groups. The use of clemastine as a rescue drug decreased total symptom scores for several hours. The time of peak plasma concentration after a single oral administration of clemastine was 3 hours. The rescue drug was administered on day 1 to 2 patients in the cetirizine group (at 4:35 PM or 11:20 PM), 5 patients in the fexofenadine group (at 3:05 PM, 3:15 PM, 5:00 PM, 8:30 PM, or 12:00 AM), 6 patients in the loratadine group (at 3:05 PM, 3:15 PM, 4:00 PM, 11:00 PM, 11:00 PM, or 12:55 AM), and 6 patients in the placebo group (at 3:00 PM, 3:30 PM, 4:30 PM, 5:15 PM, 11:15 PM, or 12:00 AM). The effect of clemastine therapy may have disappeared by morning (10 AM) on day 2. If clemastine use was still effective at the beginning of day 2, a reduction in nasal symptom scores would have been expected in the fexofenadine, loratadine, and placebo groups. However, only patients in the cetirizine group showed a significant improvement.

Changes in QOL Scores

No significant differences were observed in total QOL scores in the 4 study groups at baseline (Table 1). At the end of the study, overall QOL was significantly improved from baseline in all 3 active treatment groups, whereas patients in the placebo group exhibited significant QOL impairment. Reductions in mean total QOL scores at the end of the study were 24.7%, 19.3%, 33.2%, and −12.9% in the cetirizine, fexofenadine, loratadine, and placebo groups, respectively. No differences in QOL scores were observed among the 3 active treatment groups at the end of the study.

Observed changes in each of the 6 domains of the JRQLQ are shown in Figure 6. Administration of cetirizine on days 1 and 2 led to significant improvement in 3 items of physical functioning, 2 of activity limitations, and 1 of satisfaction with treatment compared with baseline. Administration of fexofenadine produced significant improvement in 2 items of physical functioning, 2 of activity limitations, and 1 of satisfaction. Administration of loratadine produced significant improvement in 1 item each of activity limitations and satisfaction.

Safety

All study medications were well tolerated. No serious adverse effects were reported during the study. The most frequently reported adverse effect was drowsiness, experienced by 7 participants (2 each in the cetirizine, fexofenadine, and loratadine groups and 1 in the placebo group).

DISCUSSION

The purpose of this study was to compare the efficacy, with special focus on the onset of action, and safety of cetirizine, fexofenadine, and loratadine vs placebo in Japanese patients with JCP. The results suggest that cetirizine is the most effective of these medications overall given its ability to suppress individual symptoms, improve JRQLQ scores in several domains, and reduce the need for rescue medication.

Second-generation oral antihistamines are believed to act fast. Pharmacokinetic studies of the 3 drugs used in this study demonstrate rapid absorption rates after single and multiple oral doses. Times to peak plasma concentration after single oral administrations of cetirizine, fexofenadine, and loratadine are 1, 2, and 1.4 to 1.6 hours, respectively.[9] Oral antihistamines have been demonstrated to be highly effective against eye symptoms occurring when pollen counts are high.[10–14] In the present study, a significant reduction in eye watering was found within 2 hours of administration of the first dose in all 3 active treatment groups. Antihistamines have also been reported to be highly effective against paroxysmal sneezing and nasal discharge as well as eye and nasal itching in general.[10–14] However, in the present study, only

cetirizine suppressed sneezing, nasal discharge, and nasal itching compared with placebo.

There is little reported effect of these drugs on nasal congestion. However, we demonstrated that cetirizine therapy significantly decreases nasal congestion compared with placebo use. This finding may be supported by indirect evidence; for example, it has been shown that administration of cetirizine in patients with allergic rhinitis results in a significant decrease in serum RANTES (regulated upon activation, normal T-cell expressed, and secreted), a major chemoattractant protein for eosinophils, and MCP-1.[15] Furthermore, eosinophil infiltration was significantly reduced by cetirizine treatment in patients with SAR after allergen-specific challenge.[16] In vitro, cetirizine has been shown to inhibit eotaxin-induced eosinophil transendothelial migration.[17] Furthermore, administration of cetirizine in mice reduced not only interleukin 4 (IL-4) and IL-5 expression but also eosinophil infiltration in nasal mucosa.[18] These results seem to support the effect of cetirizine on the improvement in nasal congestion observed in this study.

Intercellular adhesion molecule 1 is a transmembrane glycoprotein that promotes adhesion in inflammatory reactions. All 3 antihistamines reduce the expression of intercellular adhesion molecule 1 on epithelial cell membranes, as shown by in vitro[19,20] and in vivo[21] studies. Fexofenadine and loratadine possess anti-inflammatory properties. Treatment with fexofenadine in vitro reduces the eosinophil-induced release of IL-8 and granulocyte-macrophage colony-stimulating factor from human nasal epithelial cells[22] and inhibits the production of IL-4 and thymus- and activation-regulated chemokine by human peripheral blood lymphocytes.[23] In addition, fexofenadine treatment of sensitized mice in vivo prevented tissue eosinophilia and T_H2 cytokine production.[24]

Loratadine treatment inhibited histamine-induced P-selectin expression and IL-6 and IL-8 secretion by human endothelial cells.[25] Incubation of loratadine in vitro attenuated the nitric oxide–induced release of RANTES by human bronchial epithelial cells[26] and leukotriene B4 production by neutrophils.[27] However, the effects of fexofenadine and loratadine on nasal congestion were not found. This result might be due to the medication period. It is possible that prolonged administration of fexofenadine and loratadine results in a more comfortable nasal passage.

Natural exposure to an allergen by walking in a park is a clinical research method first used by Meltzer et al.[10] The obvious advantage of this method is that it replicates the real-life situation of patients with pollinosis. However, a disadvantage is that the drug effects can only be evaluated for short durations. The main concerns in the present study were the weather and the amount of airborne pollen. Average amounts of airborne pollen for the time of year were present (90 grains/cm^2 for >2 days). Consequently, sufficient natural exposure for assessing this study was obtained. A characteristic of JCP is that it can easily be aggravated owing to Japan's long pollen season and the large amount of airborne pollen. Nonetheless, in terms of treatment planning, screening of fast-acting drugs is an extremely important point of clinical research. In this regard, cetirizine's onset of action occurred at 1 hour, and maximum effects were seen at 2 hours, suggesting that cetirizine is a highly useful antihistamine for patients with JCP.

Recently, environmental exposure units have been used not only to provide constant exposure to pollen but also, more accurately, to determine experimental conditions rather than going outdoors during the peak pollen season.[28,29] Although innovations in outdoor park study design and methods have proved successful in the rating of antihistamines, environmental exposure units provide reproducible exposure environments for testing at any time. The onset of action and the efficacy of cetirizine therapy observed in this study are consistent with those observed in other multidrug comparative studies[28,29] using environmental exposure units and park tests.

Improving patient well-being and health-related QOL is widely recognized as an important goal in the treatment of patients with JCP. Cetirizine therapy provided the greatest number of improved items in the JRQLQ. The 3 antihistamines tested in this study provided safe and effective symptomatic relief, as shown by JRQLQ scores. Although moderate correlations between symptom severity and RQLQ score have been found in several studies,[30–32] the nasal symptom score may be more sensitive than the JRQLQ score to compare the effectiveness of anti-JCP medications in short-term studies. One reason is the duration of such studies; reliable improvement in the QOL score usually requires 1 or more weeks of treatment.[33] Another reason apparently is problems of recording symptoms using the visual analog scale used in the JRQLQ, at least in our study population. Similar studies conducted in Japanese patients using the original RQLQ, with scores ranging from 0 to 7, suggested that these patients hesitated to check the mark. Therefore, we reduced the range to 0 to 4 for the JRQLQ used in this study. Total score represented as a number seems easier for Japanese patients.

Ranking of antihistamines is important for patients with JCP and physicians in primary care. We rank cetirizine as having the best effectiveness, followed by fexofenadine and then loratadine. This supports the trend already observed by other research groups.[10,11,13,14,28,29] It is possible that in the present study the duration was too short to observe benefits in the loratadine group. However, it has been reported that cetirizine performed better than loratadine in a 7-week, double-masked study, although the results were not significant.[34] Several other reports have found that cetirizine is superior to loratadine in the suppression of nasal symptoms in patients with SAR.[10,11,28,29] In Europe, it has been reported that there are no differences in efficacy between fexofenadine and cetirizine in patients with SAR.[35,36] Furthermore, fexofenadine has been demonstrated to be significantly more effective than loratadine in relieving nasal congestion and eye symptoms and in improving QOL.[13,14]

In this study, patients treated with cetirizine hydrochloride and loratadine received the full dose of medication in the

morning, whereas those taking fexofenadine hydrochloride received 60 mg at 10 AM and 10 PM. In the United States, the customary dose of fexofenadine hydrochloride is 180 mg once daily. In general, Japanese people are of smaller stature than American people, and it has not been established whether 180 mg of fexofenadine hydrochloride once daily can be taken safely by Japanese patients. Further studies are needed to determine the optimal dose of fexofenadine in the Japanese. In this study, all 3 antihistamines tested were well tolerated. Although there were no significant differences among the treatment groups regarding adverse effects, significant differences were seen in terms of time to onset of action, duration of effect, and efficacy. This study provides some insights into the clinical responses to 3 antihistamines that may prove helpful in the management of JCP.

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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.

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).

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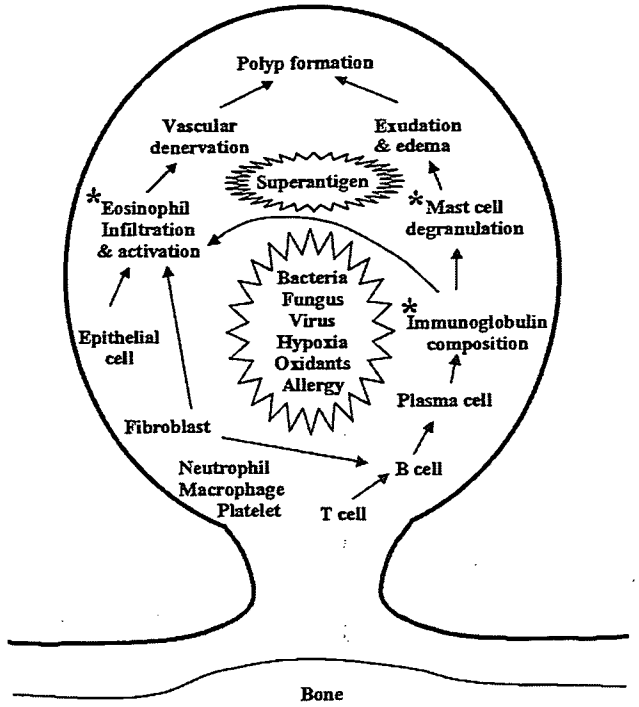

Fig. 1. Mechanism of nasal polyp formation. *Some commonly observed pathological findings in nasal polyps.

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.

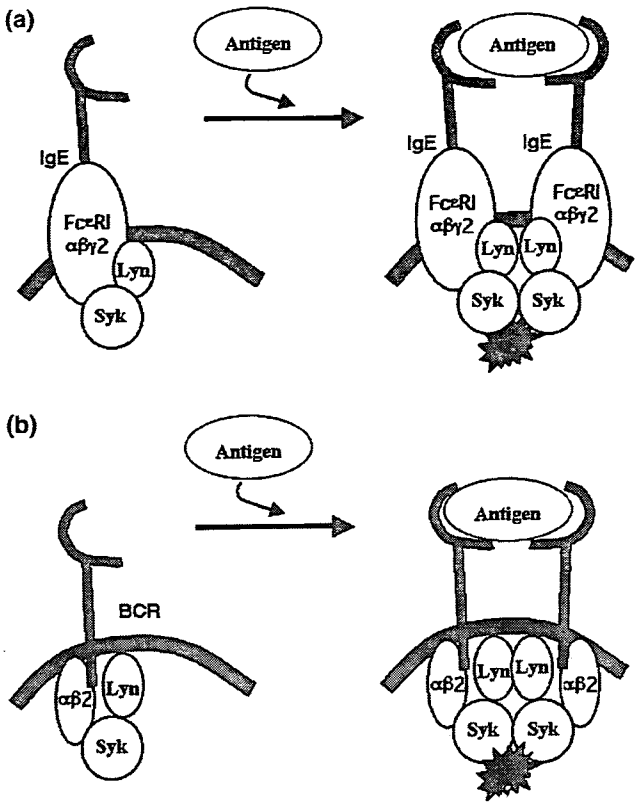

Fig. 2. Syk plays critical roles in intracellular signal transduction in human mast cells (a) and B cells (b).

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].

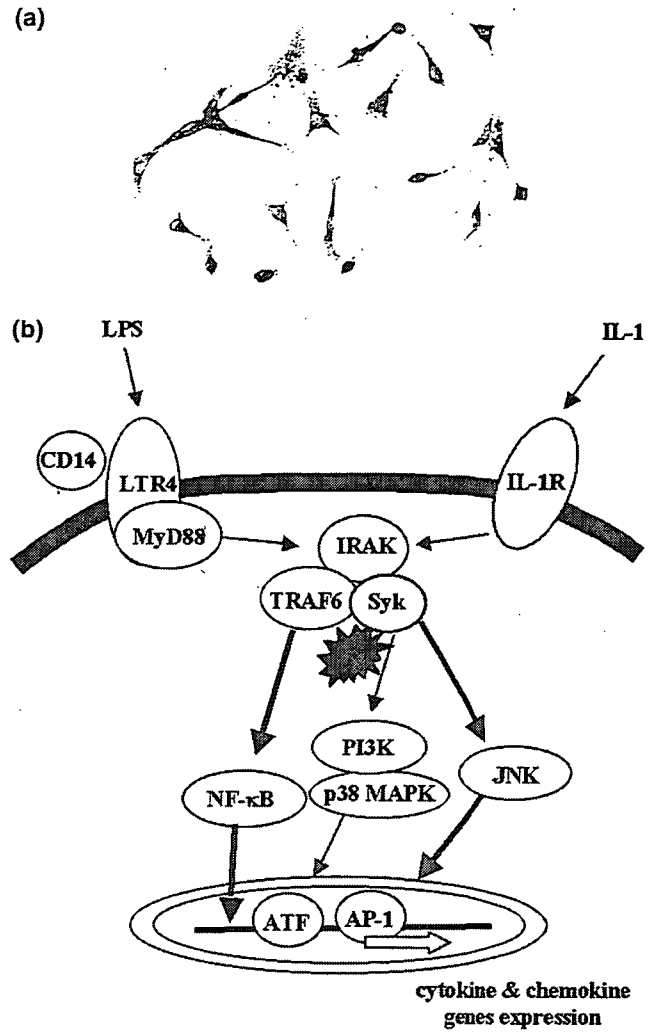

Fig. 3. Syk expressed in human nasal fibroblasts (a) and its signal transduction (b).

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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