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Current Article

IgE とアレルギー性鼻炎
—クラススイッチから遺伝子治療の可能性まで—

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はじめに

アレルギー性鼻炎の病因抗原の大部分は，花粉やダニなどの吸入系抗原である。吸入系抗原に対するIgE抗体の多くは，Fcεレセプターを介して肥満細胞や好塩基球の細胞膜上に固着している。IgEが固着した肥満細胞や好塩基球は鼻粘膜に広く分布し，抗原が鼻腔より吸入されると抗原特異的IgEと結合しIgE同士を抗原を介して架橋させ，それにより細胞内シグナルが作動し，ヒスタミンを代表とするケミカルメディエイターを放出させ，くしゃみ，鼻汁，鼻閉を引き起こすとされている。現在まで，抗原特異的IgEがどこで，どのようにして産生されるかについてはよくわかっていない。

本稿ではIgEへのクラススイッチ機構や鼻粘膜に存在するIgEの産生および供給，臨床的意義，IgEに限定した治療戦略，とりわけ遺伝子治療の可能性に関して述べる。

I. IgEへのクラススイッチ分子機構

ヒトIgE産生機構は，非特異的IgEに関してかなり判明している。しかし抗原特異的IgE産生機構は動物実験にて誘導が可能であるが，その機序についてはまだ完全な証明はなされていないのが現状である。まだいかなる免疫グロブリンが産生できるかわからないナイーブなB細胞であるIgM^+IgD^+B細胞に，interleukin-4（IL-4）もしくはIL-13の刺激とCD40リガンドの刺激の両方が入るとIgE産生細胞へクラススイッチする[1,2]。CD40リガンドはT細胞や肥満細胞，好塩基球の細胞表面上に存在し[3]，ナイーブなB細胞上のCD40と結合する。IgEにクラススイッチした細胞（IgE^+B細胞）は，さらに分化・増殖しIgEを産生する形質細胞となりIgEを産生する（図1）。

IgEへのクラススイッチの分子機構を理解するには，遺伝子レベルからの理解が必要である。ヒトB細胞の免疫グロブリンH鎖（heavy chain）遺伝子は図2に示すように，μ（IgM）－δ（IgD）－γ3（IgG3）－γ1（IgG1）－α1（IgA1）－γ2（IgG2）－γ4（IgG4）－ε（IgE）－α2（IgA2）の順に各クラスが上流から並ぶ。IgDを除くそれぞれのクラス遺伝子は5'側からintervening（I）領域と呼ばれる領域，特有の反復配列を有するスイッチに関連するスイッチ（S）領域，定常部をコードするC領域から構成される（図2）。

IgM^+IgD^+B細胞がそれぞれのクラスに分化することをクラススイッチと呼ぶが，スイッチングに先だって特有の転写（transcription）が誘導される[4~6]。これはI領域から始まりC領域に至るものであるが，転写物自体をgerm-line transcriptと呼ぶ。このgerm-line transcriptは，I領域に多数のストップコドンが存在するため蛋白質に翻訳されない（図2）。Germ-line transcriptの発現はどのクラスへのスイッチングにおいてもスイッチ領域間での遺伝子組み換え前に起こり，その発現が

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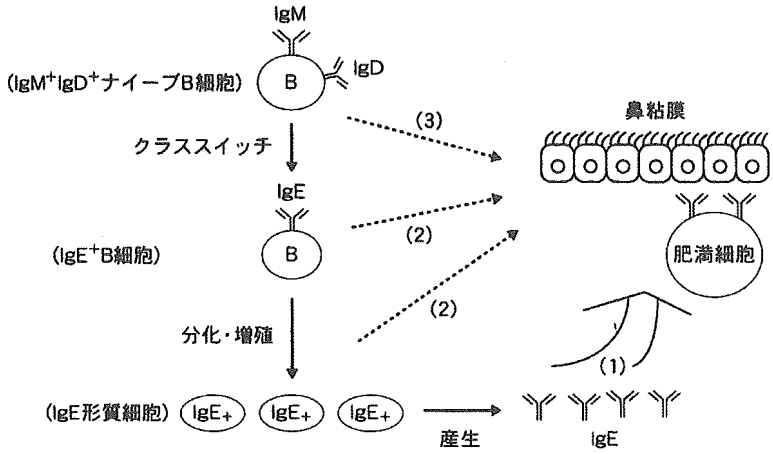

図 1　鼻粘膜への IgE 供給路
(1) 産生された IgE が血流，リンパ流によって供給される。(2) IgE 産生 B 細胞が鼻粘膜に到達し，分化・増殖して IgE を産生する。(3) ナイーブ B 細胞が鼻粘膜に到達し，IgE にクラススイッチした後，分化・増殖して IgE を産生する。

クロモゾーム構造を変える(開かせる)ことによってスイッチングに必須であると考えられている[4]。スイッチが終了した細胞ではこの germ-line transcript は消滅する。IgE へのクラススイッチの際には，ε germ-line transcript が必ず発現するが，ヒトの場合は IL-4 で誘導される[1]。

続いて CD40 リガンドの刺激が加わると IgE クラスの S 領域（Sε）と IgM の S 領域（Sμ）との間で遺伝子組み換えによる DNA 再構成が生じる[7,8]。これは図 2 に示すように 1 本の染色体上で S 領域を逆向きに並べてループを描き，環状 DNA が胎児型 DNA から欠失することによる[9,10]。ループアウトにより環状 DNA が切り出され，目的とされるクラスの遺伝子が完成する。たとえば IgE であれば Sμ と Sε が結合し，そのあとに IgE の定常領域が続く。抗原特異的領域（VDJ）より転写が始まり，Sμ と Sε の部分はスプライシングされ VDJ の転写に定常領域の転写が続き，翻訳されると抗原特異的 IgE が産生される。

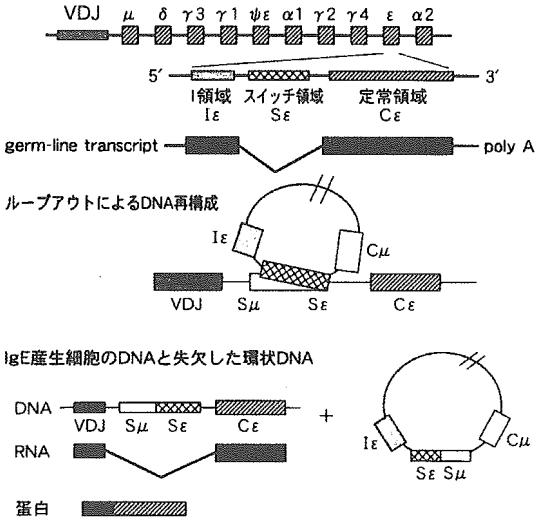

図 2　B 細胞の遺伝子とクラススイッチ分子生物学的機序

もう 1 つの変則的な IgE クラススイッチとして，トランススプライシング機構が存在する。サイトカインで誘導された 2 つの germ-line transcript の前駆型メッセンジャー RNA 間で，トランススプライシング機序を介して産生される非定型的 germ-line transcript を見いだした[11]。IgD+B 細胞において IL-4 単独刺激で，ε germ-line transcript（Iε-Cε）と γ4 germ-line transcript（Iγ4-Cγ4）が誘導される。それとともに Iε-Cγ4，Iγ4-Cε という型の germ-line transcript の存在が reverse transcription-polymerase chain reaction（RT-PCR）法にて証明された（図 3）。Iε-Cγ4 のような下流にあるクラスの I 領域が上流にあるクラスの定常領域よりも前にあるということは，トランススプライシング機序と DNA 組み換えの 2 つのメカニズム関与が考えられるが，IgD+B 細胞において IL-4 単独刺激では DNA 組み換えは起

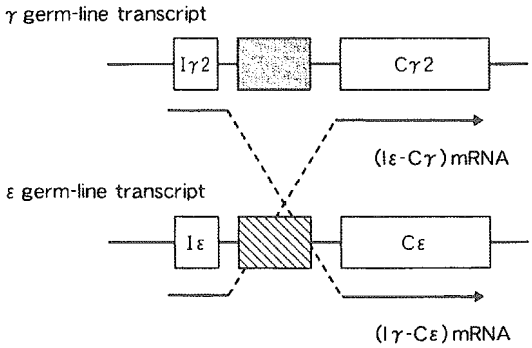

図 3　トランススプライシング機構
γ germ-line transcript と ε germ-line transcript によって非定型的な RNA が作製される。

こり得ない。すなわち，トランススプライシング機序が最も考えられるということである。今日までに完全なヒトの系でのトランススプライシングの証明は，これが初めてである。さらに，同様な Iγ-Cμ, Iε-Cμ, Iγ4-Cα1 も同じ系で RT-PCR 法で増幅可能であった。これらのことは，VDJ-Cμ の B 細胞（IgM 産生細胞）や VDJ-Cα の B 細胞（IgA 産生細胞）であっても，ε germ-line transcript (Iε-Cε) が誘導されれば，DNA の再構成なしにトランススプライシングによって，ε の成熟型 RNA (VDJ-Cε)，つまり IgE が産生されるという期待を抱かせる。ただし，この頻度（トランススプライシングによって成熟型 RNA ができる頻度）は決して高いレベルではないが，可逆的かつ一時的であり，生物学的意義はかなり高いと考えられる。実際，IgG2 産生 GM1500 細胞において，再度 NA 再構成なしに IgE 産生細胞を IL-4 刺激で認めた。RNA レベルでは，γ2 成熟型 RNA (VDJ-Cγ2) と ε germ-line transcript (Iε-Cε) を認めるとともに，ε 成熟型 RNA (VDJ-Cε) と非定型的 Iε-Cγ2 の存在が証明された[11]。

II. 鼻粘膜で検出される IgE

産生された IgE は，図 1 に示すように 3 つのストーリーの可能性に沿って，鼻粘膜に分布するようになる。

1) IgE は粘膜以外の鼻関連リンパ組織（nasal associated lymphoid tissue）で産生され，血流もしくはリンパ流によって鼻粘膜に供給される。このことはマウスに放射性同位元素で標識した IgE を投与すると，粘膜に集積することでその可能性が証明されている[12]。運ばれてきたほとんどの IgE は鼻粘膜固有層で分化した肥満細胞の Fcε レセプターに結合したり，粘膜に運ばれる途中で肥満細胞などに結合する。

2) 他の場所で IgE にスイッチした IgE 産生 B 細胞がリンパ流や血流によって鼻粘膜に動員され，鼻粘膜で IgE 産生を行う。その際，IgE 産生 B 細胞が鼻粘膜で分化・増殖する場合と，多くの分化した IgE 産生形質細胞が動員される場合と 2 通り存在する。現在この 2 つの可能性を明確に区別する方法は確立されていない。ヒト鼻腔に，ある条件下で抗原刺激を行うと鼻腔洗浄液中に IgE 産生細胞が増加し，同様に抗原特異的 IgE も増加することは証明されている[13]。

3) 鼻粘膜局所で IgE へのクラススイッチが誘導され，IgE 産生 B 細胞が鼻粘膜で分化・増殖し IgE 産生が行われる。マウスにおいては，気道内への抗原投与が germinal center を気道粘膜内に形成し，IgE 産生細胞を誘導させ，IgE の粘膜への供給源になることが証明された[14]。

実際，抗 IgE 抗体でアレルギー性鼻炎患者の鼻粘膜を免疫組織染色を行うと，遊離の IgE はほとんど染色されないが，鼻粘膜の固有層には多くの IgE 陽性細胞が認められる。IgE 陽性細胞のほとんどは IgE が固着した肥満細胞であるが，一部は B 細胞も存在する。遊離の IgE が染色されない理由は染色技術の問題の可能性もある。

III. 鼻粘膜における IgE クラススイッチ誘導の証明

鼻粘膜に存在する IgE がどのように作られたかについては，前述の 3 つの可能性がある。1) や 2) に関してはマウスやヒトにおいて証明されていたが，3) の粘膜局所での IgE クラススイッチ誘導については，クラススイッチの分子生物学的機序から，DNA および RNA レベルからのアプローチが必要であり，学術的にも魅惑的なものであった。

われわれは，まず DNA レベルよりアプローチした。それは環状 DNA を検出することで IgE クラススイッチの存在を証明しようとした。これは，

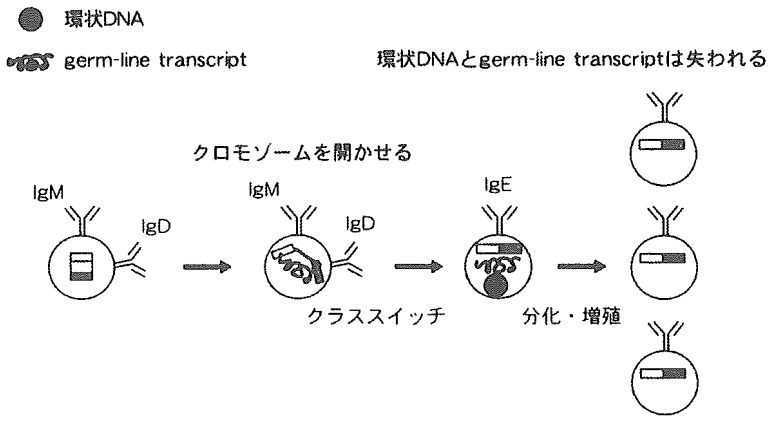

図 4　環状 DNA と germ-line transcript のクラススイッチでの意義

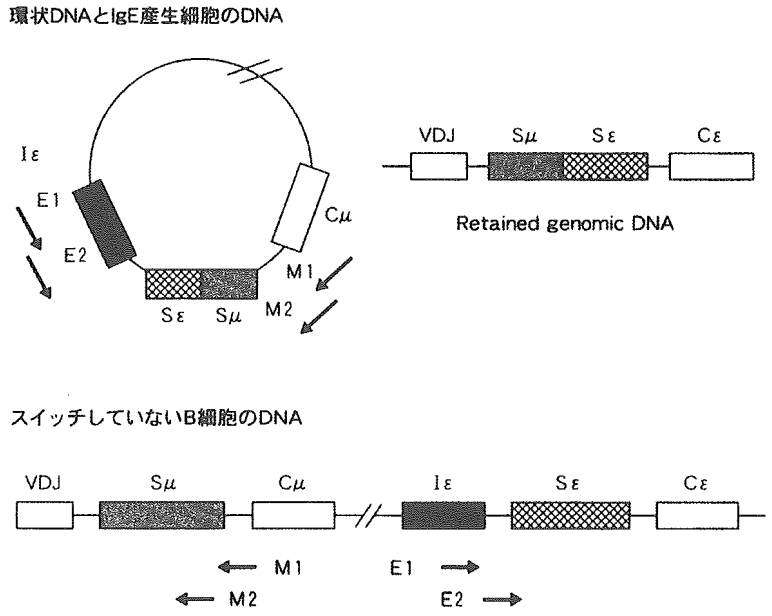

図 5　環状 DNA 検出時の nested PCR 法におけるプライマーの設定
プライマーは環状 DNA のみを増幅できる。

図 4 に示すようにクラススイッチが行われた直後の細胞内には，環状 DNA と germ-line transcript が存在する。しかし，すぐに分解されてしまい，そこから増殖・分化する細胞には存在しないからである[1,7]。すなわち，IgE にスイッチしてしまった B 細胞がいかに増えようと，いかに IgE を産生しようと，環状 DNA と germ-line transcript は存在しないからである。

われわれは，花粉アレルギー患者増大の原因であろうと最近注目を浴びているディーゼルエンジン排出粒子（DEP）を利用して以下の実験を行った。なぜなら，環状 DNA の量は極めて微量であり，IgE へのクラススイッチは同一抗原に対する IgG へのクラススイッチに比べ 100 分の 1 以下である。そのため，ヒト鼻粘膜における環状 DNA の存在を証明することを抗原刺激のみで行うことは無理であったためである。DEP は *in vitro* での B 細胞からの IgE 産生や *in vivo* での IgE 産生を増強することが既に報告されていた[15,16]。

そこで，ブタクサ花粉アレルギー患者を対象患

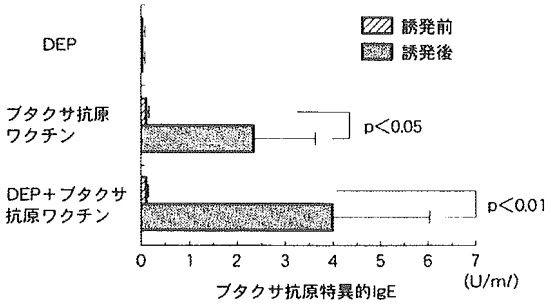

図 6　DEP とブタクサ抗原ワクチンの鼻腔内投与による抗原特異的 IgE

者として用い，以下の実験を行った。8 人に対して，①ブタクサ抗原ワクチン誘発，②DEP による誘発，③ブタクサ抗原ワクチンと DEP 併用誘発を行い，各々前後計 6 回の鼻腔洗浄を行った[17]。それぞれの誘発は 3 週間以上の間隔をあけて行った。投与した DEP の量は，ロサンゼルスのバス停で 1 日立っていたときに曝露される量であるとされる 0.3 mg とした。回収した鼻腔洗浄液の total IgE，ブタクサ特異的 IgE を測定し，nested PCR 法により環状スイッチ DNA の存在を検索した（図 5）。その結果，ブタクサ抗原ワクチンと DEP 併用投与後からのサンプルのみ，6 つの環状スイッチ DNA を得ることができた。同時に鼻腔洗浄液中非特異的 IgE，鼻腔洗浄液中ブタクサ特異的 IgE も高値を示した（図 6）。以上のことは，鼻粘膜局所での IgE へのクラススイッチ誘導を証明したことになる。DEP は，抗原とともにヒト鼻腔内に入ると抗原刺激を修飾し，抗原特異的 IgE 産生増強に働いた。

他方，アンチセンスの RNA プローベを用いて *in situ* hybridization を行い，ε germ-line transcript 発現を検出し，鼻粘膜でのクラススイッチ誘導が証明された[18]。つまり IgE クラススイッチの前に必ず起こり，かつスイッチしてしまった細胞においては消失してしまう RNA からのアプローチである。季節性の花粉アレルギー性鼻炎患者からシーズン前とシーズン中に鼻粘膜を採取し，ε germ-line transcript 発現を調べると，花粉シーズン中に有意に上昇していた。さらにステロイド点鼻によって治療効果が認められ患者では，ε germ-line transcript は低い発現を維持していることもみつかった。IgE を産生する成熟型 RNA の発現も ε germ-line transcript 同じ発現パターンを示し，IgE 産生に必須の IL-4 の発現も同様であった[18]。次に，季節外に花粉アレルギー性鼻炎患者に対して抗原刺激を行った結果，抗原刺激を行った鼻粘膜では有意に ε germ-line transcript の発現と IL-4・IL-13 の発現が亢進していた[19]。

IV. 鼻粘膜におけるサイトカイン環境

以上より，鼻粘膜局所での IgE クラススイッチを証明できたが，実際の IgE クラススイッチを誘導する環境はどうであろうか。根本的に，IgE クラススイッチに必須なものは IL-4 もしくは IL-13 と CD40 リガンドである。アレルギー性鼻炎患者に対して抗原刺激を行うと，鼻粘膜における IL-4 の RNA 発現が有意に上昇することが以前より証明されている[20]。抗原刺激によって誘導される IL-4 の産生細胞は，約 70%が T 細胞であり残り約 30%が肥満細胞であった[21]。CD40 リガンドは，T 細胞も肥満細胞自身ももっており，*in vitro* の実験では IL-4 存在下でこれらの細胞で IgE を誘導できる。アレルギー性鼻炎患者の肥満細胞は抗原刺激によって有意に IL-4 や IL-13，CD40 リガンド，IgE リセプターの発現が上昇する[22]。鼻粘膜で産生された IgE は，さらに肥満細胞の IgE リセプター発現を亢進させ，さらなる肥満細胞の活性化と IgE 産生を誘導するドグマを形成するとの仮説である。実際，鼻粘膜に存在する IgE のほとんどは肥満細胞と結合しており[21]，肥満細胞は鼻粘膜での IgE クラススイッチに極めて重要だと思われる。実際の花粉飛散期に，花粉症患者の鼻腔洗浄液中サイトカイン量を蛋白レベルで測定すると，IL-4，IL-5，IL-10 が対照群に比較して有意に高値を示していた。しかし，かなりの症例で検出感度以下であった[23]。一方で，花粉飛散期に IgE 誘導に抑制的に働く IFN-γ の量が低下し，この Th1/Th2 のアンバランスが花粉アレルギーの病因であり，増悪因子であろうと推測している報告もある[24]。

V. 実地臨床での IgE

では，臨床的には IgE はどうであろうか。ア

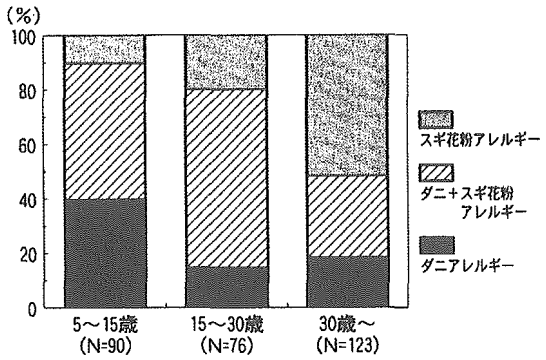

図 7　福井医科大学耳鼻咽喉科受診者によるダニとスギ花粉陽性者の年齢別割合

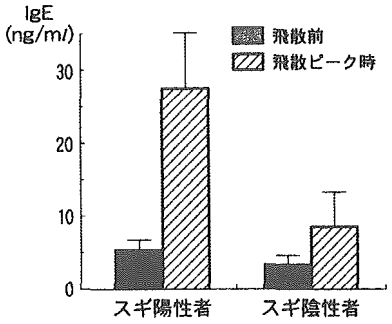

図 8　鼻腔洗浄液中の非特異的 IgE の変化
スギ陽性者においてはスギ飛散ピーク時に非特異的 IgE の有意な上昇を認める（$p<0.01$）。

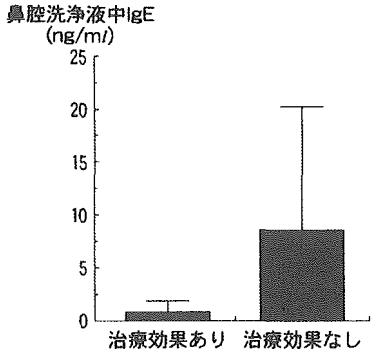

図 9　スギ飛散ピーク時における初期治療の効果による鼻腔洗浄液中の非特異的 IgE
治療効果がなかった群では，有意に鼻腔洗浄液中の非特異的 IgE が高値を示した（$p<0.05$）。

レルギー性鼻炎の診断には血清中抗原特異的 IgE が有効である。しかし，非特異的 IgE に関しては，あまり有効な報告はされていない。大きな集団になると，高 IgE 血症群と低 IgE 群では症状発現に有意差が認められる[25]。

われわれは，1994～1998 年の間に福井医科大学耳鼻咽喉科を受診し，アレルギーの精査を行った 361 名について検討を行った。特異的 IgE 陽性者は全体の 80%，289 名であった。特徴的であったことは，加齢により非特異的 IgE は低下し，ヤケヒョウダニに対する RAST スコアー値も有意に低下したことであった。一方，年齢が 30 歳を超えると花粉に対するアレルギー性鼻炎患者が増加した（図 7）。

次にスギ花粉飛散前後で鼻腔洗浄液中の非特異的 IgE を測定した。するとスギ花粉陽性者の鼻腔洗浄液中には，スギ花粉飛散ピーク時に有意に高い非特異的 IgE が存在し，スギ花粉陰性者には非特異的 IgE の上昇が認められなかった（図 8）。スギ花粉症の治療として，抗ヒスタミン剤や抗サイトカイン産生阻害剤などの初期治療を行うことが一般化している。そこでスギ花粉飛散ピーク時に鼻腔洗浄を行い，洗浄液を回収した。その結果，初期治療で飛散ピーク時の症状が十分に抑制された群では，スギ花粉飛散ピーク時の非特異的 IgE の上昇が認められず，効果の認められなかった群では有意に非特異的 IgE が上昇していた[26]（図 9）。同様の報告が，最近われわれ以外からもなされるようになり，鼻腔洗浄液の重要性が指摘されている[27]。これらのことは，鼻腔洗浄液中の非特異的 IgE が，薬物治療の客観的効果判定に使用できる可能性を示している。さらに，このスギ花粉飛散ピーク時の非特異的 IgE の上昇を抑制することを指標に，今後の治療法の改善，確立が期待される。スギ花粉飛散ピーク時の鼻腔洗浄液中非特異的 IgE の上昇がスギ特異的 IgE であれば最も理想的であるが，測定系の問題から何に対する IgE なのかわからないのが現状である。しかし筆者の個人的な意見では，ある程度の親和性さえもてば，とりわけスギ特異的な IgE の必要性はなく，大量に多くの IgE があれば，進入する多くの抗原に自然に結合・架橋しヒスタミンを放出するのではないかと推測する。今後の検討項目であろう。

VI. IgE 抑制への戦略

IgE 産生抑制は，IgE 誘導初期に IFN-γ を作用

させると起こる。同様にIL-12にもIgE産生抑制を認める。この現象から当初，外因性のIFN-γやIL-12を用いたアレルギー性鼻炎の治療が計画された。しかし臨床応用となると，IFN-γとIL-12ともにいろいろな問題点が存在した[28,29]。アレルギー性鼻炎患者に対し，リコンビナントIFN-γの全身的静脈内投与を施行したところ，特異的IgEおよび抗原特異的IgEの産生には全く影響を及ぼさず，抗アレルギー作用も認めなかった[28]。逆に，全身倦怠感や発熱の副作用をかなり高頻度に認めた[29]。IL-12に関しても，多くの臨床家が期待をよせたが，癌治療における臨床試験ではかなりの副作用が認められ，詳細な報告は未だなされていない[29]。唯一，この2つのサイトカイン臨床応用の可能性は，ネブライザーによる経鼻腔・経気道的投与によるアレルギー性鼻炎，気管支喘息などの治療である。マウスのアレルギーモデルでは，IFNγやIL-12のネブライザーによる経鼻腔・経気道的投与により，気道内でのアレルギー反応の低下や，好酸球の集積とIgE産生亢進の阻止などが報告されている[30,31]。

そこで考えたのが，内因性のIFNγを誘導し，IgE産生を抑制使用とする試みである。われわれは，結核菌由来DNA（MY-1）を使用した。このMY-1は，BCGの抗腫瘍効果を検討していた際にみつけられ，マウスの実験ではIFNγを代表とするIFNの産生を誘導する[32]。同様にヒトの末梢リンパ球（PBMC）においてもIFNγ産生亢進を認めている[33]。MY-1をヒトIgEを誘導する*in vitro*の系に添加して，IgE産生にいかなる影響を及ぼすか検討した。MY-1は，濃度依存的にIgE産生を有意に抑制した。MY-1自身はPBMCに対して細胞毒性を示さず，培養後の細胞生存率や細胞増殖率に影響を及ぼさなかった。MY-1の添加はPBMCのIFNγ産生を亢進させ，抗IFNγ抗体処理によって約60%のIgE産生抑制が解除された。抗IL-12抗体の添加は抗IFNγ抗体の添加と同様に，MY-1のIgE産生抑制を部分的に解除させた。以上のことから，①MY-1はIgE誘導を抑制する。②その機序として，MY-1によって産生亢進したIFNγが関与する。③IL-12もMY-1のよるIgE産生抑制機序の一端を担っていることが判明した[34]。

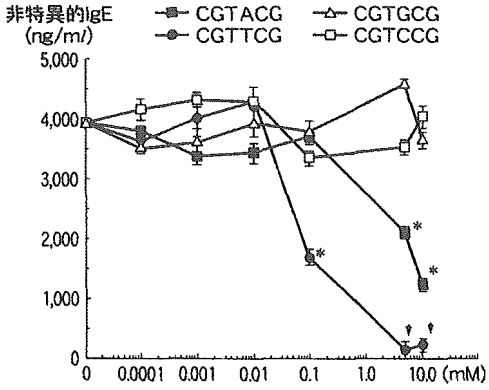

図 10　合成DNAの濃度依存的IgE産生抑制
（文献36を改変）

より高率にIgE産生を抑制する目的で，MY-1を細分化し最もIgE産生を抑制する塩基配列を求めることとした。幾つかの実験の結果，最もマウスの脾細胞において効果のあったものは，6塩基が3つの対になった構造（パリインドローム構造）を中心にもつ30塩基であった[35]。その後，パリインドローム構造の前半3塩基のバリエーション，すなわち計64（4×4×4）種類の30塩基でNK活性の増強を調べたところ，10種類にて著しくNK活性の増強が認められた[35]。これはヒトの末梢血を用いた実験でも同様であり，その際，IFNγ産生も平行に上昇していた。そこで，その10種類と増強しなかった1種類の計11種類でIgE産生を検討した。合成DNAによるIgE産生抑制は，個人差がかなり認められたが，その中でCGTACGを含む30塩基はほとんどの症例で抑制を認めていた。同症例のIFNγ産生を検討したが，やはりIgE産生抑制とほぼ平行であり，CGTACGを含む30塩基は高いIFNγ産生を示した[36]。そこで，パリインドローム構造が本当に重要であるのかどうか，4番目の塩基Aを他の塩基に変えてIgE産生を調べてみた。するとCGTTCGの塩基配列が最もIgE産生を抑制した（図10）。抗IFNγ抗体と抗IL-12抗体の添加によって，MY-1と同様に完全にIgE産生抑制を阻止できなかったが，約60～70%のIgE産生抑制を阻止した。すなわち，MY-1とこのCGTTCGの30塩基は同様の機序でIgE産生を抑制しているものと考えられ

た。さらに興味深いことに，CpG モチーフには種特異性があり，マウスに効果のある CpG モチーフではヒト B 細胞ではあまり反応しなかった。

VII. 遺伝子治療の可能性

われわれが計画しているアレルギー性鼻炎に対する遺伝子治療は，この CGTTCG の塩基配列を含んだ合成単鎖 DNA を用いた治療である。単純に，合成単鎖 DNA をアンチセンスに用いられるようにリン酸エステル化することによって，細胞内で安定化し鼻腔投与を行った場合，内因性の IFNγ を産生したり，抗原刺激に対してサイトカイン産生パターンを Th2 型より Th1 型にシフトさせることができる可能性がある。しかし，かなりの炎症反応がマウスの下気道にて報告されているので[37]，その予防がポイントであろうと思われる。われわれは，細胞内シグナルの幾つかをさらに過剰発現させたり，抑制することによってコントロールできないかと考えている。

さらに抗原特異性をもたせるために，抗原蛋白発現ベクターと合成単鎖 DNA を組み合わせて，遺伝子版減感作療法を行いたいと思っている。現在 *in vitro* の系を作製している。鼻構成初期培養細胞株に抗原蛋白発現ウイルスベクターを transfection し，合成単鎖 DNA の添加にて抗原特異的 T 細胞のサイトカイン産生がどのように変化するかを確認したいと思っている。そして B 細胞を添加し，ε germ-line transcript の定量を行い，IgE クラススイッチに影響を及ぼせるか検討したいと思っている。鼻腔粘膜局所での IgE 産生抑制という観点から全身的投与に比べて副作用軽減が可能であり，本モデルが完成すると臨床への可能性もかなり近づくと思われる。

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Analysis of Natural History of Japanese Cedar Pollinosis

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Key Words

Japanese cedar pollinosis · Japanese cedar pollen · Remission, spontaneous · Aging · Serum IgE antibody · Cross-sectional study · Vertical-sectional study

Abstract

Background: The marked increase in the incidence of Japanese cedar (*Cryptomeria japonica;* JC) pollinosis is a social problem in Japan. Elucidation of its natural history is, therefore, essential. ***Methods:*** Cross-sectional and vertical-sectional studies were performed regarding the effects of aging on sensitization to Japanese cedar pollen (JCP) and development of JC pollinosis by measuring serum IgE titers to JCP and by oral examination of residents of the Maruyama Town, Chiba, Japan from 1995 to 2001. We also studied the incidence of its spontaneous remission and the background factors. ***Results:*** In a vertical-sectional study, the serum IgE titer to JCP was strongly influenced by the amounts of pollen scattered. An increase in age by 6 years did not reduce the IgE titer to JCP in subjects in their 40s. However, in subjects aged 60 or more, annual differences in the JCP count did not affect serum IgE titer to JCP, which remained low even after a season with a high pollen count. In subjects with JC pollinosis aged over 40 showing a CAP RAST score of more than 2 to JCP in 1995, spontaneous remission of JC pollinosis was observed in 16.1% over a period of 6 years. Factors affecting spontaneous remission include sex, age at the time, serum IgE titer to JCP and age at first onset of JC pollinosis. ***Conclusions:*** The CAP RAST score was strongly associated with spontaneous remission in the multivariable model.

Introduction

Allergic diseases are increasing worldwide [1–5]. In particular, increases in Japanese cedar (JC) pollinosis are becoming a social problem in Japan. Accordingly, we conducted cross-sectional research on sensitization to Japanese cedar pollen (JCP) and prevalence of JC pollinosis among residents including children and adults in a town located on the Boso Peninsula of the Chiba Prefecture. Subjects were followed up over a period of 6 years from 1995 to 2001 to study the effect of aging on the serum IgE antibody titer to JCP, the development of JC pollinosis in those who had a positive serum IgE antibody titer to JCP, the incidence of spontaneous remission and the background factors. Because only 40 years have passed since the first report on JC pollinosis, there has been almost no report on the effect of aging on sensitization to JCP and the development of JC pollinosis. To take long-term measures against JC pollinosis, it is necessary to understand the natural history of the disease.

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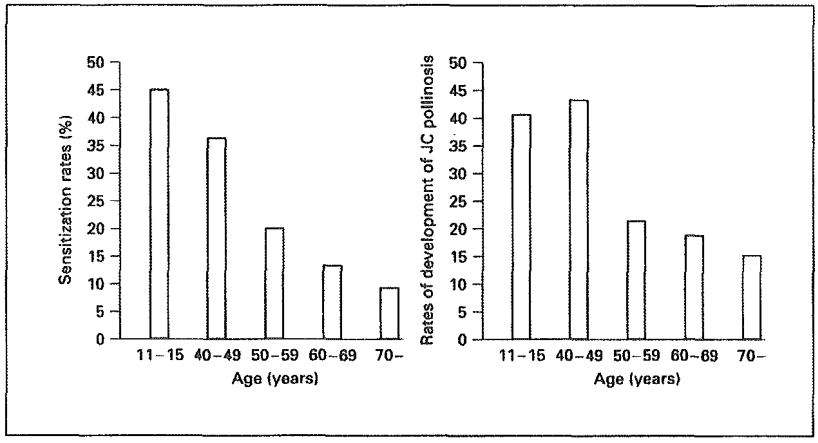

Fig. 1. Positive rates for serum IgE antibody to JCP (percentage of subjects with CAP RAST score of ≥2) and rates of development of JC pollinosis in subjects positive for the antibody (1995).

Subjects and Methods

As part of the medical examination of adult residents of the Maruyama Town, Awa-gun, Chiba Prefecture, located towards the southern tip of the Boso Peninsula, a questionnaire survey was conducted to determine whether they had developed JC pollinosis. This study was performed in June 1995. In this area, the JCP season is from early January to April. In 1995, the pollen count was high in this area. Among the local residents, 1,560 adults (647 men and 913 women) participated in the study; written informed consent was obtained after having adequately explained the objective of the study and its procedures. Serum IgE antibodies to JCP and to mites *(Dermatophagoides pteronyssinus)* were determined to investigate the sensitization rates of JC pollinosis and of mite allergic rhinitis to these antigens in all residents and any difference in the prevalence rate by age (cross-sectional research). Similar investigations were conducted with 292 children aged 11–15 years as part of the medical examination at elementary schools and junior high schools in the same district.

In June 1996, when the JCP count was low, medical examination, questionnaire survey and determinations of serum IgE antibody titer antibodies to JCP were conducted in the same 135 junior high school students who were examined in 1995, a year with a high pollen count.

In the same adult subjects who were examined in 1995, medical examination, questionnaire survey and measurement of serum IgE antibody titers to JCP and to mites were also conducted in 1999, 2000 and 2001 to investigate the changes in the above antibody titers as well as changes in rates of development of JC pollinosis and mite allergic rhinitis with aging (vertical-sectional research). Incidences of multiple sensitization to *Dactylis glomerata* and *Artemisia* were also examined in 2000.

Incidences of spontaneous remission and background factors of JC pollinosis were examined in subjects who had developed JC pollinosis symptoms in at least three successive seasons by 1995 and showed serum CAP (Pharmacia CAP System) radioallergosorbent test (RAST) score above 2 in 1995 and whose serum IgE antibody titers to JCP were measured more than once during the 3 years from 1999 to 2001. The specific antibody level (CAP RAST score) was classified as follows: score 6 = higher than 100 UA/ml; score 5 = 50.0–99.9 UA/ml; score 4 = 17.5–49.9 UA/ml; score 3 = 3.50–17.4 UA/ml; score 2 = 0.70–3.49 UA/ml; score 1 = 0.35–0.69 UA/ml; score 0 = less than 0.34 UA/ml. Generally a CAP RAST score of 2 or more is regarded as positive, a score of 0 is negative, and a score of 1 is equivocal.

A follow-up survey was conducted from 1995 to 2001 in subjects above the age of 40 who had a CAP RAST score to JCP of above 2 but did not develop JC pollinosis in 1995 during the period of the high JCP count to investigate the incidence and background factors of newly developing JC pollinosis after the age of 40 years in addition to the measurement of changes in serum IgE antibody titer to JCP.

Statistical Analysis

A significant difference was assessed by the Fisher exact test for categorical variables and the Mann-Whitney U test for continuous variables. Multiple logistic regression models were employed in order to analyze factors associated with remission and onset. Statistical analysis was performed with the JMP 5.0 (SAS Institute, Cary, N.C., USA).

Results

Changes in Serum IgE Antibody Titers to JCP and to Mites with Aging

Results of Cross-Sectional Research

Figure 1 shows positive rates for serum IgE antibody to JCP (CAP RAST score of ≥2) by age as well as rates of development of JC pollinosis by age in 292 elementary school and junior high school students aged 11–15 years measured after the JCP season in 1995. Sensitization rates reached 44.9% at this age. The rate of development of JC pollinosis in children with positive antibody to JCP reached 40.5%. Figure 1 indicates sensitization rates to JCP and rates of development of JC pollinosis compared

Table 1. Comparison of the number of children with JC pollinosis and serum IgE antibody titer to JCP in the same junior high school students between 1995 (high JCP count year) and 1996 (low JCP count year)

IgE antibody titer	Subjects	Mean serum IgE antibody titer to JCP, UA/ml		Number of children with JC pollinosis	
		1995	1996	1995	1996
1995 ≥ 1996	121	9.31	5.42	30	43
1995 < 1996	14	2.72	4.35	3	8
Total	135			33	51

with each age group covering 10 years in 1,560 residents over the age of 40. A sharp decrease in sensitization rates with age was observed in subjects over the age of 40. The rate of development of JC pollinosis in subjects positive for IgE antibodies to JCP decreased rapidly over the age of 50 years.

Results of Vertical-Sectional Research

Chronological changes with age in serum IgE antibody titer to JCP were investigated in 162 adult subjects who showed a CAP RAST score ≥2 to JCP in 1995 and whose serum IgE antibody titers to JCP were measured in 1999, 2000 and 2001. In the Chiba Prefecture, the JCP count was high in 1995, low in 1999, moderate in 2000 and high in 2001. In subjects in their 40s, serum IgE antibody titers to JCP fluctuated depending on the pollen count and there was no effect of aging on the serum antibody titer to JCP. In subjects in their 60s and 70s, no correlation was observed between fluctuations of serum IgE antibody titers to JCP and the pollen count, while a variation of the antibody titer in subjects in their 50s was intermediate compared to subjects in their 60s and 40s (fig. 2).

Because of the markedly decreased pollen count in 1996 compared to that in 1995, an increase of serum IgE antibody titer to JCP was observed in only 14 children and a decreased titer was seen in 121 of 135 junior high school students who were examined both in 1995, a year with a high pollen count and in 1996, a year with a low pollen count. Despite the above facts, JC pollinosis symptoms were observed in 1996 in all children except 1 of 33 who developed JC pollinosis symptoms in 1995, a year with a high pollen count. Moreover in 1996, 18 children newly developed JC pollinosis of 102 who did not develop the symptoms in 1995 (table 1).

Multiple Sensitization to Other Allergens in Subjects Positive for Serum IgE Antibody to JCP

In the investigation performed in 2000, children corresponding to 76.3, 59.3 and 16.9% of those who showed a CAP RAST score ≥2 to JCP were shown to have also been sensitized to mites, *D. glomerata* and *Artemisia*, with a CAP RAST score of ≥2 to each allergen. On the other hand, adult subjects aged over 40 years corresponding to 36.8, 54.7 and 23.6% of those who showed a CAP RAST score ≥2 to JCP were shown to have also been sensitized to mites, *D. glomerata* and *Artemisia* with a CAP RAST score of ≥2 to each allergen. Subjects who had been sensitized with 3 and 4 allergens including JCP were observed in 40.7 and 13.6% of children, respectively, and 25.7 and 10.4% of adults, respectively (fig. 3).

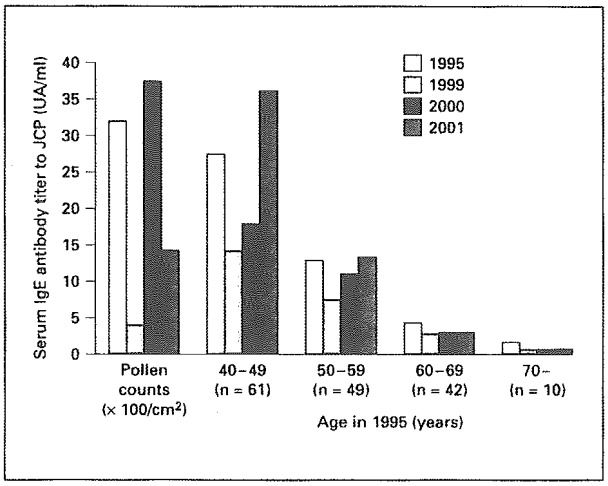

Fig. 2. Fluctuations of serum IgE antibody titer to JCP by age group over the 6 years from 1995 to 2001 in 162 subjects with a CAP RAST score to JC of ≥2 in 1995.

Spontaneous Remission of JC Pollinosis

Among 280 subjects aged over 40 years who showed a CAP RAST score ≥2 to JCP in the first examination performed in 1995, 56 subjects developed symptoms of JC pollinosis in more than three successive seasons including

Table 2. Background factors in subjects in whom spontaneous remission of JC pollinosis occurred compared with those who continue to have the disease

	Subjects with spontaneous remission (n = 9)	Subjects without spontaneous remission (n = 47)	p
Age (in 1995), years	61.1	48.7	0.002
Males	7/9	13/47	0.007
Age at the onset, years	50.8	40.2	0.027
Serum IgE antibody titer to JCP in 1995, UA/ml	4.7	40.7	<0.001
Complications by other allergic diseases	5/9	16/47	0.272
Predisposition	0/9	7/47	0.583

There were 280 subjects over the age of 40 in 1995 with a serum CAP RAST score to JCP of ≥2. Of these, 9 were asymptomatic in the last 3 successive pollen seasons (including 2001).

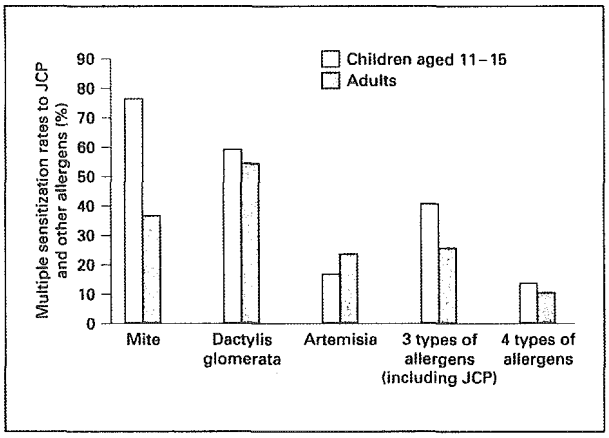

Fig. 3. Multiple sensitization rates (CAP RAST score of ≥2) in adult residents (n = 144/1,451) and children (n = 59/158) positive for JC antibody in 2000.

1995, and the serum IgE antibody titers to JCP were measured more than twice by 2001 in these 56 subjects. In 9 subjects among them, no JC pollinosis symptoms were observed during the last three seasons or more including 2001, a year with a high pollen count. These subjects were regarded as cases of spontaneous remission and examined regarding their background factors including age and serum IgE antibody titer to JCP at first examination, age at onset of JC pollinosis, age at disappearance of JC pollinosis symptoms, complications by other allergic diseases including perennial allergic rhinitis and their correlation with the incidence of predisposition (history of JC pollinosis in their parents, brothers and sisters). Serum IgE antibody titers to JCP in 1995 were 4.7 UA/ml in the spontaneous remission group and 40.7 UA/ml in the non-remission group. The comparison of their background factors with those of 47 subjects who continued to show JC pollinosis symptoms successively over the same periods of time demonstrated significant differences in sex, age at onset of JC pollinosis, age at first examination, and serum IgE antibody titer to JCP (table 2). In addition, the CAP RAST score was strongly associated with spontaneous remission in the multivariable model [odds ratio (OR) 48.99; 95% confidence interval (CI) 4.17–2,204.96]. In multivariate analysis, there was no significant effect on sex, age (in 1995) and age at the onset (table 3).

Onset of JC Pollinosis in Middle-Aged and Elderly Persons

Showing a CAP RAST score ≥2 to JCP in 1995, a year of an abundance of scattered JCP, 182 subjects over the age of 40 did not develop JC pollinosis. Among these subjects, serum IgE antibody to JCP was measured in a total of 151 subjects over the years 1999, 2000 and 2001, and 19 of these subjects developed JC pollinosis in the 6 years since 1995. Comparison of age, sex, complication, predisposition and serum IgE antibody titer to JCP of these subjects with those of subjects who did not develop JC pollinosis in these periods of time revealed that the mean age of the former is 50.7 years and that of the latter is 58.7 years, with the former age being significantly lower (table 4). In a multivariate analysis, age was associated with a lower risk of onset (OR 0.49 per 10 years; 95% CI 0.26–0.86). Predisposition was related to a higher risk (OR 5.06; 95% CI 0.94–22.80) (table 5). Since the number of

Table 3. Factors of spontaneous remission of JC pollinosis

Factor	OR	95% CI	p
Age (in 1995; per 10 years)	1.31	0.36–5.50	0.681
Male	9.49	0.90–286.80	0.097
Age at the onset (<40/≥40)	0.26	0.01–8.89	0.469
CAP RAST score (2/>2)	48.99	4.17–2,204.96	0.009
Complications by other allergic diseases	0.31	0.01–4.17	0.384

Table 4. Background factors in subjects who newly developed JC pollinosis after reaching 40 years of age compared with subjects positive for serum IgE to JCP who had not developed the disease

	Subjects who developed the disease (n = 19)	Subjects who did not develop the disease (n = 163)	p
Age (in 1995)	50.7	58.7	0.003
Males/females	10/19	99/163	0.622
Serum IgE antibody titer to JCP in 1995, UA/ml	24.5	4.8	0.118
Complications by other allergic diseases	5/19	9/163	0.008
Predisposition	3/19	7/163	0.073

There were 280 subjects over the age of 40 in 1995 with a CAP RAST score to JCP of ≥2. A total of 182 subjects had not developed symptoms of the disease by 1995. Of these, 19 subjects developed JC pollinosis in 1996 or thereafter.

Table 5. Factors of onset of JC pollinosis

Factor	OR	95% CI	p
Age (in 1995; per 10 years)	0.49	0.26–0.86	0.019
Male	1.03	0.33–2.95	0.963
CAP RAST score (2/>2)	0.55	0.18–1.73	0.296
Complications by other allergic diseases	4.08	0.91–17.09	0.057
Predisposition	5.06	0.94–22.80	0.040

subjects who newly developed the disease was larger than that of those in whom spontaneous remission occurred in their 40s, the prevalence rate of JC pollinosis in subjects positive for antibody to JCP increased with age. On the other hand, in subjects in their 60s or older, the incidence of subjects developing the disease newly decreased and the number of subjects in whom spontaneous remission occurred increased. Accordingly, a prevalence rate of JC pollinosis in subjects over the age of 60 positive for serum IgE antibody to JCP in 1995 decreased with age or remained low at the same level. The variation of the prevalence rate of JC pollinosis in patients in their 50s was intermediate in its pattern compared with that in patients in their 60s and 40s (fig. 4).

Discussion

In childhood, the sensitization rate to JCP and the prevalence rate of JC pollinosis increased with age, and subjects who developed the symptoms of JC pollinosis once continued to have it in the next pollen season even

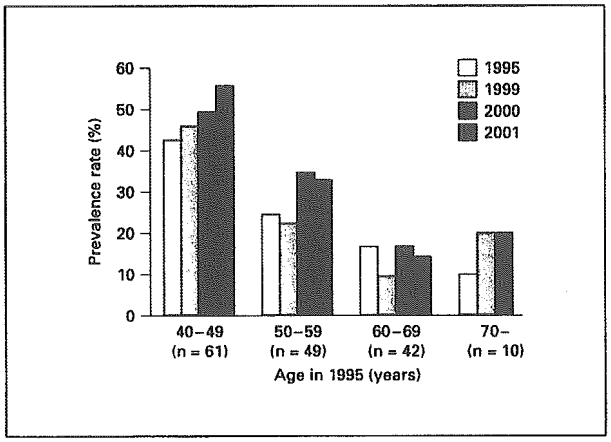

Fig. 4. Fluctuations of prevalence rates of JC pollinosis over a 6-year period in subjects with a serum CAP RAST score to JC of ≥2 by age group.

when the amounts of scattered JCP were remarkably reduced. Spontaneous remission hardly occurred at this age. In vertical-sectional research where the same adult subjects were followed up for 6 years, serum IgE antibody titer to mites significantly decreased with age in subjects over the age of 40 who were positive for serum IgE antibody to mites but this was not accompanied by double sensitization to JCP. It is considered that the potential of IgE production in B lymphocytes to mites antigen decreases with age from the age of 40 onward [6–9]. However in subjects with JC pollinosis serum IgE antibody titers to JCP did not decrease over a 6-year period in subjects in their 40s, and the IgE antibody titers to JCP are strongly influenced by the amount of pollens scattered. The difference in the variation of antibody titers to different antigens might have arisen from the difference in the amounts of antigen exposed and in the degree of their antigenic potency between patients with JC pollinosis and those with perennial allergic rhinitis due to mites. After the age of 60 years, differences in the amounts of JCP scattered did not affect the IgE antibody titer to JCP, while at the age of 50, the variation of the titer was intermediate in its pattern compared to those at the age of 60 and 40. The above data are in agreement with the fact that spontaneous remission of JC pollinosis was observed in subjects from the age of their late 40s and at the highest incidence in those in their 60s.

There have been very few reports on spontaneous remission of pollinosis, while there are large differences in the spontaneous remission rates given by different authors. From the results of a follow-up study in 738 university students with pollinosis conducted 23 years after graduation from the university, Greisner et al. [10] reported that the symptoms of pollinosis disappeared in 22.9% of the subjects, while they remained but were improved in 32%, remained unchanged in 33.3% and were exacerbated in 9.2%. For evaluating the results, however, changes in the environment as regards exposure to pollens due to changing their place of residence after graduation should be taken into consideration. Broder et al. [11] reported that spontaneous remission of pollinosis was observed in 5% of females and 10% of males during a 5-year follow-up period in examinations of 6,563 residents with a high incidence of spontaneous remission in subjects aged 45 years or more. On the other hand, Danielsson and Jessen [12] reported after their 12-year follow-up of 82 subjects that a disappearance of JC pollinosis symptoms was observed only in 1.2% (1/82), while the symptoms were improved, remained unchanged and worsened in 39, 39 and 21% of subjects, respectively. According to Smith [13], 10 (8.9%) of 112 children became asymptomatic 5 years later. Since there are significant differences in the incidence of spontaneous remission of allergic rhinitis for all types of antigen, the amount of antigen to which the subjects are exposed, age distribution, severity of the disease and the length of the follow-up period, and a consolidation of the definition itself of the term of spontaneous remission are considered necessary.

When the subjects studied were limited to those whose ages at first examination were over 40 and whose CAP RAST scores to JCP were more than 2, spontaneous remission was observed in 16.1% of subjects in the 6-year follow-up period. Although spontaneous remission rates of JC pollinosis are significantly higher in subjects over the age of 60, the overall rate in subjects including children was several percent lower than reported until now. Cases in which spontaneous remission cannot be expected will likely increase, as children with JC pollinosis and patients with a predisposition to JC pollinosis increase.

Since there might be large differences in environmental factors from childhood to the age of onset of pollinosis, including diet, residence and many other factors between patients with JC pollinosis aged 10–20 at present and those aged 60 or more, it is difficult to predict and compare future incidences of spontaneous remission in these two groups in a similar straight line. Further long-term vertical-sectional follow-up research is required to understand the natural history of JC pollinosis.

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Original Article

Effect of ramatroban, a thromboxane A_2 antagonist, in the treatment of perennial allergic rhinitis

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ABSTRACT

Background: It is known that the symptoms of allergic rhinitis can significantly reduce the quality of life of the patient. One of such typical symptoms of allergic rhinitis is nasal obstruction. Nasal obstruction is currently thought to be closely related to the presence and abundance of lipid mediators, such as leukotriene and thromboxane (TX) A_2. The novel drug ramatroban, a TXA_2 receptor antagonist, which has been developed by Bayer Yakuhin Ltd. (Osaka, Japan), has been demonstrated, in clinical trials, to improve nasal obstruction in the treatment of patients with allergic rhinitis and it has recently become commercially available.

Methods: In the present study, ramatroban was administered for 28 days to 10 patients who were diagnosed with perennial allergic rhinitis but were untreated. Changes in self-reported symptom scores and *in vivo* allergic reaction parameters were assessed during three observational periods.

Results: From baseline scores, all three symptom scores after 28 days treatment with ramatroban declined clearly in all patients, except for one patient who suffered a cold during the study period and had aggravated rhinorrhea and nasal obstruction as a result. The concentrations of histamine and TXB_2 (a metabolite of TXA_2) in the nasal fluid induced by antigen challenge after the 28 day treatment period also decreased in most subjects compared with concentrations during the pretreatment period. The symptom scores for nasal obstruction during the pretreatment period were correlated with the concentration of TXB_2 in antigen-induced nasal fluid.

Conclusions: The present study reconfirmed the clinical efficacy of a post-marketed drug, namely ramatroban, in the treatment of allergic rhinitis. In addition, the results suggest that ramatroban suppressed the secretion of chemical mediators in nasal that are thought to be involved in the allergic reaction in patients with perennial allergic rhinitis.

Key words: allergic, histamine, nasal obstruction, perennial, ramatroban, rhinitis, thromboxane A_2 receptor.

INTRODUCTION

The first step in an allergic reaction is the release of histamine and various lipid mediators derived from arachidonic acid via antigen-stimulated mast cells in mucous membranes. Although histamine is predominantly responsible for sneezing and nasal discharge, various substances, such as lipid mediators, are involved in nasal obstruction in a complex manner.[1] The reaction site is considered to be blood vessels in the nasal mucosa.[2]

One study[3] that investigated the mediators involved in the development of nasal obstruction reported that when various mediators were sprinkled on the nasal mucosa, the resistance of the nasal cavity started to increase. The major mediators that were identified in the study as contributors to nasal obstruction included histamine, leukotriene, platelet-activating factor and prostaglandin D_2. In other studies,[4,5] it was reported that thromboxane (TX) A_2 also played an important role in the development process of nasal obstruction. It is also known that lipid mediators are released not only from mast cells, but also from eosinophils and that they are involved in the late-phase inflammatory reaction.[1]

Ramatroban is a selective TXA_2 receptor antagonist that was developed by Bayer AG (Leverkusen, Germany)

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and is now licensed for use in the treatment of allergic rhinitis by Bayer Yakuhin Ltd (Osaka, Japan) in Japan. Pharmacologically, ramatroban suppresses the promotion of vascular permeability and infiltration of eosinophils and its efficacy has been proven clinically for treating patients with allergic rhinitis.[6]

METHODS

The clinical study was conducted at Sekino Clinical Pharmacology Institute (CPI). Prior to the study, the study protocol was reviewed and approved by the institutional review board at Sekino CPI.

Subjects

Ten patients were enrolled into the study. These patients were diagnosed with perennial allergic rhinitis but were untreated at diagnosis. All patients gave written informed consent to participate in the study. The diagnosis of perennial allergic rhinitis was confirmed on the basis of a positive history of typical symptoms of allergic rhinitis, +2 RAST scores and a positive allergic response to house dust mite in any of an anterior rhinoscope examination, nasal eosinopilia test, skin test or nasal provocation test.

Methodology

The study consisted of three observational periods: (i) a period of 7 days before initiating medication (pretreatment period: days 1–7); (ii) a period of 21 days with study medication (treatment period: days 8–28); and (iii) a period after completing medication (post-treatment period: days 29–32). During the treatment period, ramatroban

Table 1 Evaluation criteria for determination of the symptom score

	Score (points)				
	4	3	2	1	0
Sneezing attacks*	≥ 21	20–11	10–6	5–1	0
Nasal discharge†	≥ 21	20–11	10–6	5–1	0
Nasal obstruction	Complete nasal obstruction all day	Nasal obstruction severe and breathing through the mouth performed for a considerable time	Nasal obstruction marked and breathing thorough the mouth performed several times a day	No breathing through the mouth, but nasal obstruction present	No nasal obstruction

*Mean no. attacks per day.

†Mean no. nose blows per day.

Table 2 Symptom score

	Sneezing attack or secretion score				
	4	3	2	1	0
Nasal obstruction score					
4	4	4	4	4	4
3	4	3	3	3	3
2	4	3	2	2	2
1	4	3	2	1	1
0	4	3	2	1	0

Table 3 Evaluation criteria for the reactivity of nasal antigen challenge test

	Reactivity			
	+++	++	+	–
No. symptoms	3	3	2	0
No. sneezing episodes	≥ 6	≤ 5	Not counted	

Reactivity is evaluated based on the number of symptoms (sneezing, nasal discharge and nasal obstruction) and the number of sneezing episodes.

In determining the number of sneezing episodes, a single sneeze in 5 min was denoted as one episode in the challenge test.

(75 mg tablet, p.o.) was administered twice daily, once after breakfast and once in the evening. No concomitant drugs were allowed during the study period.

Evaluations

By using a patient diary, the severity of nasal symptoms, such as sneezing, rhinorrhea and nasal obstruction, was scored by patients each day during the study period in accordance with the Severity Classification of the Clinical Practice Guideline for Nasal Allergy and the Symptom Score (Table 1).[7] The average scores for the three evaluation periods (days 1–7, 22–28 (the last 7 days of the treatment period) and 29–32) were used for evaluation analyses as representations of the symptom severity during the pretreatment, treatment and post-treatment periods, respectively. Changes in average scores for individual patients during the three evaluation periods were determined.

In addition to the patient diary for daily symptom scores, nasal antigen challenge tests were performed using house dust mite-extracted paper discs (dry weight 250 μg/mL). The tests were conducted with all subjects at the beginning of the pretreatment period (day 1) and at the end of the post-treatment period (day 32) to assess the effect of ramatroban on nasal reactions. Assessments were performed by comparing the two test results in terms of reactivity (Table 2), the volume of nasal fluid, nasal cavity resistance (Table 3) and the concentration levels of histamine and TXB_2 in the nasal fluid.

Five minutes after challenge, reactivity was assessed, nasal fluid was collected directly from both nostrils by suction and the resistance of the nasal cavity was measured with a rhinomanometer (Rhinorrheograph MPR-3100; Nihon Kohden, Tokyo, Japan).

The nasal fluid sampled was mixed with dithiothreitol (500 μL of 0.05% dithiothreitol in 0.01 mol/L phosphate-buffered saline) in order to resolve mucin and samples were centrifuged at 400 g for 10 min. Then, the cell-free supernatant was extracted and stored at –20°C for future analyses of histamine and TXB_2.

Histamine and TXB_2 assays[8,9]

Histamine was quantified using radioimmunoassay by competitively binding acylated histamine and [^{125}I]-labeled acylated histamine to stabilized antihistamine mouse monoclonal antibody.

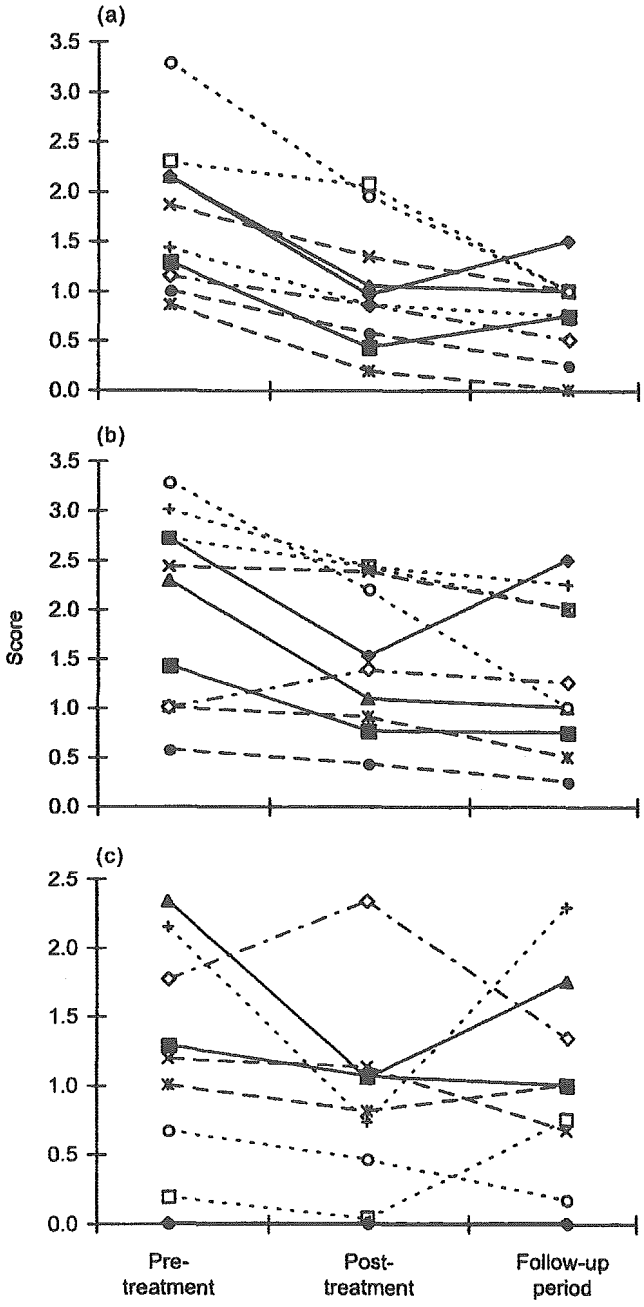

Fig. 1 Average symptom scores during the three observational periods for each individual patient for (a) sneezing, (b) rhinorrhea and (c) nasal obstruction. The three observational periods during the study consisted of: (i) a period of 7 days before initiating medication (pretreatment period: days 1–7); (ii) a period of 21 days with the study medication (treatment period: days 8–28); and (iii) a period after completing medication (post-treatment period: days 29–32).

The prostanoid in the samples was bound to an octadesilsilsyl silica carrier under acidic conditions and protein and lipids were removed.[10,11] Prostanoid was