

〔原著〕

成人喘息受診中断例の予後の検討

—無治療無症状継続群と有症状群との比較検討—

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【背景】成人喘息の予後に関する報告は少なく、特に長期寛解が可能かどうかについては明確な解答は得られていない。

【方法】当院アレルギー科外来で平成2~4年まで気管支喘息として受診していたが、以降、受診していない患者1168名に対しアンケートを実施した。

【結果】行き先不明での戻り分370件(31.7%)を除いた798名中430名から回答を得た(回収率53.9%)。430名中、86名(20.0%)が過去3年以上全く喘息の治療を必要とせず無症状であると回答した(寛解群：無治療無症状継続群)。

【考察】このことから、成人喘息においても長期的に無治療で無症状である寛解予備群が存在することが示唆された。寛解予備群導入への予測因子としては初診時重症度が軽症であること、発症より早期に受診していること、初診時呼吸機能が良好、特にアセチルコリン気道過敏性が軽度である、などが考えられた。

Key words : adult asthma — outgrow — prognosis — remission

緒言

小児喘息と成人喘息では自然歴が大きく異なり、小児喘息の約70%程度は思春期から20歳に至る間に、無治療でも症状が認められなくなり、いわゆるアウトグロウ(治癒/寛解)と呼ばれる状態になる。一方残りの約30%程度は成人喘息に移行すると考えられている。小児喘息ガイドラインではアウトグロウには臨床的治癒、機能的治癒、寛解(緩解)があり、患者の治療、管理を行った後、最低2~3年以上経過観察した時点で、その患者の症状の変化によって以下の判定が可能であると記されている¹⁾。臨床的治癒とは無治療で無症状状態が5年以上継続することを指し、寛解(緩

解)は無症状になってからの年数で表す。機能的治癒は呼吸機能検査、気道過敏性試験が健常人と同等に回復している状態をいう。アウトグロウした症例の多くは長期的にみても無治療で無症状の状態を維持できているという報告は多い²⁾³⁾。また、一度アウトグロウという寛解状態にあったものが成人で再発し治療が必要になる症例も存在することが知られている⁴⁾。このことは小児喘息におけるアウトグロウには真の意味の喘息治癒状態から、気道過敏性や気道炎症が存続しているにも拘わらず、見かけ上、無症状状態が続いている臨床的治癒(機能的治癒ではない)が存在することを示している。一方、成人喘息は小児と比較して寛解する率は低く、再発率も高い。小児のような自然治癒傾向も時にはみられるが、その頻度は低く治癒しないと報告するものが多い。成人喘息はその背景が多彩であり、年齢層も幅広く、さまざまなファクターが関係することから予後に関する疫学調査の報告は少ない。そのため、治癒とはいかないまでも長期寛解が可能

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Table 1 A contents of a questionnaire

[1] 薬（経口または吸入）を
(1) 全く服用（常用、頓服ともに）せず、
①過去5年以上発作がない
②過去3-5年以上発作がない
③過去1-3年以上発作がない
④過去1年以内に発作がある
(2) 服用（常用、頓服ともに）して、
①過去5年以上発作がない
②過去3-5年以上発作がない
③過去1-3年以上発作がない
④過去1年以内に発作がある
(3) その他
①死亡（内喘息死）
②不明
[2] 減感作療法、変調療法を
①している
②していない
③不明
[3] 現在定期的に病（医）院を
①受診している
②受診していない
③不明
[4] 当科を受診しなくなった理由
①発作がなくなったため
②他院受診中のため
③その他

か否かについても明確な解答は得られておらずその実態は不明である。

過去に我々は当院を受診していた成人喘息患者で、ある時期（昭和63年～平成2年）から受診しなくなった患者のその後の喘息状態を知ることを目的として当該患者を対象としてアンケート調査を実施した⁵⁾。その結果、自己申告に基づく結果であるが3年以上無治療、無症状の状態にある患者が少なからず存在し、その背景因子は臨床症状が軽症、気道過敏性が軽度であることが判明した。その結果を踏まえて、今回は平成2～4年において前回と同一の調査を行い、再現性を確認するとともに無治療無症状群と有症状群とについて比較し、通院中の臨床症状や臨床所見から成人喘息の予後の予知因子が推定できるかどうかについて検討した。

対象と方法

当院アレルギー科外来に平成2～4年まで気管支喘

息として受診していたが、以後受診していない患者1168名に対して、平成10年8月封書でアンケートを依頼しはがきで回答を得た。アンケート内容は(1)くすり（経口または吸入）を全く服用せずに、①過去5年以上発作がない、②過去3～5年間以上発作がない、③過去1～3年間発作がない、④過去1年以内に発作がある。(2)くすり（経口または吸入）を服用して上記同様①②③④を質問した(Table 1)。くすり（経口または吸入）を全く服用せず過去3年以上発作がない群((1)の①+②)を寛解（無治療無症状）群、くすりの服用の有無にかかわらず過去1年以内に発作がある群((1)の④)を非寛解（有症状）群と定義した。寛解の定義については前述の小児喘息のガイドラインにおける小児気管支喘息予後（転帰）判断基準すなわち「その患者の症状ならびに長期管理薬治療ステップの変化によって判定が可能である」を参考にした。背景調査はカルテ調査により、外来初診時の問診より発症年齢、家族歴を聴取し、発作状況、治療状況、検査データより重症度を判断した。重症度は日本アレルギー学会重症度分類に準拠した⁶⁾。末梢血より好酸球数、血清総IgE値を測定した。また喀痰（自然痰）より喀痰中の好酸球数を測定し、Hanselの基準に基づいて半定量した。スパイログラムではVC、FEV1などを測定した。また約35種類のアレルゲンを用いた皮内反応を行い、一つ以上の抗原が陽性である症例をアトピー型と定義した。ただし血清総IgE値低値かつカンジダのみ陽性症例は非アトピー型と定義した。気道過敏性検査はアセチルコリンを用いた標準法で行った。以上の項目について種々の背景因子を比較検討した。

結果

行き先不明での戻り分370件(31.7%)を除いた798名中430名から回答を得た(回収率53.9%)。アンケートの結果内容をTable 2に示す。これらより、430名中、86名(20.0%)が過去3年以上全く喘息の治療を必要とせず喘息症状が出ていない寛解群（無治療無症状群）であった。一方、122名(28.4%)は治療薬を必要、不必要に関わらず、1年以内に発作を認めた非寛解群（症状継続群）であった。発症年齢は寛解群平均29.8歳、非寛解群平均28.4歳と有意差を認めなかった(Fig. 1(A))。また分布をヒストグラムで見ると非寛解群では10歳未満発症、60歳以上発症が多かった(Fig. 1

Table 2 The result of a questionnaire

[1] 薬（経口または吸入）を		
(1) 全く服用（常用，頓服ともに）せず，	117 [114]	(27.2 [26.5] %)
①過去5年以上発作がない	70 [67]	(16.3 [15.6] %)
②過去3-5年以上発作がない	19 [19]	(4.4 [4.4] %)
③過去1-3年以上発作がない	13 [13]	(3.0 [3.0] %)
④過去1年以内に発作がある	15 [15]	(3.5 [3.5] %)
([] : 減感作療法，変調療法なし)		
(2) 服用（常用，頓服ともに）して，	246	(57.5%)
①過去5年以上発作がない	70	(16.3%)
②過去3-5年以上発作がない	24	(5.6%)
③過去1-3年以上発作がない	45	(10.5%)
④過去1年以内発作がある	107	(24.9%)
(3) その他	67	(15.6%)
①死亡（内喘息死）	42 [2]	(9.8 [0.5] %)
②不明	25	
[2] 減感作療法，変調療法を		
①している	23	(5.3%)
②していない	315	(73.3%)
③不明	92	(21.4%)
[3] 現在定期的に病（医）院を		
①受診している	191	(44.4%)
②受診していない	185	(43.0%)
③不明	54	(12.6%)
[4] 当科を受診しなくなった理由		
①発作がなくなったため	152	(35.3%)
②他院受診中のため	77	(17.9%)
③その他	142	(33.0%)

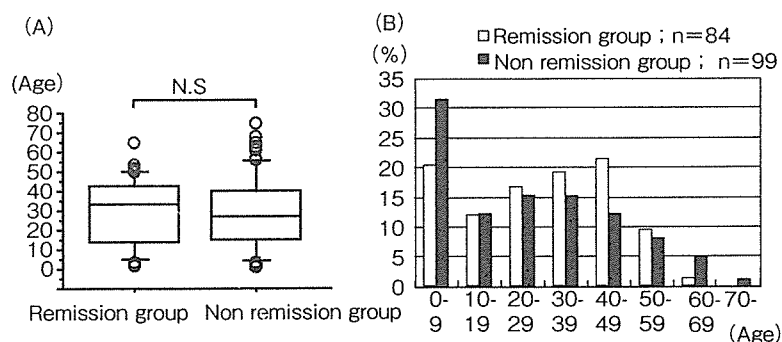


Fig. 1. (A) The comparison of age of onset in remission group and nonremission group. (B) The distribution of age of onset in adults asthmatics.

(B)). さらに発症年齢から小児期から寛解のない小児発症，成人発症，成人再発の3つに分類すると，成人発症は寛解群 65.5%，非寛解群 45.5% であり寛解群が多かった。非寛解群では小児発症および成人再発と合わせると 54.5% と半数以上を占めている。このことから非寛解群では小児喘息の既往を有する症例が多いこ

とが明らかである。発症から初診までの期間は寛解群平均 7.4 年，非寛解群平均 8.0 年と寛解群で早期受診していた ($p=0.021$, Fig. 2 (A)). しかも，ヒストグラムでは寛解群の半数近くは 1 年以内に受診していた (Fig. 2(B)). 通院期間では寛解群平均 11.4 年，非寛解群平均 11.9 年とほぼ同じであった (Fig. 3 (A)). ヒス

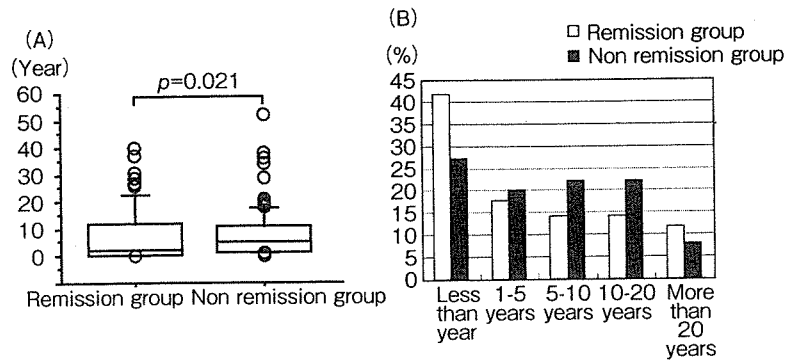


Fig. 2. (A) The comparison of the period from onset of asthma to the first hospital visit in remission group and non remission group. (B) The distribution of the period from onset of asthma to the first hospital visit.

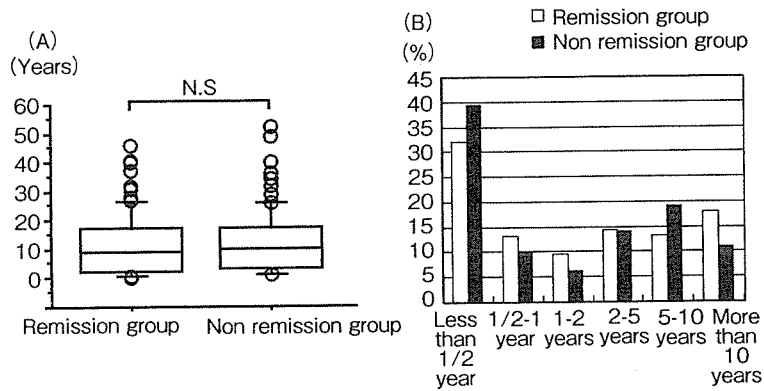


Fig. 3. (A) The comparison of the period under the hospital care in remission group and non remission group. (B) The distribution of a period under the hospital care.

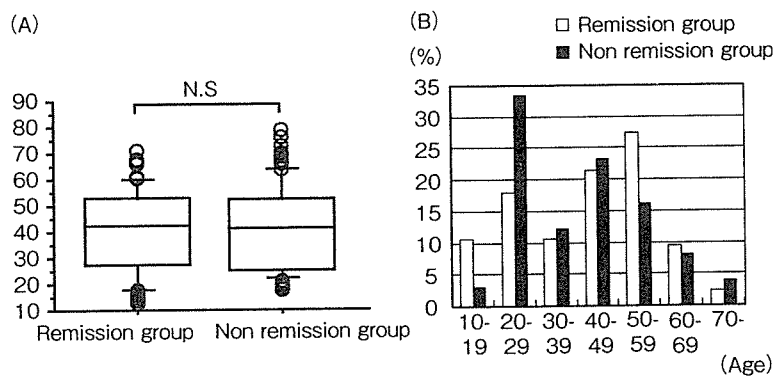


Fig. 4. (A) The comparison of the age of the last hospital visit in remission group and non remission group. (B) The distribution of age of the last hospital visit.

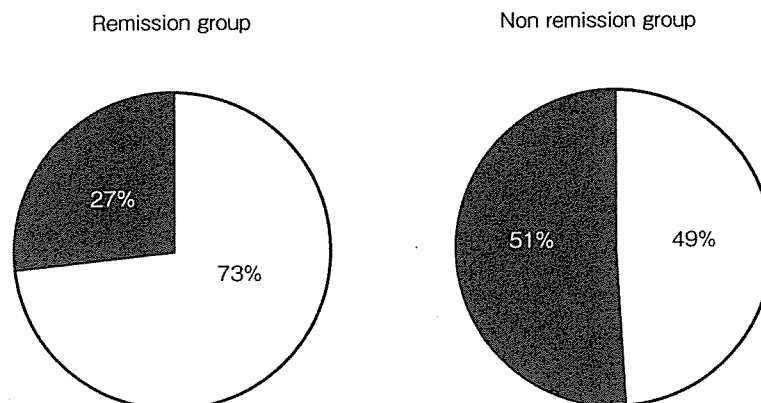


Fig. 5. The comparison of family history of atopic disease in remission group and non remission group. □ Positive family history ■ Negative family history

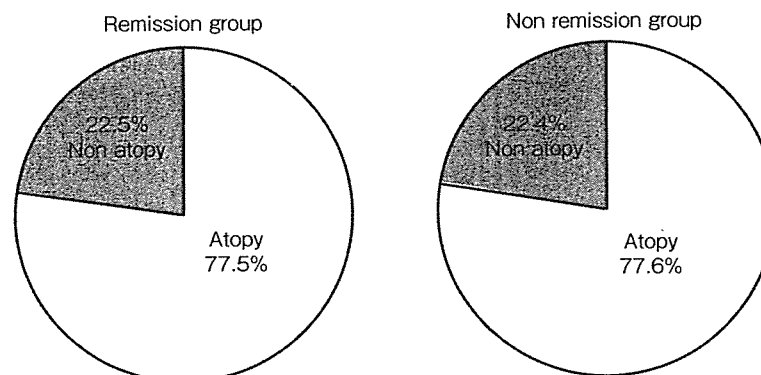


Fig. 6. The type of bronchial asthma. The comparison between remission group and non remission group.

トグラムでは寛解群、非寛解群ともに差はなく、寛解群、非寛解群ともに半年以内の短期間の通院症例が多かった (Fig. 3 (B)). 最終受診年齢は寛解群平均 41.1 歳、非寛解群 40.3 歳と差を認めず (Fig. 4 (A)), ヒストグラムでは非寛解群では 20 歳代が多く、寛解群では 50 歳代が多かった (統計学的有意差はない.) (Fig. 4 (B)). 20 歳代での非寛解群が多いのは主に転勤やそれに伴う転居、また学生の就職などであり、その中には転医も含まれている。また家族歴では寛解群では家族歴ありとする症例は 73% と多い ($p < 0.01$) が、非寛解群では家族歴には差は認めなかった (Fig. 5). 病型に関する検討では皮内反応の結果によりアトピー型、非アトピー型に分類し寛解群、非寛解群を比較した。両群ともに 78% がアトピー型であり有意差は認めなかつ

た (Fig. 6). 平成 7 年度の国病国療調査⁴⁾における一般頻度ではアトピー型 30.2%, 混合型 30.1%, 感染型 37.1% であり、当院ではアトピー型が若干多い傾向があると考えられる。初診時重症度では寛解群は軽症が 89.3% と圧倒的に多かったが重症は 0% であった ($p < 0.01$) (Fig. 7). ここでの重症度判定は日本アレルギー学会に基づくものであるが喘鳴、呼吸困難を症状の程度に用いている。咳が主体の喘息、いわゆる咳喘息の頻度はこの分類上明らかではないが、実際に存在していた可能性はある。一方、非寛解群は軽症が 52.6% と半数以上存在するものの、重症が 14.1% と寛解群と比較して多かった ($p < 0.01$). また一般喘息の重症度は軽症 43.5%, 中等症 37.8%, 重症 10.2% であり寛解群、非寛解群ともに軽症が多い傾向が認められた。初診時

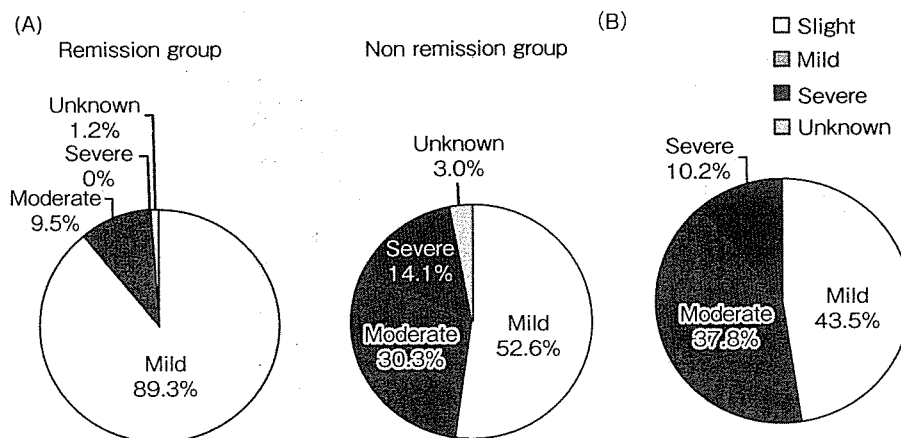


Fig. 7. (A) The severity of bronchial asthma on the first hospital visit. (B) The distribution of the severity of bronchial asthma obtained from the survey by the joint research group of national hospital and sanatorium.

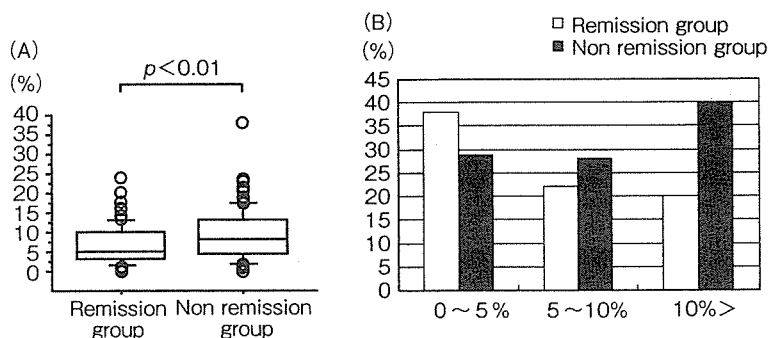


Fig. 8. (A) The comparison of the ratio of eosinophils in whole blood on the first visit in remission group and non remission group. (B) The distribution of the ratio of eosinophils in whole blood.

の末梢血好酸球数は寛解群 6.5%, 非寛解群 9.4% と非寛解群で有意に多かった ($p < 0.01$) (Fig. 8 (A)). またヒストグラムでは非寛解群では 10% 以上の好酸球数を呈した症例が約 40% であった (Fig. 8 (B)). 初診時の血清中総 IgE 値は寛解群, 非寛解群ともに有意差はないが (Fig. 9 (A)), ヒストグラムで検討すると IgE 値 ≥ 1000 IU/ml を越えるアトピー素因の強い症例は非寛解群で多かった (Fig. 9 (B)). 初診時の喀痰 (自然痰) 中の好酸球数は Hansel の基準を用いて半定量したが, 寛解群, 非寛解群ともに Score 4 と中等度以上の好酸球数を認めた症例が多かった (Fig. 10). 初診時の FEV1% は寛解群 73.2%, 非寛解群 66.9% と寛解群で有意に高値であった ($p < 0.01$, Fig. 11 (A)). またヒ

ストグラムでは寛解群は FEV1% $\geq 70\%$ が 60% 以上存在するのに対し, 非寛解群では FEV1% $\leq 50\%$ の低肺機能症例が 10% 以上存在していた (Fig. 11 (B)). 初診時アセチルコリン気道過敏性 (AchPC₂₀) では寛解群では AchPC₂₀ = 3565 $\mu\text{g}/\text{mL}$, 非寛解群では 1455 $\mu\text{g}/\text{mL}$ であり寛解群では有意に気道過敏性が軽度であった ($p < 0.01$, Fig. 12 (A)). またヒストグラムでは初診時 Ach 閾値 > 10000 $\mu\text{g}/\text{mL}$ である症例は寛解群で約 30% 存在した. ここで初診時 Ach 閾値 > 10000 $\mu\text{g}/\text{mL}$ の症例では気道過敏性は正常域であるが可逆性および喀痰中の好酸球を認め, 臨床的にも喘息であると診断し得た症例を対象としている. 逆に初診時 Ach 閾値 < 313 $\mu\text{g}/\text{mL}$ 以下の症例からは寛解症例は

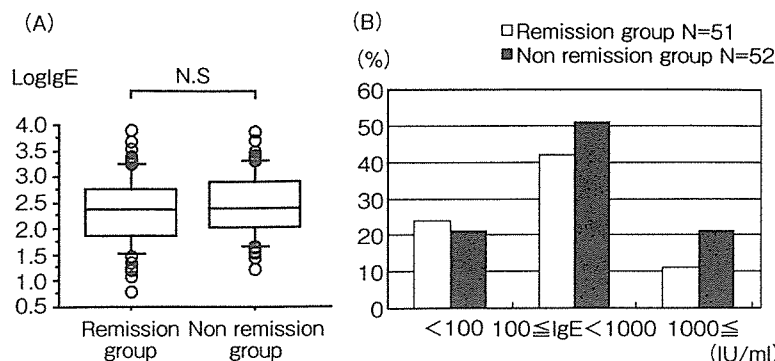


Fig. 9. (A) The comparison of the total IgE level in serum on the first hospital visit in remission group and non remission group. (B) The distribution of the total IgE level in serum.

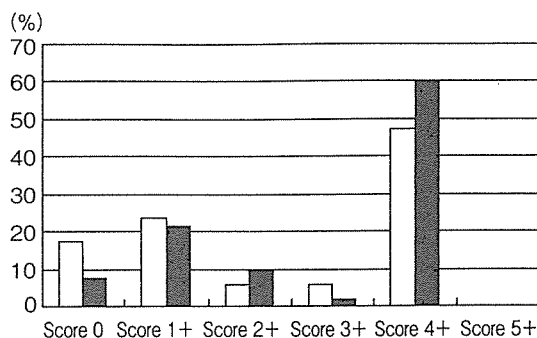


Fig. 10. The distribution of score of the number of eosinophils in sputum in remission group and non remission group. □ Remission group: N=51 ■ Non remission group: N=52

出ておらず、初診時 Ach 気道過敏性が軽度であるか高度であるかは成人喘息患者の予後をある程度規定する可能性があると考えられる。

また治療内容については初診時、徐放性テオフィリン製剤を使用した症例は寛解群で 71.3%、非寛解群で 82.7% であり、終診時にはそれぞれ 56.3%、78.8% と統計学的有意差は認めないものの寛解群では徐放性テオフィリン製剤使用が少ない傾向が認められた。このことは通院中の症状軽減によるテオフィリン製剤の使用の中止、また regular use が rescue use へ変化したことなどが推定される。非寛解群と比較して臨床症状が軽度であることを示唆する所見である。また本調査は平成 2~4 年度までに受診中の患者対象のため吸入ステロイド併用症例の頻度は全体的にみても少なく、寛解群では初診時 1.3%、終診時 7.5% であり、非寛解群

では初診時 3.8%、終診時 24.0% であった。非寛解群では初診時より吸入ステロイド使用が多く、終診時でも使用症例が多かった。また対症療法を必要としない、無治療であった症例は寛解群において初診時 8.8%、終診時 23.4% であった。一方、非寛解群では初診時 1.9%、終診時 8.7% が無治療であった。以上のことから治療内容については寛解群、非寛解群ともに差は認めなかった。

考 案

成人喘息の予後に関する疫学調査は少ない。小児喘息と比較し、発症年齢が幅広く、患者個々の background が多彩であり、一定条件での追跡調査が難しいためであろうと思われる。従って成人喘息に治療が期待できるのかどうかについていまだ明確な解答は得られていない。また治療とはいかなくとも臨床的な長期寛解状態が可能かどうかについても明らかではない。本研究はいわゆる成人喘息の寛解率調査ではなく、そもその研究の動機はかつて当院に成人喘息患者として通院していたが、その後通院しなくなった患者がその後無症状でいるのか、あるいは通院治療を続けているのかを調査することが目的であった。アンケート調査はあくまでも患者自身の症状把握であるため、「症状がない」の中に軽度の咳嗽や本人が発作と自覚しない呼吸困難の症状が含まれている可能性は十分にある。従ってくすりを必要とせず症状がないという判断はあくまでも患者の主観による臨床的寛解としなければならない。しかし、くすりを必要とせず 3 年あるいは 5

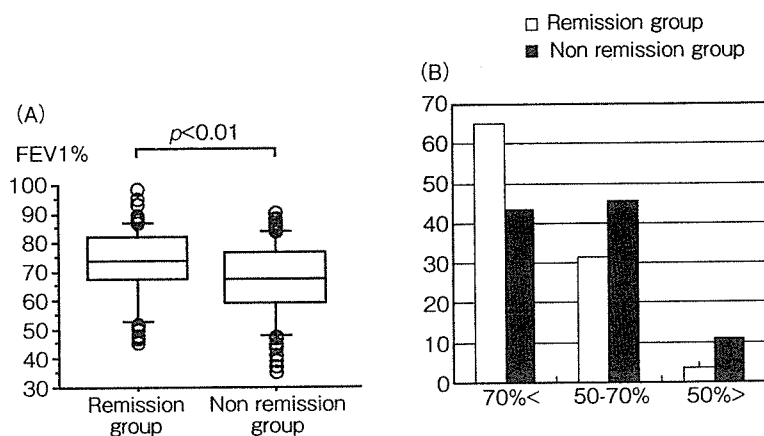


Fig. 11. (A) The comparison of FEV1% on the first hospital visit in remission group and non remission group. (B) The distribution of FEV1%.

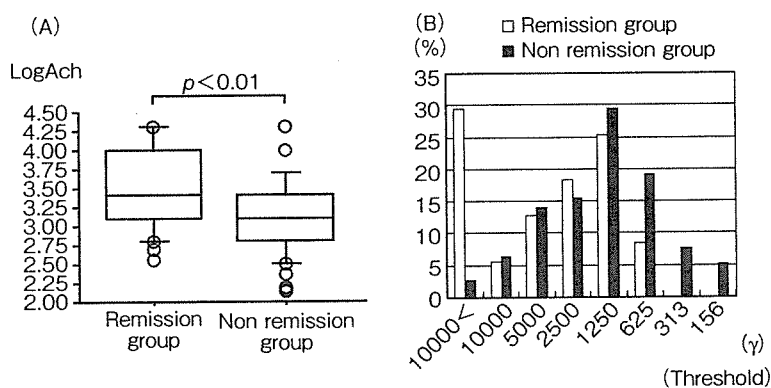


Fig. 12. (A) The comparison of bronchial reactivity to Ach (AchPC₂₀) on the first hospital visit in remission group and non remission group. (B) The distribution of bronchial reactivity to Ach (threshold amount of Ach).

年症状のない症例が少なからず存在することが明らかであり、この症例の中に成人喘息でも治癒し得る症例が存在する可能性があると考えられる。当院では過去に平成7年厚生科学研究疫学研究班調査において同様の調査を行った。それは昭和63年度から平成1年度まで当院受診歴があるがその後受診していない成人喘息患者を対象とし、アンケート調査を今回と同様に行ったものである。結果、954名中、462名より回答を得た(回答率48.4%)。くすりを服用しなくとも過去3年以上発作がないと答えた症例は462名中74名(16.0%)であり、今回の調査と類似していた⁵⁾。これらの結果から成人喘息患者の中には臨床的長期寛解症例が存在する可能性が示唆され、その中に将来の治癒患者が含まれ

る可能性がある。

成人喘息では寛解の頻度や寛解導入の予測因子に関する報告は少ないが、Vonk⁷⁾らによると0~39歳発症(中央値4歳)の喘息患者の26年後を追跡調査し、閉塞性障害が消失した症例では閉塞性障害が残存している症例と比較し、臨床症状が軽く、その内30.1%の症例ではヒスタミン気道過敏性が消失(HistPC₂₀>32 mg/mL)していると報告している。

一方、小児に関する予後の報告は多く、小児喘息119症例の30年後を追跡した調査では吸入ステロイドを使用せず臨床症状が消失しているいわゆる臨床的寛解症例は51.7%存在し、その中でもさらに%FEV1が90%以上、ヒスタミン気道過敏性が正常域である寛解

症例は22%であるという報告⁸⁾、101症例の小児喘息を対象とした調査では20年後の臨床的寛解率は57%であり、その中で93.1%の症例がヒスタミン気道過敏性が消失していたという報告⁹⁾、budesonide治療後臨床症状が消失した40症例を対象とし、治療を中止後も悪化を認めない症例が52%存在するという報告¹⁰⁾などがある。

小児喘息の再発、非寛解に関する因子は1・HD, miteに対する感作, 2・気道過敏性, 3・女性, 4・current smoking, 5・early age at onsetが報告されている¹¹⁾¹²⁾。アウトグロウに関する予測因子として、気道過敏性の残存が関与する¹³⁾¹⁴⁾、閉塞性障害の程度が関与する⁸⁾と双方があり未だに結論は出ていない。

また末梢血¹⁵⁾、および気道¹⁶⁾の好酸球性炎症の程度は小児喘息の寛解に関与しないという報告がある一方で、今回の我々の成人を対象とした調査では喀痰中の好酸球数は有意差を認めないものの、末梢血では非寛解群で多いという結果であった。この結果から成人と小児では気道過敏性に関わる好酸球性炎症の意味が異なることを示唆する可能性を有するものと考えられる。

小児では抗原感作、アトピー素因を乳幼児期に獲得し、成長とともにアトピー素因が減少する症例がアウトグロウに関与する⁸⁾¹⁴⁾¹⁷⁾と報告されているが今回の結果では病型や総IgE値、アレルギー疾患についての家族歴について有意差を認めなかったことから成人喘息においてはアトピー素因の有無は予後に影響しない可能性が示唆された。さらに喫煙歴についてはnon-smokerでは寛解群59.5%、非寛解群55.9%、ex-smokerは寛解群20.3%、非寛解群35.7%、current smokerは寛解群20.3%、非寛解群8.3%であり統計学的有意差($p=0.024$)を認めた。喫煙が喘息発作、予後に影響を与えることはすでに知られていることであり、寛解群にnonsmokerが多く、一方、非寛解群でex-smokerが多いことは予想された結果であった。一方、寛解群にcurrent smokerが20.3%も存在することは驚くべき結果であった。しかし、寛解群にcurrent smokerが多いのは、成人喘息患者調査で小児発症成人喘息群にcurrent smokerが多いのと意味合いは似ているのではないと思われる。すなわち、小児発症は成人発症に比べて軽症が多いことが患者調査で知られているが、current smokerが多いことも小児発症に軽症が多いことの裏付けなのかもしれない。故に、寛解群で初診時にcurrent smokerが多いのは、やはり、もともと軽症だからという理解も可能であろう。ある

いは、寛解群の一部は喫煙に影響されないという可能性も示唆される結果である。

成人喘息は発症年齢により、小児喘息が寛解せずに成人まで継続して続いている「小児発症喘息」、成人になって初めて発症した「成人発症喘息」、小児喘息が一度寛解し、成人になってから再発した「成人再発喘息」、さらに思春期に発症した「思春期発症喘息」に分類される¹⁸⁾²⁰⁾。1992年の厚生省成人喘息実態調査研究班の秋山¹⁹⁾らの報告では発症年齢別の割合は小児発症成人喘息11.1%、成人再発喘息3.7%、成人発症喘息77.7%であった。今回の調査では全国調査と比較して寛解群、非寛解群ともに小児発症喘息と成人再発喘息が多く、特に非寛解群では小児発症喘息が多かった。この事実は小児発症喘息の長い病歴が予後を左右するとも考えられる。しかし、発症年齢、通院期間、最終受診年齢にも差は認めず、服薬内容にも寛解群と非寛解群では差を認めないことから治療内容や通院期間が必ずしも予後を規定するわけではないという可能性も考えられる。これらのことから初診時より臨床症状や気道過敏性が軽度である症例が治療内容に影響されず寛解しうる可能性を有する群ともいえるかもしれない。また過去に秋山²¹⁾は初診時気道過敏性が非常に亢進している、すなわち閾値が低い(Ach閾値156 $\mu\text{g}/\text{mL}$ 以下)患者群16例につき、その背景因子、初診時および現在の重症度、入院・挿管歴、最近の治療状況について検討した。初診時の重症度は軽症、中等症、重症とさまざまであるが、これらの症例のなかでは寛解に導入された例は一例もみられなかった。この結果から初診時アセチルコリン気道過敏性が高度亢進症例では寛解する可能性は低く、軽度亢進例が寛解予備群であることが示唆された。

また寛解に治療内容が関与するかどうかについてはKoh²²⁾らの思春期喘息を対象とし、臨床症状が消失したが気道過敏性が残存する喘息群と臨床症状および気道過敏性の残存する喘息群にbudesonide(800 μg)を6カ月間使用し、その結果、有症状の喘息症例では有意に気道過敏性が改善したが、臨床的寛解症例では気道過敏性は改善しなかった。このことは小児喘息における気道過敏性が好酸球性炎症のみを反映しないこと、また臨床的寛解に治療内容が影響しないことなどが推測される。我々の結果でも寛解群では吸入ステロイド導入率が少なく、徐放性テオフィリン製剤の使用も外来通院中に減少したことなどから、臨床的寛解に治療内容は影響しない可能性も示唆される。

以上より、今回のアンケート調査で対象となった無治療無症状喘息症例はいわゆる臨床的治癒、あるいは、機能的治癒か否かは明らかではない。しかし、成人喘息においても治療を必要とせず、臨床症状を認めない症例がわずかながらにも存在することはアンケート調査から伺えると考えられる。そして無治療無症状継続群は将来の寛解予備群の可能性を有すると推定される。寛解予備群の予測因子としては初診時重症度が軽症であること、発症より早期に受診していること、初診時呼吸機能が良好、特にアセチルコリン気道過敏性が軽度である、ことなどが考えられる。アトピー素因、治療内容などは寛解の可能性の有無には直接影響を与えないと考えられた。この事実からは軽症喘息の一部は治療によらない、すなわち自然寛解する要素を持ち得ている可能性が示唆される。

成人喘息の予後に関する報告は少ない。今後はこれらの無治療無症状群が客観的所見からも真の寛解状態にあるか否かを検討していく必要があると思われる。

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PROGNOSIS OF BRONCHIAL ASTHMA IN ADULTS

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Background : The prognosis of adult asthma, whether a long-term remission is available, is still unknown. In this paper we investigated the prognosis of adult asthma patients.

Methods : We sent a questionnaire by mail to 1168 patients who had been taken care in our clinic until 1990-1992, but disappeared afterward. Those patients were asked their present status of clinical condition of asthma including symptoms, medication, etc.

Results : Delivery of mail was failed in 370 patients because of changed address. 430 of 798 patients replied the mail and 86 patients out of 430 patients were in remission state with no symptom without any medications. The characteristics of these patients in remission are early hospital visit after developing asthma, mild in severity, mild in obstructive lung function and mild in bronchial hypersensitivity to acetylcholine at the first visit hospital.

Conclusion : We concluded that some of adult asthmatic patients might become in clinical remission.

Association of Transforming Growth Factor- β 1 Single Nucleotide Polymorphism C-509T with Allergy and Immunological Activities

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Key Words

Immunological activities · Single nucleotide polymorphisms · T-cell proliferation · TGF β 1

Abstract

Background: A single nucleotide polymorphism (SNP) C-509T within the tumor growth factor β 1 (TGF β 1) gene has been associated with atopic asthma and asthma severity. To further understand the mechanisms involved, the association of C-509T with allergy, T-lymphocyte proliferation and plasma TGF β 1 concentration has been explored in a case-control study with allergic and non-allergic subjects. **Methods:** The recruited subjects including allergic (n = 38) and nonallergic (n = 25) participants have been genotyped for C-509T using allele discrimination assay. Association of C-509T with allergy status was examined using logistic regression analysis in both dominant and recessive models. Association of C-509T with T-cell proliferation in control and antigen-stimulated peripheral blood mononuclear cells (PBMCs), plasma TGF β 1 and total IgE level were tested by multiple regression analysis. **Results:** Individuals with homozygous mutant TT genotype showed a higher risk of allergy (TT: odds ratio = 5.099, 95% confidence limit: 1.355–19.190, p = 0.016) after covariates were adjusted. A trend

to increased plasma TGF β 1 in subjects with T allele has been discovered. In the meantime, the T allele is associated with lower T cell proliferation in controls and maximum response to above antigens. A low T-cell proliferation is correlated with higher plasma TGF β 1 concentration (p < 0.01). The in vitro studies confirmed the suppressing effect of TGF β 1 on T-cell proliferation at physiological range. A significant inhibitory effect on IL-4 production was also observed. **Conclusions:** A C to T base change in TGF β 1 SNP C-509T has been associated with a higher risk of allergy. The mechanisms are not clear. Elevated TGF β 1 levels associated with the C-509T polymorphism might suppress immune activation as well as Th2 cytokine production.

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Introduction

Allergic diseases such as asthma, allergic rhinitis and atopic dermatitis have been considered as complex polygenic disorders resulting from a combination of genetic and environmental factors. In past years, a lot of efforts have been pursued to look for possible genetic components contributing to allergies and asthma [1–4]. Studies from our group and others have indicated that tumor

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growth factor $\beta 1$ (TGF $\beta 1$) is a promising candidate gene whose genetic polymorphisms may play a key role in allergic diseases [5–10]. Among reported genetic polymorphisms in the TGF β gene, a C to T base exchange at –509 position in the promoter region was associated with elevated levels of total IgE in allergic asthma patients in our previous study [8]. Two subsequent independent case-control studies in relatively large populations reported the association of variant T allele with asthma, and even with asthma severity [5, 6]. The exact role and possible mechanisms involved are still not clear.

TGF $\beta 1$ is a 25-kDa disulfide-linked homodimeric multifunctional cytokine. With respect to its diverse activities in normal cell growth and development, TGF $\beta 1$ might be the most important growth factor in immunomodulatory effects because knockout mice died of massive inflammatory lesions [7]. Both anti-inflammatory and inflammatory properties have been reported [8]. In the present case-control study including allergic and non-allergic subjects, we have been able to further study the significances of the TGF $\beta 1$ C-509T genetic polymorphism in allergic diseases, its association with TGF $\beta 1$ production, immune modulation activity reflected from antigen-stimulated T-cell proliferation in peripheral blood mononuclear cells (PBMCs), and even regulatory effects on cytokine production.

Patients and Methods

Subjects

Adult volunteers either with a history of atopy or allergic diseases, e.g. allergic rhinitis, atopic dermatitis, and asthma, or no allergy (healthy individuals) have been recruited. Atopy or allergy status was determined based on identified allergen through positive skin test or allergen-specific IgE test, and allergic symptoms. To avoid possible confounding effects for T-cell proliferation to antigen, patients with other diseases especially an immune deficiency, allergic patients with a history of immunotherapy, and patients who have taken steroids or other immunosuppressive agents at least 3 days before blood drawing met the exclusion criteria. All subjects signed informed consent with National Jewish Institutional Review Board approval before being enrolled and donating blood.

Genotyping

Peripheral venous blood was obtained from each subject. Genomic DNA was extracted from whole blood using the QIAamp blood kit (QIAGEN, Santa Clarita, Calif., USA). Briefly, blood cells were first lysed, and supernatant containing genomic DNA was mixed with ethanol and applied to QIAamp spin column. After washing several times, purified genomic DNA attached in column was eluted finally in TE buffer.

The allelic discrimination assay using the ABI PRISM 7700 Sequence Detection System (AB Applied Biosystems, Foster City,

Calif., USA) has been developed for genotyping of TGF $\beta 1$ C-509T (HUgn0007040). Primers used for amplification of DNA fragments are: forward primer at position –572~–551 (-CATGGGAGGT-GCTCAGTAAAGG-), reverse primer at position –437~–454 (-GGTAGGAGAAGAGGGTCTGTCAAC-). Probe 1 containing allele C at position –519~–494 was linked with reporter dye FAM (6-carboxyfluorescein) at the 5' end and black hole quencher (Biosearch Technologies) at the 3' end: -CCCTTCCATCCCT-CAGGTGTCCTGTT-. Probe 2 containing allele T instead of C was labeled with reporter dye 6-JOE (6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein). Each reaction contains 25 μ l of Taqman Universal Master Mix (AB Applied Biosystems), 900 nM of forward and reverse primers in concentration, 100 nM of probe 1 and probe 2 in concentration, and 200 ng of genomic DNA in 50 μ l total volume. Thermal cycling conditions for allelic discrimination assay are: 2 min at 50°C, 10 min at 95°C for AmpliTaq Gold Activation, and 40 cycles of 15 s at 95°C for denaturing and 1 min at 60°C for annealing/extension. Rn for a given reaction tube, defined as the ratio of the emission intensity of the reporter dye over the emission intensity of the passive reference, was obtained. The relative Rn value of FAR to JOE reporter dye for each reaction was utilized to separate CC (1.1–1.43), CT (0.74–1.1) and TT (0.30–0.74) after adjustment with non-template control based on the 95% confidence limits in each group. Duplicate experiments have been performed confirming reproducibility. Three distinct groups for CC, CT and TT have been recognized based on our setup thresholds.

Cell Culture and Cell Proliferation

PBMCs were isolated from human heparinized venous blood by density gradient centrifugation on Ficoll-Paque (Amersham, Uppsala, Sweden), and washed three times in HBSS (Gibco, Grand Island, N.Y., USA). Purified PBMCs were suspended in complete RPMI 1640 medium (Gibco), which was supplemented with 5% heat-inactivated fetal calf serum (Gemini Bio-Products, Calabasas, Calif., USA), 200 U/ml penicillin, 200 μ g/ml streptomycin, 50 μ g/ml gentamicin, 4 mM L-glutamine (Gibco), 10 mM HEPES (Sigma), 1 mM sodium pyruvate (Gibco), 0.1 mM nonessential amino acid (Gibco), and 50 μ M 2-mercaptoethanol.

To assess lymphocyte proliferation response, PBMCs were cultured at a concentration of 10^5 cells/well in 96-well flat-bottom plates in a volume of 200 μ l. PBMCs were incubated with medium control; tetanus toxoid (Aventis Pasteur, Swiftwater, Pa., USA) at concentrations of 0.02, 0.07, and 0.2 lac-forming units (LFU)/ml; timothy (pollen) *Phleum pratense* allergic extract (Bayer, Elkhart, Ind., USA) at concentrations of 4, 40, and 400 PNU/ml, and standardized cat hair allergic extract (Fel d1; ALK, Horsholm, Denmark) of 1, 10, and 100 BAU/ml. On day 6, 1.0 μ Ci/well [3 H] thymidine (Amersham, Arlington Heights, Ill., USA) was added to 100 μ l of cell culture and incubated for 6 additional hours. Cells were finally harvested onto filter paper disks via a multiwell harvester and radioactivity (counts/min) counted with a 1450 Microbeta Plus Liquid Scintillation Counter (Wallace, Orange, Calif., USA). All samples were run in triplicate.

To assess the effect of TGF $\beta 1$ on cell proliferation, PBMCs were also cultured at a concentration of 10^5 cells/well in 96-well flat-bottom plates in a volume of 200 μ l for 6 days. Human recombinant TGF $\beta 1$ (R&D Systems, Minneapolis, Minn., USA) was added as indicated in the Results. Radioactivity was counted as described above.

To assess the effect of TGF β 1 on cytokine production, PBMC were cultured at a concentration of 10^6 cells/well and treated as described in the Results. Supernatant was collected at 48 h, and IL-2, IFN γ , IL-4 were measured using ELISA assays.

Plasma TGF β 1 Measurement

Plasma was first isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque and further centrifuged at 3,000 *g* for 10 min to eliminate platelets, and then frozen at -70°C . Plasma TGF β 1 was measured using the TGF β 1 ELISA Kit (Biosource, Nivelles, Belgium). Based on the manufacturer's instruction, latent TGF β 1 was first extracted from platelet-depleted plasma, and incubated with biotinylated anti-TGF β 1 in TGF β 1-antibody-coated wells for 3 h as well as TGF β 1 standard and diluent control. Streptavidin-HRP working solution was added for 30 min after thorough washing. Stabilized chromogen solution was finally added to each well for 30 min and terminated by stopping solution before absorbance (optical density, OD) was detected at a wavelength of 450 nm. The concentration of plasma TGF β 1 was calculated after deriving from a standard curve. The minimum detectable dose of TGF β 1 is 15.6 pg/ml. Duplicate experiments have been performed.

Plasma Total IgE Measurement

Plasma total IgE was measured using the ELISA kit from ALerCHEK (Portland, Maine, USA). According to the manufacturer's instructions, 20 μl of standard IgE and plasma sample were diluted with 80 μl of specimen diluent and incubated in anti-human IgE-coated microwells for 60 min at room temperature. After thorough washing with washing buffer, 100 μl of HRP-conjugated goat anti-human IgE was added for another 60 min. Finally, 100 μl of TMB/peroxide substrate were incubated for 30 min after another thorough washing and terminated by 0.5 *N* sulfuric acid before OD at 450 nm was determined. Total IgE concentration (IU/ml) in plasma was derived from the constructed standard curve. Duplicate experiments have been performed.

Cytokine Assays

IL-2, IFN γ and IL-4 production in cell culture supernatants was measured using ELISA kits (Biosource) with a sensitivity of <5 pg/ml, 0.03 IU/ml, and 2 pg/ml, respectively. According to the instructions, standard, control and samples from supernatant were incubated in specific-antibody-coated microwells together with antibody conjugate for 2 h at room temperature on a horizontal shaker set. After thorough washing, 200 μl of freshly prepared chromogenic solution were pipetted into each well and incubated for 15 min at room temperature on a horizontal shaker set before 50 μl of stop solution were added. The OD value at 450 nm was read, and plasma concentrations of cytokines were calculated based on standard curves finally.

Statistical Methods

Statistical analyses were performed using SAS software version 9.1.3. Balance of categorical variables regarding gender, race, ethnicity, genotype and allele frequency between allergy and non-allergy groups was tested using the χ^2 test or Fisher's exact test in PROC FREQ. The Hardy-Weinberg equilibrium for TGF β 1 C-509T allele frequency was calculated according to Thompson et al. [9]. Logistic regression was utilized to find the association between the TGF β 1 C-509T genotype and allergy status, while gender (male

or female), race (White, Asian or Black) and TGF β 1 C-509T genotype (CC and CT, or TT only) has been considered in model building. Backward selection with slentry = 0.2, slstay = 0.2 options was used to remove low-risk factors. To adjust for possible confounding influences in the association of the C-509T genotype with numerical variables such as T-cell proliferation, stepwise-wise selection with slentry = 0.2 and slstay = 0.2 has been run with race, gender, allergy status, and TGF β 1 C-509T genotype entered in multiple regression models. Regression diagnostics have been performed to make sure that no violation of assumption occurs. Pearson's correlation coefficient has been used to estimate correlations between two numerical variables. Nonparametric approaches (the Kruskal-Wallis test and Wilcoxon's two-sample test) have been applied for multiple comparison among groups. *p* values less than 0.05 are considered as statistically significant.

Results

We recruited 63 volunteers including 38 with a history of allergic or atopic diseases and 25 normal subjects. All of them are nonsmokers. The properties of the recruited population are summarized in table 1. There is no significant difference between allergy and non-allergy groups regarding the composition of gender, race, ethnicity (χ^2 test or Fisher's exact test, $p > 0.05$) and age (Wilcoxon's rank sum test, $p > 0.05$). The allele frequency of TGF β 1 C-509T is consistent with the Hardy-Weinberg equilibrium ($\chi^2 = 3.93$, $p > 0.1$). Except for medium and phytohemagglutinin (PHA) control, T-cell proliferation stimulated by tetanus toxoid and timothy allergy is significantly higher in the allergy group than in the non-allergy group. There is a trend towards an increase in T-cell proliferation for cat hair allergen in the allergy group although it is not statistically significant ($p = 0.0710$).

Association of the TGF β 1 C-509T Genotype with Allergy

The association of the TGF β 1 C-509T genotype with allergy was examined using logistic regression. The possible covariates such as gender (male or female) and race (White, Asian or Black) have been considered and were removed according to the selection criterion (see Statistical Analysis). In the recessive model, the allergy status was associated with the TT genotype (odds ratio = 5.099, 95% confidence limits 1.355–19.190, $p = 0.016$). However, there is no significant association between allergy status and CC in the dominant model (odds ratio = 0.823, 95% confidence limits 0.26–2.383, $p = 0.7199$). This suggests that the TT variant of the TGF β 1 gene at promoter -509 is associated with increased risk of allergy in humans.

Table 1. Subject characteristics (mean and SD)

	Allergy	No allergy	Total	p value
Subjects	38	25	63	
Age, years	36 (10)	38 (12)	36 (11)	0.5737
Sex, %				
Female	58	52	56	0.6450
Race, %				
White	79	68	75	
Asian	16	32	22	
Black	5	0	3	0.2725
Ethnicity, %				
Non-hispanic	89	100	94	
Hispanic	11	0	6	0.0937
TGFβ1 C-509T genotype, %				
CC	37	44	40	
CT	53	20	40	
TT	11	36	20	0.0158
TGFβ1 C-509T allele frequency, %				
C	63	54	60	
T	37	46	40	0.9114
Timothy allergy, %				
Yes	84	0	51	<0.0001
Cat allergy, %				
Yes	58	0	35	<0.0001
CPM for control	8.49 (1.64)	8.12 (1.34)	8.34 (1.52)	0.2243
CPM for tetanus toxoid	9.70 (1.13)	9.04 (1.12)	9.44 (1.16)	0.0216
CPM for cat hair allergen	9.19 (1.36)	8.59 (1.13)	8.95 (1.30)	0.0710
CPM for timothy allergen	9.36 (1.15)	8.79 (1.08)	9.13 (1.15)	0.0409
CPM for PHA	11.45 (0.66)	11.45 (0.51)	11.45 (0.60)	0.8088
Total IgE	1.58 (0.75)	0.84 (0.72)	1.32 (0.82)	0.0009
Plasma TGFβ1, ng/ml	3.10 (0.47)	3.16 (0.41)	3.12 (0.45)	0.6014

Racial and ethnic classification is according to the standard used in Census 2000. For age and other numerical data, means (SD) are shown. Values for CPM and total plasma IgE are natural logarithmic transformed. Wilcoxon's rank sum score test was used for comparisons between allergy and non-allergy groups.

Association of TGFβ1 C-509T with Plasma TGFβ1 Level

To understand the possible mechanisms associated with the above finding, the association of C-509T with plasma TGFβ1 levels has also been estimated using multiple regression. The results indicated that there is a trend to higher plasma TGFβ1 concentration in the presence of T at the C-509T allele (CC: 3,008.34 ± 369.96, CT: 3,143.76 ± 529.17, and TT: 3,312.66 ± 386.55 pg/ml). However, statistically significant differences could not be shown in either the dominant model (CC only: p = 0.1198) or the recessive model (TT only: p = 0.1346) while possible covariates such as allergy, gender, age and race have been adjusted for. The hypothesis that a higher concentration of plasma TGFβ1 in the TT genotype accounts

for the association of C-509T with allergy may need to be verified in larger studies in the future.

Association of the TGFβ1 C-509T Genotype with T-Cell Proliferation in Human PBMCs

With respect to the importance of TGFβ1 in the immunomodulation of allergen-induced cell proliferation, the association of the TGFβ1 C-509T genotype with antigen-stimulated cell proliferation in human PBMCs has also been examined using multiple regression analysis. Based on selection rules, the adjusted variables are listed in table 2 as indicated. Outcomes including unstimulated cell proliferation (medium control) and cell proliferation induced by maximum tetanus toxin, cat hair allergen and timothy allergen extracts showed a negative relationship

Table 2. Association of the TGFβ1 C-509T genotype with T-cell proliferation and IgE (n = 63)

Outcomes (ln value)	Adjusted variables	Regression coefficients for TT or CC (mean ± SE)	t value	p value
TT (recessive)				
CPM for medium control	White	-1.26 ± 0.48	-2.64	0.0127
CPM for tetanus toxoid	Black	-1.04 ± 0.34	-3.08	0.0031
CPM for cat hair allergen	cat allergy, White	-1.02 ± 0.41	-2.50	0.0152
CPM for timothy allergen	grass allergy, White	-0.93 ± 0.36	-2.55	0.0134
CPM for PHA	age, White	-0.07 ± 0.192	-0.37	0.7132
Plasma IgE	allergy	0.003 ± 0.26	0.01	0.9902
CC (dominant)				
CPM for medium control	age, White, Asian	1.20 ± 0.37	3.25	0.0019
CPM for tetanus toxoid	age, allergy	0.70 ± 0.28	2.48	0.0159
CPM for cat hair allergen	White, cat allergy	0.67 ± 0.32	2.07	0.0429
CPM for timothy allergen	White, grass allergy	0.84 ± 0.29	2.95	0.0047
CPM for PHA	age, Asian	0.03 ± 0.16	0.19	0.8464
Plasma IgE	allergy	0.07 ± 0.20	0.34	0.7376

with TT ($p < 0.05$) in the recessive model except for PHA. Conversely, the T-cell proliferation had a positive relationship with CC in the dominant model ($p < 0.05$) except for PHA. These results indicate that the C-T mutation is associated with reduced T-cell proliferation in general. We have used the stimulation index (ratio of antigen-stimulated cell proliferation over control) as response variable for tetanus toxin, cat allergen and timothy allergen. Neither the TT nor the CC genotype was correlated with antigen-specific cell proliferation (data not shown), suggesting that suppression might not be antigen specific.

The analysis for the association of the TGFβ1 C-509T genotype with plasma total IgE indicates that IgE is apparently not associated with the TGF C-509T genotype ($p = 0.9902$ for TT and $p = 0.7376$ for CC). The C-509T genotype does not exhibit an allergy-status-dependent difference ($p = 0.5676 > 0.05$ for TT and $p = 0.6718 > 0.05$ for CC) in IgE level.

Effect of TGFβ1 on T-Cell Proliferation

We also tested whether higher concentration of plasma TGFβ1 is correlated with lower T-cell proliferation capacity (fig. 1). From the graphs, negative associations of the plasma TGFβ1 concentration with natural log-transformed value of control, tetanus toxoid, cat allergen, timothy allergen, and even PHA-stimulated cell proliferation were found. Total IgE seems not to be associated with plasma TGFβ1 amounts. After adjusting for possible covariates such as gender, race and age in multiple regres-

Table 3. Regression analysis of T-cell proliferation and IgE with plasma TGFβ1 concentration adjusted for gender, age, and race (n = 63)

Outcomes (ln value)	Regression coefficients for TGFβ1 (mean ± SE), $\times 10^{-3}$	t value	p value
CPM for medium control	-1.573 ± 0.4251	-3.70	0.0005
CPM for tetanus toxoid	-0.808 ± 0.3354	-2.41	0.0196
CPM for cat hair allergen	-1.239 ± 0.3711	-3.34	0.0016
CPM for timothy allergen	-0.970 ± 0.3317	-2.92	0.0051
CPM for PHA	-0.356 ± 0.1924	-1.85	0.0705
Plasma IgE	-0.138 ± 0.2291	-0.6	0.5485

sion models, the linear correlation still exists (table 3). These results support our hypothesis that human subjects with the T allele in the TGFβ1 C-509T genetic polymorphism suppressed immunological activity resulting probably from higher TGFβ1 production.

Furthermore, recombinant human TGFβ1 was added at three different concentrations (32 and 3.2 ng/ml and 32 pg/ml) simultaneously in in vitro studies while PBMCs were cultured in the presence of tetanus toxoid (0.02 LFU/ml) as a stimulator. TGFβ1 significantly inhibited cell proliferation at 32 ng/ml ($p = 0.012$) with a dose-response trend (fig. 2) consistent with a suppressing effect of TGFβ1.

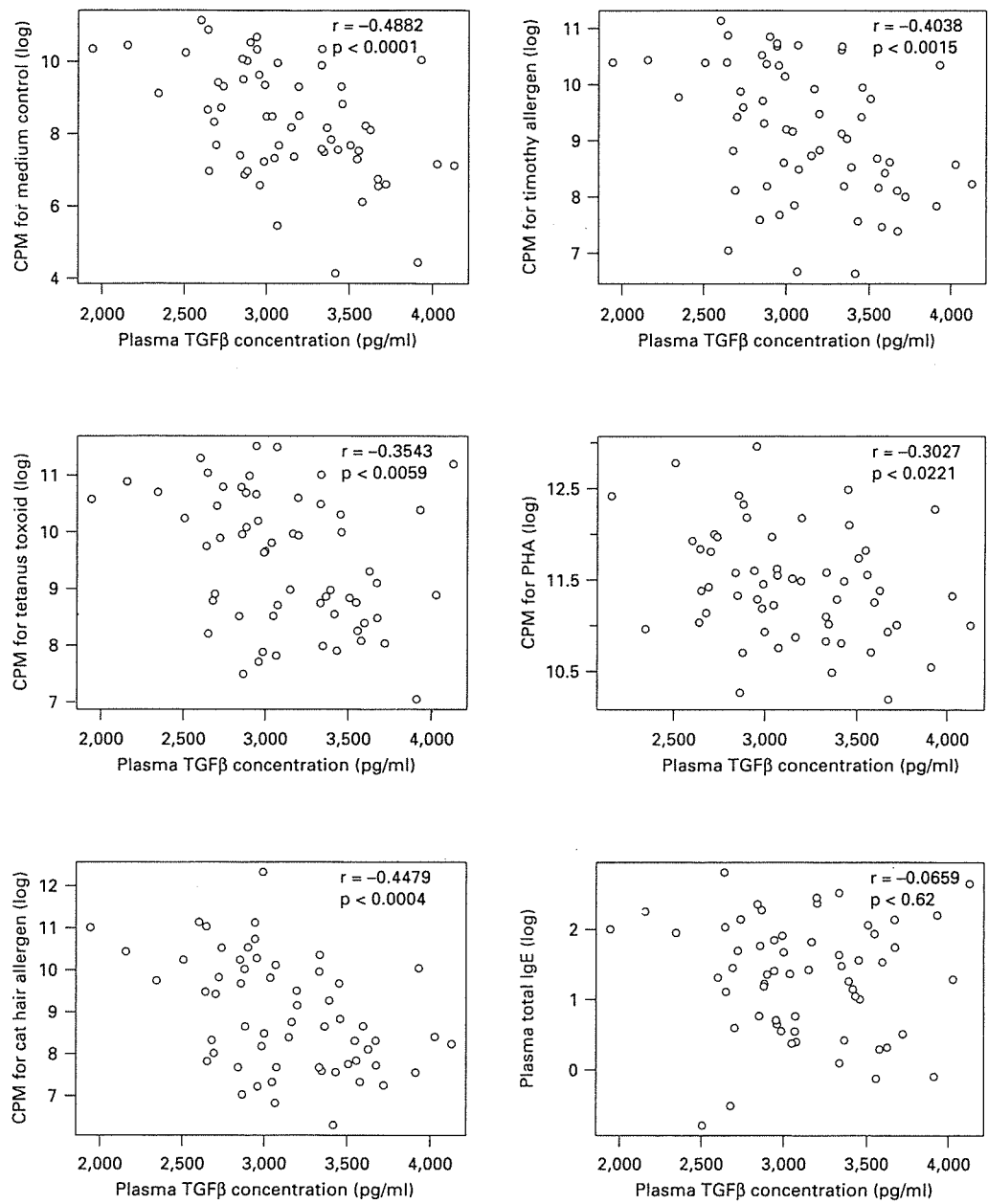


Fig. 1. Scatter plot of plasma TGFβ1 with plasma total IgE and T-cell proliferation in response to antigens in 63 subjects. Counts per minute (CPM) and total IgE are the natural log-transformed value of maximum T-cell proliferation in medium control, tetanus toxoid, cat allergen, timothy allergens, PHA-stimulated PBMCs and plasma IgE level. r = Correlation coefficient.

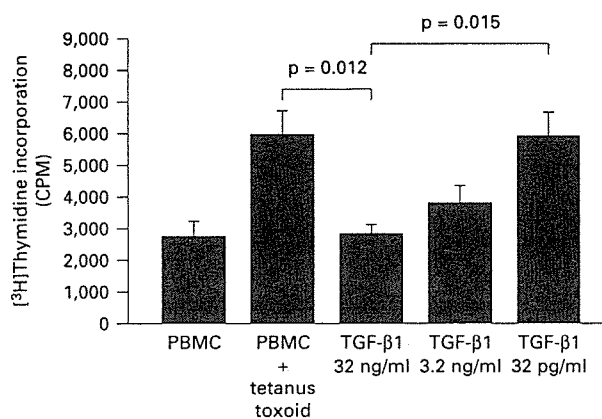


Fig. 2. Inhibition of T-cell proliferation by TGFβ1. [³H]thymidine uptake (counts/min, CPM) of T-cells stimulated with 0.02 LFU/ml tetanus toxoid in the presence of TGFβ1 for 6 days (n = 8).

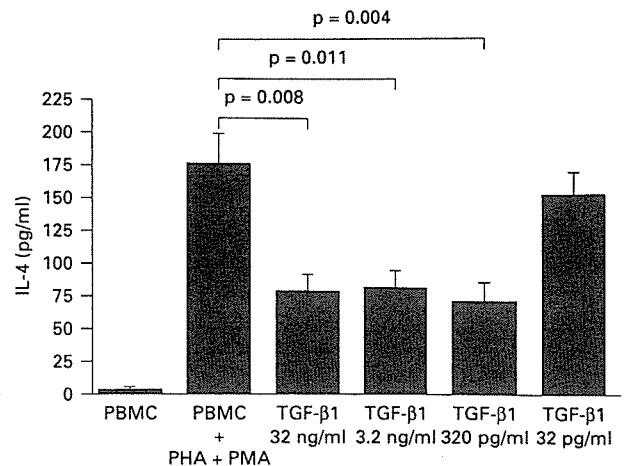


Fig. 3. Inhibition of IL-4 synthesis by TGFβ1. PBMCs were stimulated with PHA 10 μg/ml and 10 ng/ml phorbol 12-myristate 13-acetate (PMA) in the presence of TGFβ1 (n = 5).

Inhibiting Effect of TGFβ1 on Cytokine Production

Cytokines have been considered to be very important in regulating T-cell proliferation. The inhibitory effect of TGFβ1 on cytokine production was further studied *in vitro*. PBMCs were incubated in the presence of 10 μg/ml PHA and 10 ng/ml phorbol 12-myristate 13-acetate with TGFβ1 at four different concentrations (32 and 3.2 ng/ml, and 320 and 32 pg/ml) for 48 h. Supernatant was collected and measured for IL-4, IL-2, and IFNγ using ELISA. Figure 3 showed that TGFβ1 significantly inhibited IL-4 synthesis even at 320 pg/ml (p = 0.004). However, the effects of TGFβ1 on IL-2 and IFNγ production were not statistically significant (data not shown). The suppressing effect of TGFβ1 on cytokine production, especially of the Th2 type, may be involved in the association of the TGFβ1 genotype with reduced immunological activities.

Discussion

TGFβ belong to a super family of about 40 structurally related polypeptide growth factors [10]. Perturbation of the TGFβ superfamily has been implicated in various human diseases, e.g. cancer, angiogenesis, fibrotic diseases, and atherosclerosis, due to their mediation of many key events in normal growth and development including cellular differentiation, proliferation, motility, organization and death [11]. TGFβ1 is one of the best-studied

factors in mammalian tissues. The role of TGFβ1 in asthma and allergic diseases has also been well documented in the literature [12]. Increased TGFβ1 expression in asthma has been repeatedly reported. Overexpression of TGFβ1 mRNA and proteins in bronchial biopsies from subjects with severe and moderate asthma has been observed compared with controls without asthma [13–15]. In asthmatic airways, TGFβ1 mRNA expression mainly produced from eosinophils was related to the degree of disease severity, subepithelial fibrosis and airway remodeling [13, 14, 16].

The genetics of atopy and asthma has been a very interesting research area in past years. Deciphering secrets in genetic variations related to allergy and asthma development may greatly benefit our efforts in the prevention and treatment of asthma and allergic diseases. The candidate gene approach, in which genes of relevance to the physiological changes of the disease process are tested for direct involvement with the disease, has been successfully applied to link genetic variation in the promoter region of a number of cytokine genes to asthma and allergic diseases. The promoter region of the TGFβ1 gene has been characterized showing regulatory impacts on transcriptional processes [17]. Our previous work demonstrated that a TGFβ1 promoter polymorphism at C-509T with the TT homozygote genotype is linked to allergic and asthmatic patients with elevated serum IgE [18]. Two case-control independent studies in relatively large populations of white individuals also found the association of

variant T allele with asthma, even with severity of asthma [5, 6, 19]. In our present study, the T variant of the genetic polymorphism TGF β 1 C-509T is significantly associated with allergy status. These results consistently support our hypothesis that the TGF β 1 T allele genotype is an important risk factor in allergy development.

C to T base change is located in the region of the promoter associated with Yin Yang-1 activator consensus sequence. It has been reported to increase promoter function using luciferase constructs [20]. The T allele has been demonstrated to enhance the YY1 binding site on the TGF β 1 promoter region and may account for the increased transcription of TGF β [6]. The C-509T polymorphism was significantly associated with increased concentration of latent TGF β 1 in serum in a human study [21]. Our present results also showed an increased trend of plasma TGF β 1 to the T allele. Limited sample sizes and differences in population properties may explain the lack of statistical significance in this study. It is reasonable to deduce that subjects with the T allele may display a higher level of TGF β 1 in other tissues such as respiratory airways or skin. The observed overexpression of TGF β 1, e.g. in bronchial biopsies of asthmatic patients as discussed before, may at least partly result from their enhanced capacity to produce TGF β 1 determined by their genetic components such as the T allele mutation in C-509T, although a feedback mechanism from the inflammatory reaction in asthmatic patients cannot be excluded.

It has been well known that immunological activity plays a crucial role in initiating and developing allergic reactions in response to allergens. In mice, knockout studies with TGF β 1 indicated that TGF β 1 might be one of the most important growth and regulatory factors in the immune system because the knockout mice died of massive inflammatory lesions [22]. However, the evidences about its effects in the immune system seem confusing and contradictory. Its anti-inflammatory effect has been demonstrated by inhibiting the proliferation of T and B lymphocytes [23, 24]. On the other hand, TGF β 1 has been found to be a growth factor by inducing proliferation of macrophages, dendritic cells, and even T cells [25, 26]. Our present study indicates that a C to T mutation at the TGF β 1 genetic polymorphism C-509T is correlated with suppressed T-cell proliferation *in vivo*. This suggests that subjects with the T allele in the absence or presence of allergy demonstrated reduced lymphocyte response after exposure to allergen, even at the basic level. Increased production of TGF β 1 seems to be one of the reasons, since the plasma TGF β 1 concentration is negatively correlated with the degree of T-cell proliferation, and TGF β 1

suppresses T-cell proliferation *in vitro* at physiological concentrations. Human subjects with the T allele may own a relatively lower immune activity due to more TGF β 1 inside their body.

Allergic diseases are aberrant immune responses to antigen mainly mediated by IgE. Our previous study indicated that the C to T base exchange is associated with elevated IgE in atopic asthmatic patients. However, other studies did not confirm our findings [6]. Our present study failed to show a significant association with the total IgE level while other possible covariates have been considered. Lack of adjustment of covariates, e.g. the allergy status, in our previous report might be one reason for the elevated IgE in atopic asthma patients. These results implied a complicated relationship between TGF β 1 and total IgE. As we know, TGF β 1 is believed to inhibit IgE predominately in immunity. It might be related to its downregulatory effect on various cell surface molecules including CD23 and MHC class II antigen in human cells, which may be very important in promoting IgE synthesis [27, 28]. TGF β 1 could also inhibit e-germline transcription [29] directly through transcription factors or through inhibiting Th2 cytokines such as IL-4. Elevated TGF β 1 in TT allele subjects is expected to reduce the IgE level. However, as IgE synthesis is a very complicated process involving lots of cytokines (e.g. IL-13 and IL-10), even suppression of IgE is environment directed. It has been reported that at a later stage of differentiation, IgE B cells are refractory to the inhibitory effect of TGF β , as shown by the slight but significant increase in the spontaneous secretion of IgE by PBMC of atopic patients [30]. Finally, TGF β 1 downregulation of IgE may be a mechanism where Th2 inflammation may still be prominent, with no increase in IgE, a situation that might be seen in intrinsic asthma for example. Therefore, we may not be surprised to see the lack of association of this genetic polymorphism with the IgE level in plasma in our present study.

Both T-cell proliferation and production of IgE and cytokines, including Th2 and Th1, are believed to play an important role in allergic inflammation. In our study, the Th2 cytokine IL-4 was depressed rather than IL-2 and IFN γ , implying that Th2 cytokines are crucial in explaining the observed suppressing effect of TGF β 1 on T-cell proliferation. However, the suppressing effect of TGF β 1 on the Th1-type reaction or initiation of T-cell reactions may still exist. This is because the TGF β 1-induced downregulation of the IL-2 receptor at both protein and mRNA level has been reported [29]. TGF β 1 has been shown to inhibit IL-2-induced tyrosine phosphorylation, activation of Jak-1 and Stat 5, and IL-2-induced expression of

α and β chains of IL-2R [30]. Interaction of IL-4 and IFN γ has been reported. Reduction of IL-4 by TGF β 1 may reduce the forces to inhibit IFN γ production. These may be some reasons that we failed to observe the significant effect on IFN γ . With respect to the cytokine IL-4, T-cell proliferation in the allergic reaction, an increased TGF β 1 concentration in T-allele subjects may play a role in the suppression of the allergic reaction or inflammatory effect rather than an increase in the risk of allergic status. The mechanisms involved are still not clear. More studies in larger patient cohorts and on the mechanisms regarding its role in allergic development may be worth of much more attention in the future. Other roles than cell proliferation for TGF β 1 may be hidden behind the association of TGF β 1 C-509T with allergy. As an example, TGF β 1 is an important stimulant of fibrosis, inducing formation of the extracellular matrix, and promoting wound healing and scar formation. Therefore, the profibrotic effect of TGF β 1 may play a more important role in asthma development. Another important function for TGF β 1 is their regulatory activity in cell differentiation. IL-3 and M-CSF in cooperation with TGF β induces granulocyte macrophage colony-stimulating factor-independent differentiation of human CD34+ hematopoietic progenitor cells into dendritic cells with features of Langerhans' cells. TGF β 1 may play an important role in the

differentiation and maturation of dendritic cells and monocyte differentiation. TGF β 1 may play an important role in regulating some cell cycle arrest rather than others, as indicated in CD34+ CD38-c-kit(high) vs. c-kit(low/-) cells. Due to the complexity in the development of allergic diseases, the T allele may be combined with other genetic variations, which produce a different environment leading to the allergic status.

In summary, our study demonstrated the modulatory effects of TGF β 1 and its polymorphism on cell proliferation and cytokine production. Further investigations with larger sample sizes could further solidify our findings. Clarification of the association of the TGF β 1 C-509T polymorphism with allergic diseases may raise new and better methods for diagnosis, and more importantly, the potential for new pharmacological interventions related to the products of the candidate genes.

Acknowledgments

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