厚生労働科学研究費補助金

免疫アレルギー疾患予防・治療研究事業

皮膚アレルギー炎症発症と治療における サイトカイン・ケモカインとその受容体に関する研究

平成 14 年度～16 年度　総合研究報告書

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平成 17（2005）年 4 月

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皮膚アレルギー炎症発症と治療におけるサイトカイン・ケモカインとその受容体に関する研究

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研究要旨　アレルギー性皮膚疾患は多数にのぼるが、アトピー性皮膚炎(AD)、水疱症類天疱瘡(BP)、菌状息肉症(MF)は、血清 IgE の上昇、末梢血好酸球数の増加など共通した病態を示す特徴がある。本研究ではこれらの病態に対するサイトカイン・ケモカインの関与について検討した。これまでの研究によって、ケラチノサイト（KC）から TNF-αや IFN-γなどによる刺激で産生誘導ないし増強される TARC/CCL17, MDC/CCL22 に加えて、Eotaxin-3/CCL26, CTACK/CCL27, MEC/CCL28 などがアレルギー性皮膚疾患に重要な働きをしていることが示された。そして in vitro によるこれらケモカイン産生制御の研究から in vivo における炎症への関わり合いも示唆されたと考えている。動物モデルとして作成した TARC Tg の解析から、TARC はそれのみでは炎症を引き起こさないが、一旦炎症がおこると CCR4 陽性 Th2 細胞を遊走させ、Th2 優位な状態を誘導して炎症を修飾すると考えられた。VEGF Tg の解析から、VEGF が炎症細胞浸潤、血管増生を誘導するうえで重要な分子であることが明らかとなった。また、CCR10 の発現細胞として形質細胞を同定したことは CCR10 に作用するケモカイン CTACK, MEC の生理機能に関して新たな地平を開いた。皮膚におけるこれらのケモカインの産生と形質細胞の遊走との関係が注目される。CCR4 を介したシグナル伝達系では、MEK1/2 を介した経路が細胞遊走に重要な役割を果たしていることが示唆された。CCR4 を発現する T 細胞の皮膚への浸潤・遊走が大きな一因となっている AD や MF に対して、シグナル伝達阻害剤の臨床応用・効果が期待される。以上のような皮膚の病態解析に関する検討からアレルギー性皮膚疾患の理解が進み、それに基づいた治療戦略の開発が期待される。

A. 研究目的

皮膚アレルギー炎症には患者数の増加や難治化、治療の混乱などで社会的問題化しているアトピー性皮膚炎(AD)や、環境の変化によって新たな物質による皮膚障害として現れる接触皮膚炎など厚生労働行政上問題となるものが多い。このようなアレルギー炎症が皮膚に現れる機序は他臓器に比べて研究が進んでおり、最近はサイトカインのうち白血球に対する遊走能を有するケモカインについての研究が注目をあつめている。本研究班では、このサイトカイン、ケモカインとその受容体発現細胞に焦点をあてて皮膚のアレルギー炎症機序を明らかにし、発症予防を含めた治療の可能性を示そうというものである。具体的には Th2 ケモカインと呼ばれている TARC/CCL17、MDC/CCL22 とその受容体である CCR4、皮膚特異的ケモカインとされ

る CTACK/CCL27 とその受容体 CCR10、好酸球浸潤に関与する Eotaxin/CCL11, Eotaxin-2/CCL24, Eotaxin-3/CCL26 とその受容体 CCR3、主に粘膜免疫に関与する MEC/CCL28 とその受容体 CCR10, CCR3 を中心に研究を進めた。

B. 研究方法

(1) 皮膚アレルギー疾患におけるケモカインの関与に関する研究では、これまで玉置らは AD 患者血清中で TARC が高値を示しかつ病勢と相関し、TARC 産生細胞としては表皮ケラチノサイト（KC）であろうとする結果を報告してきた。義江らは AD 患者における TARC 値を血清と血漿を用いて比較し、MDC, Eotaxin についても比較検討した。玉置らは AD 患者を中心に血清中の MDC, Eotaxin-2, Eotaxin-3, CTACK, MEC の値を ELISA で検討した。また、末梢血好酸球数増多や血清 IgE 値上昇などで AD と共通した病態を示す水疱性類天疱瘡（BP）や菌状息肉症（MF）患者でも、同様に血清 TARC 値を検討した。中村らは AD 病変部皮膚における TARC と CCR4 の発現について in situ hybridization 法を用いて検討した。(2) KC からの TARC, MDC 産生制御について義江らは正常ヒト培養表皮細胞（NHKC）を、玉置らは細胞株 HaCaT 細胞を各々用いて検討した。中村らは HaCaT 細胞からの TARC 産生について AD の治療で用いられる紫外線（UVA）と TGF-β1 による影響について検討した。古江らは線維芽細胞からの TARC 産生制御を HaCaT 細胞と比較検討した。玉置らは KC からの Eotaxin-3, CTACK, MEC 産生制御について、HaCaT 細胞を用いて検討した。(3) 動物モデルとしては KC に特異的に発現するトランスジェニックマウス（Tg）を玉置らは TARC, CTACK について、中村らは VEGF (vascular endothelial cell growth factor) について作成し解析した。(4) 義江らは皮膚指向性メモリー/エフェクター細胞で選択的に発現している CCR10 の発現を B 細胞で検討した。(5) 師井らは恒常的 CCR4 発現細胞（マウスリンパ腫細胞 EL4/CCR4）を用いて細胞遊走試験を行い、各種のシグナル伝達阻害剤を用いた実験により CCR4 シグナル伝達経路を解析した。(6) 中村らは AD 患者末梢血より採取した単核球をダニ抗原と培養し、単核球が産生する TARC 値を健常人のそれと比較した。また、AD の治療に使われる各種薬剤の影響も併せて検討した。(7) 玉置、師井らは血中 TARC 値の臨床におけるマーカーとしての可能性について検討した。

（倫理面への配慮）

患者血清、末梢血リンパ球などについては、充分な理解と同意を得た上で研究を行なった。マウスに対する実験は、動物愛護の観点から充分に配慮して行なった。

C. 研究結果

(1) 義江らは TARC に関しては血漿と比べ血清の値が 5-20 倍程度高くなること、特に AD 患者で差が大きいことを見いだした[1]（文献番号は G.研究発表 1.論文発表に対応）。また eotaxin についても血清では値が若干上昇したが、MDC については血漿と血清で大きな違いはなかった。玉置らは、AD 患者において血清 MDC 値が高値を示し、病勢を反映することを示した[2]。また、Eotaxin-2 値は上昇しないが、Eotaxin-3 値は上昇し病勢と相関した[3]。CTACK に関しては、AD および Th1 優位の皮膚疾患である尋常性乾癬（PsV）患者の血清中で上昇しており、特に AD 患者では病勢と相関した[4]。MEC に関しては、AD, PsV, BP 患者の血清中で上昇していた。TARC に関しては、BP 患者[5]では血清および水疱液中で、MF 患者[6]では血清中で上昇しており、各々病勢を反映することを明らかにした。中村らは皮膚病変部の KC

に TARC mRNA 発現を、真皮浸潤細胞に TARC, CCR4 mRNA 発現を認めた[7]。(2) 義江らは NHKC について TARC, MDC の mRNA 発現はともに IFN-γで誘導され、IL-4 は効果がないことを明らかにした[8]。玉置らは HaCaT 細胞からの TARC[9], MDC[10]産生が TNF-αと IFN-γによって増強され、IL-4 により抑制されることを示した。中村らは HaCaT 細胞からの TARC 産生が、TGF-β[11]や紫外線（UVA）[12]により抑制されることを明らかにした。古江らは KC と異なり線維芽細胞では、TNF-α＋IL-4 の存在下で TARC 産生が誘導され、IFN-γはそれをさらに増強させることを明らかにした[13]。玉置らは HaCaT 細胞からの Eotaxin-3 産生が IL-4 や IL-13 により増強されること、また CTACK や MEC 産生が TNF-αと IL-1βによって増強されることを明らかにした。またロキシスロマイシンが表皮 KC からの TARC 産生を抑制することを示し、臨床上の有用性についての機序を明らかにした。(3) 動物モデルとして玉置らが作成した TARC Tg では皮膚炎の自然発症はみられなかったが、接触皮膚炎反応を行なうと Th1 型反応は抑制され、Th2 型反応は増強された。また、Th2 型接触皮膚炎反応において、Tg マウスでは Non-Tg マウスと比較して肥満細胞数が有意に増加し、血清中 IgE 値も有意に増加した。中村らが作成した VEGF Tg では加齢に伴い口囲，顔面，耳介に皮膚炎を生じた。組織学的に表皮 KC の増殖、真皮乳頭層の血管増生、血管周囲性に細胞浸潤を認めた。また、TNCB による接触皮膚炎反応により著明な皮膚炎の増悪を認めた。(4) 義江らは CCR10 が B 細胞では形質細胞の段階で選択的に発現することを見出し、MEC は CCR10 を介して形質細胞の骨髄や粘膜組織への帰巣を促進することを明らかにした[14]。(5) 師井らは EL4/CCR4 を用いた細胞遊走試験において、MEK1/2 阻害剤である U-0126 では遊走能が 50%以下にまで抑制されることを示した[15]。(6) 中村らは AD 患者末梢血由来の単核球が産生する TARC は、健常人のそれと比較して有意に高いことを示した。また、ステロイド、シクロスポリン、タクロリムスなどの薬剤が TARC 産生を抑制することを明らかにした[16]。(7) 玉置、師井らは血清 TARC 値が日常臨床で AD のマーカーとして用いられる可能性を示唆する結果を得た。

D. 考察

これまでの研究によって、(1) KC からサイトカインによる刺激で産生増強される TARC, MDC に加えて、KC から産生される Eotaxin-3, CTACK, MEC などもアレルギー性皮膚疾患に重要な働きをしていることが示された。TARC, MDC では CCR4 発現 Th2 細胞の、Eotaxin-3, MEC では CCR3 発現好酸球の、CTACK, MEC では CCR10 発現 T 細胞の皮膚への遊走に関与していると考えられる。そして(2) in vitro によるこれらケモカイン産生制御の研究から in vivo における炎症への関わり合いも示唆されたと考えている。(3) 動物モデルとして作成した TARC Tg の解析から、TARC はそれのみでは炎症を引き起こさないが、一旦炎症がおこると CCR4 陽性 Th2 細胞を遊走させ、Th2 優位な状態を誘導して炎症を修飾すると考えられた。その際、肥満細胞数の増加、血清 IgE 濃度の増強といった AD に似た状態がみられ、こういった AD の病態に TARC が関与していることが示唆された。現在、抗炎症作用を有するシグナル伝達阻害剤（CX-659S など）の軟膏を用いて、動物モデルで惹起された炎症の抑制効果を検討中である。また、別に作成した CTACK Tg の解析も現在進めている。また VEGF Tg の解析から、VEGF はアレルギー炎症で血管増生、炎症細胞の浸潤を誘導することが明らかとなった。これらの動物モデルに関する検討からアレルギー性皮膚疾患

の理解が進み、それに基づいた治療戦略の開発が期待される。(4) CCR10 の発現細胞として形質細胞を同定したことは CCR10 に作用するケモカイン CTACK, MEC の生理機能に関して新たな地平を開いた。皮膚におけるこれらのケモカインの産生と形質細胞の遊走との関係が注目される。(5) CCR4 を介したシグナル伝達系では、MEK1/2 を介した経路が細胞遊走に重要な役割を果たしていることが示唆された。CCR4 を発現する T 細胞の皮膚への浸潤・遊走が大きな一因となっている AD や MF に対して、シグナル伝達阻害剤の臨床応用・効果が期待される。(6) AD 患者末梢血由来の単核球から産生される TARC が健常人に比べて極めて多いことが示され、Th2 優位な病態に重要であることが示唆された。(7) 日常臨床で使用可能なマーカーを見つけ出すことは、皮膚アレルギー疾患の診療において極めて有用である。

E. 結論

我々は AD、PsV、BP、MF などのアレルギーが関与する皮膚疾患患者の血清中ケモカイン濃度の測定に始まり、培養 KC を用いた in vitro でのケモカイン産生機序に関する解析、さらに表皮 KC にケモカイン・サイトカインを過剰発現させた in vivo での動物モデルの解析へと研究を進めてきた。その結果、皮膚アレルギー炎症発症には TARC/CCL17, MDC/CCL22 とその受容体 CCR4、CTACK/CCL27, MEC/CCL28 とその受容体 CCR10、Eotaxin/CCL11, Eotaxin-3/CCL26 とその受容体 CCR3 や、VEGF, IL-4, TNF-αなどのサイトカインが重要な働きをしていることを明らかにした。このような皮膚の病態解析に関する検討からアレルギー性皮膚疾患の理解がより深まり、さらに新たな治療戦略の開発へと結びついていくものと期待される。

F. 健康危険情報　なし

G. 研究発表

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H. 知的財産権の出願、登録状況

1. 特許取得　特許出願平 9-513294
　　特許出願 2004-210465
2. 実用新案登録　なし
3. その他　なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Kagami S, et al.	Significant elevation of serum levels of eotaxin-3/CCL26, but not of eotaxin-2/CCL24, in patients with atopic dermatitis: serum eotaxin-3/CCL26 levels reflect the disease activity of atopic dermatitis.	Clin Exp Immunol	134	309-13	2003
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（倫理面への配慮）

本研究の過程で取扱った個人情報については、漏洩することのないように主任研究者が責任を持って保護致します。

Presence of high contents of thymus and activation-regulated chemokine in platelets and elevated plasma levels of thymus and activation-regulated chemokine and macrophage-derived chemokine in patients with atopic dermatitis

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Background: T_H2 cells and eosinophils selectively express CC chemokine receptor 4 and CCR3, respectively, and their chemokine ligands are likely to play important roles in the pathogenesis of atopic dermatitis (AD).
Objective: The purpose of this study was to demonstrate the presence of thymus and activation-regulated chemokine (TARC) in platelets and its release during clotting and to evaluate the circulating levels of TARC, macrophage-derived chemokine (MDC), and eotaxin in control subjects and patients with AD.
Methods: We compared plasma and serum contents of TARC, MDC, and eotaxin. We measured TARC contents in platelet lysates. We analyzed the correlation of plasma levels of TARC, MDC, and eotaxin with various clinicolaboratory parameters in patients with AD.
Results: Serum contents of TARC rapidly increased during clotting, whereas those of MDC and eotaxin increased only slightly. We demonstrated that platelets contained TARC, and its levels were dramatically elevated in patients with AD. Platelets also released TARC on stimulation with thrombin. We therefore evaluated circulating levels of these chemokines in control subjects and patients with AD by using plasma samples. Plasma TARC levels were significantly increased in patients with AD ($P < .0001$) and showed significant correlations with severity scoring of atopic dermatitis (SCORAD) index ($r = 0.665$, $P < .00001$), serum lactate dehydrogenese levels ($r = 0.696$, $P = .00001$), eosinophil counts ($r = 0.381$, $P = .007$), and platelet counts ($r = 0.562$, $P < .0001$). Similarly, plasma MDC levels were significantly increased in patients with AD ($P < .0001$) and showed significant correlations with SCORAD index ($r = 0.727$, $P < .0001$), serum lactate dehydrogenese levels ($r = 0.861$, $P < .0001$), eosinophil counts ($r = 0.505$, $P = .005$), and platelet counts ($r = 0.370$, $P = .01$). On treatment, plasma TARC and MDC levels were dramatically decreased in accordance with improved SCORAD scores ($P = .0012$ and $P = .0007$, respectively). On the other hand, plasma eotaxin levels did not show any significant increase or correlation with any of the clinical parameters in patients with AD.
Conclusion: Platelets from patients with AD contain high levels of TARC. Thus platelets might play an important role in AD pathogenesis by releasing T_H2-attracting TARC on activation. Furthermore, circulating levels of TARC and MDC, but not those of eotaxin, correlate well with the disease activity of AD. (J Allergy Clin Immunol 2002;110:139-46.)

Key words: Atopic dermatitis, chemokine, chemokine receptor, platelet, T_H2, eosinophil

Atopic dermatitis (AD) is a chronic inflammatory disease of the skin that is frequently associated with high serum IgE levels and eosinophilia.[1] Acute lesions

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Supported by grants from the Ministry of Health, Labor and Welfare, and from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
Received for publication November 14, 2001; revised April 1, 2002; accepted for publication April 26, 2002.

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0091-6749/2002 $35.00 + 0 1/85/126079
doi:10.1067/mai.2002.126079

Abbreviations used
AD: Atopic dermatitis
CLA: Cutaneous lymphocyte antigen
LDH: Lactate dehydrogenese
MDC: Macrophage-derived chemokine
SCORAD: Severity scoring of atopic dermatitis
TARC: Thymus and activation-regulated chemokine

are characterized by marked perivascular infiltration of $CD4^+$ memory-effector T cells expressing high levels of cutaneous lymphocyte antigen (CLA).[2] Memory-effector T cells of the CD4 lineage are now subdivided into T_H1 and T_H2 types in accordance with their cytokine profiles. T_H1 cells produce IL-2, IFN-γ, and TNF-α, for example, and are responsible for cell-mediated immunity, whereas T_H2 cells produce IL-4, IL-5, IL-6, and IL-13, for example, and are involved in humoral immunity and allergic diseases.[3] T_H2-dominant immune responses to environmental allergens in the skin on the basis of undefined genetic predispositions are the central features of AD.[2]

Chemokines are a group of cytokines that regulate migration and activation of various types of leukocytes through a group of 7 transmembrane G protein–coupled receptors.[4] Recent studies have revealed that T_H1 and T_H2 cells differentially express chemokine receptors. T_H1 cells selectively express CXCR3 and CCR5, whereas T_H2 cells express CCR4 and, less frequently, CCR3.[4] Furthermore, essentially all CLA^+ skin-seeking memory-effector T cells were found to express CCR4.[5,6] On the other hand, eosinophils mainly express CCR3.[7,8] Thus the CCR4 ligands, as well as those of CCR3, are likely to play important roles in the pathogenesis of AD through selective recruitment of T_H2 cells and eosino-phils into inflamed skin.

CCR4 is the receptor for 2 CC chemokines, thymus and activation-regulated chemokine (TARC)/CCL17 and macrophage-derived chemokine (MDC)/CCL22, whereas CCR3 is the receptor for eotaxin/CCL11 and many other CC chemokines known to act on eosinophils.[4,8] Increased expression of TARC and MDC in the skin was demonstrated in the NC/Nga strain of mouse, a murine model of AD.[9] Serum levels of TARC and MDC were shown to be increased in patients with AD but not in patients with psoriasis and other T_H1-type diseases.[10,11] Enhanced infiltration of CCR4-expressing T cells in AD skin lesions was also demonstrated.[12] Thus it is likely that TARC and MDC are produced in large quantities in AD lesional skin, leading to skin infiltration of T cells expressing CCR4 and to increased levels of these chemokines in the circulation.

The use of serum samples in the previous studies for the evaluation of circulating levels of TARC and MDC,[10,11] however, might have potential problems because (1) some chemokines are known to be secreted from platelets,[13] (2) many chemokines can be released from Duffy antigen receptor for chemokines (ie, the chemokine scavenger receptor expressed on erythrocytes),[14,15] and (3) some chemokines are even adsorbed to newly formed clotting matrix.[16] Here we compared the contents of TARC, MDC, and eotaxin in plasma and serum samples simultaneously obtained from the same donors. We found that platelets contained TARC and released it during clotting. We therefore reevaluated the circulating levels of TARC, MDC, and eotaxin in control subjects and patients with AD using plasma samples.

METHODS

Subjects and samples

A total of 29 patients with AD (22 children <16 years of age and 7 adults ≥16 years) and 29 healthy age-matched donors (19 children <16 years of age and 10 adults ≥16 years) were examined. Mean age ± SD was 8.0 ± 5.0 in children with AD, 9.9 ± 3.8 in control children, 24.6 ± 4.7 in adults with AD, and 27.8 ± 3.8 in control adults. The healthy control subjects had no history of allergic diseases, and their serum IgE levels were less than 160 IU/mL, with no detectable specific IgE antibodies to common inhaled allergens, as determined with Phadiatope (Pharmacia Upjohn, Uppsala, Sweden). The patients with AD met the clinical diagnostic criteria for the disease[1]; that is, they showed high serum IgE levels and were sensitive to more than one inhaled or food allergen, as demonstrated by a positive RAST result. Severity of AD was evaluated by using the severity scoring of atopic dermatitis (SCORAD) index.[17] Serum IgE and lactate dehydrogenese (LDH) levels and peripheral blood eosinophil and platelet counts were routinely determined. Blood samples were obtained on 35 occasions from 22 children with AD and on 18 occasions from 7 adults with AD in the course of treatment for AD. For control donors, blood sampling was done once for each individual. Venous blood was collected in EDTA-containing tubes to prepare plasma, and plasma was separated within 30 minutes by means of centrifugation at 1800*g* and 4°C for 10 minutes twice and stored at −20°C until assay. Venous blood was taken in Vacutainner tubes containing SST gel and clot activator (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 1800*g* and 4°C for 10 minutes after incubation and for 1 hour at room temperature, unless otherwise indicated, to prepare serum. Plasma chemokine levels were also determined before and after treatment for 15 patients with severe AD (SCORAD >70). After the first sampling of blood, the patients were treated with topical corticosteroids with high to medium potency, skin hydration with bathing, application of an occlusive agent (eg, petroleum jelly after bathing), and oral antihistamines (eg, diphenhydramine and ketotifen). None of the patients received systemic steroids. After 1 to 2 weeks of treatment, the patients were evaluated for disease severity, and the second blood sampling was performed. This study was carried out with the approval of the Ethics Committee of National Mie Hospital. Informed consent was obtained from all subjects.

ELISA for chemokines

Eotaxin and MDC levels were determined by using the sandwich-type ELISA, as described previously.[10,15] A sandwich-type ELISA for TARC with a detection limit of 0.6 pg/mL was developed by using 2 newly generated mouse monoclonal anti-TARC antibodies and will be described elsewhere (Morita et al, in preparation). A sandwich-type ELISA for RANTES with a detection limit of 2 pg/mL was purchased from Amersham Pharmacia Biotech (Piscataway, NJ).

Platelets

Platelets were purified from platelet-rich plasma, as previously described.[18] The purity of platelets was routinely greater than 99%. Purified platelets were mechanically lysed by means of ultrasonic treatment. TARC and RANTES contents in platelet lysates were measured with ELISA, and total protein contents were measured with a protein assay kit (Bio-Rad Laboratories, Hercules, Calif). Stimulation of platelets with thrombin was also carried out, as previously described.[18] Platelets at 1×10^8/mL were incubated at 37°C for 60 minutes with 1 U/mL thrombin (Sigma, St Louis, Mo). TARC contents in the supernatants were measured with ELISA.

Statistical analysis

Data on serum IgE levels, blood eosinophil counts, and plasma contents of TARC, MDC, and eotaxin were expressed as geometric means because logarithmically transformed values of the data followed normal distribution. Differences were analyzed with the Mann-Whitney *U* test for unpaired samples and with the Wilcoxon signed-rank test for paired samples. Spearman correlation coefficients between 2 parameters were calculated.

RESULTS

Rapid increases in serum TARC/CCL17 levels during blood clotting

We simultaneously collected plasma and serum samples from control subjects and patients with AD and determined the contents of TARC/CCL17, MDC/CCL22, and eotaxin/CCL11. As shown in Fig 1, TARC contents were dramatically increased in the serum samples. Furthermore, the serum/plasma ratio was much greater in patients with AD than in control subjects (16.2 ± 9.2 [n = 14] vs 6.0 ± 5.0 [n = 21], respectively; $P < .01$). On the other hand, serum MDC contents were only slightly increased from those in plasma samples, and a significant increase was only seen in control subjects. In the case of eotaxin, there were also slight but significant increases in serum contents in both control subjects and patients with AD.

We next determined the time course of serum chemokine levels by collecting serum samples at various time points during the clotting reaction at 37°C. Blood samples were obtained from 6 patients with AD. TARC contents rapidly increased during the clotting reaction, reaching maximal levels at 10 minutes (data not shown). On the other hand, MDC contents showed small increases, and those of eotaxin increased gradually during the 2 hours of incubation (data not shown). The latter was most probably caused by the release of eotaxin from Duffy antigen receptor for chemokines, the chemokine scavenger receptors expressed on erythrocytes,[14] during incubation, as described previously.[15] Consistent with this idea, we observed release of large amounts of eotaxin by washing erythrocytes at 4°C with a citrate buffer, pH 3.0 (data not shown).

Elevated contents of TARC/CCL17 in platelets from patients with AD

The rapid increases in serum TARC contents during clotting were likely caused by release of TARC from platelets. Furthermore, patients with AD generally

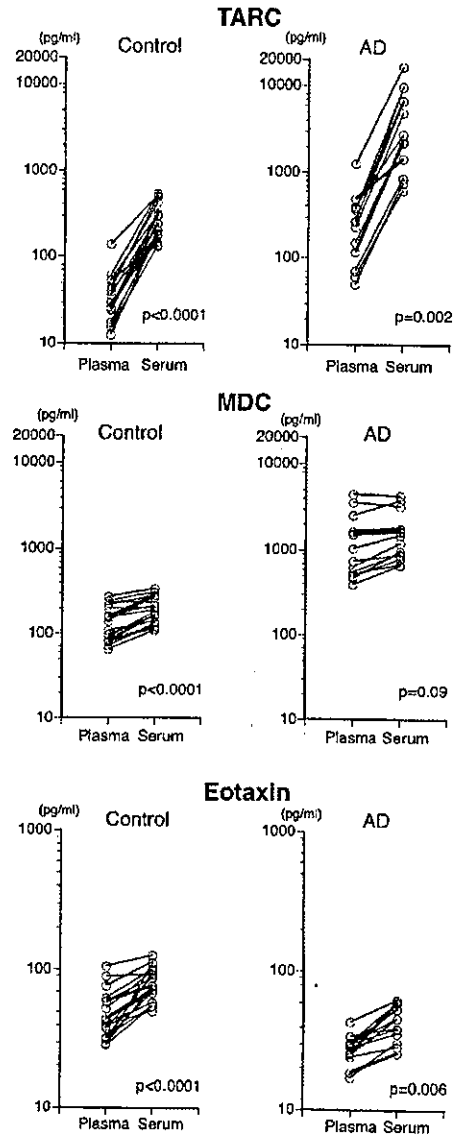

FIG 1. Comparison between plasma and serum for contents of TARC, MDC, and eotaxin. Plasma and serum samples were simultaneously obtained from control subjects (n = 21) and patients with AD (n = 14). Amounts of TARC, MDC, and eotaxin were measured with ELISA. All measurements were done in triplicate, and mean values were obtained. The mean values of TARC were as follows: control subjects, 35.2 pg/mL in plasma (range of SD, 19.3-64.1 pg/mL) and 240.5 pg/mL in serum (range of SD, 150.8-383.3 pg/mL); patients with AD, 253.2 pg/mL in plasma (range of SD, 80.3-793.4 pg/mL) and 3225.9 pg/mL in serum (range of SD, 1174.7-8859.0 pg/mL). The mean values of MDC were as follows: control subjects, 132.3 pg/mL in plasma (range of SD, 82.1-213.0 pg/mL) and 195.1 pg/mL in serum (range of SD, 137.1-277.6 pg/mL); patients with AD, 1158.3 pg/mL in plasma (range of SD, 505.1-2656.5 pg/mL) and 1590.9 pg/mL in serum (range of SD, 754.9-3352.7 pg/mL). The mean values of eotaxin were as follows: control subjects, 45.9 pg/mL in plasma (range of SD, 31.1-67.8 pg/mL) and 79.1 pg/mL in serum (range of SD, 60.1-103.9 pg/mL); patients with AD, 29.8 pg/mL in plasma (range of SD, 20.4-43.6 pg/mL) and 44.1 pg/mL in serum (range of SD, 33.2-58.5 pg/mL).

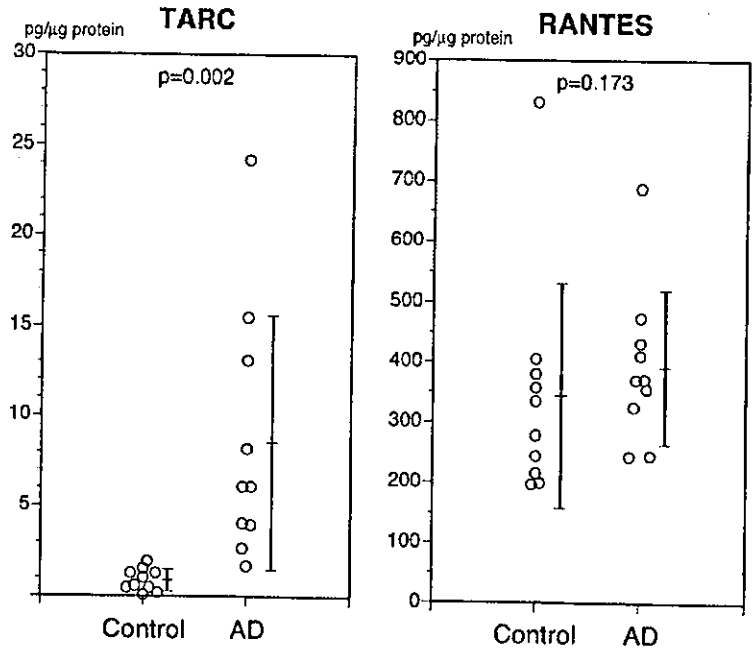

FIG 2. Presence of TARC in platelets. Highly purified platelets were obtained from control subjects (n = 10) and patients with AD (n = 10). Contents of TARC and RANTES in platelet lysates were measured with ELISA. All measurements were done in triplicate, and mean values were obtained.

showed much steeper increases in serum TARC contents than control subjects (Fig 1). We, however, found no significant difference in blood platelet counts between control subjects and patients with AD ($30.7 \pm 5.2 \times 10^4/mm^3$ vs $35.8 \pm 9.8 \times 10^4/mm^3$, respectively). We therefore determined TARC contents in platelet lysates prepared from 10 control subjects and 10 patients with AD. As shown in Fig 2, platelets indeed contained TARC. Furthermore, platelets from patients with AD consistently contained much larger amounts of TARC than those from control subjects (mean ± SD values of control subjects and patients with AD: 0.9 ± 0.6 pg/μg protein and 8.6 ± 7.1 pg/μg protein, respectively; $P = .002$). On the other hand, no such significant difference was seen in the platelet contents of RANTES/CCL5 between control subjects and patients with AD (mean ± SD values: 344.9 ± 187.2 pg/μg protein and 392.4 ± 127.9 pg/μg protein, respectively; $P = .173$). To further confirm the biologic significance of TARC in platelets, we treated platelets with 1 U/mL thrombin. Platelets released 79.2% ± 3.6% of TARC on thrombin stimulation, whereas spontaneous release was 32.7% ± 10.7% (n = 6).

Elevated plasma levels of TARC and MDC in patients with AD

The observed differences between serum and plasma in terms of chemokine contents prompted us to reevaluate the circulating levels of TARC, MDC, and eotaxin in control subjects and patients with AD by using their plasma samples. As shown in Fig 3, the plasma levels of TARC and MDC were significantly elevated in patients with AD ($P < .0001$). On the other hand, there were no significant differences in the plasma eotaxin levels between patients with AD and control subjects ($P = .121$). Notably, there were significant age-related changes in the plasma MDC levels among the control subjects; those younger than 16 years (n = 19) and those 16 years of age or older (n = 10) had mean MDC values of 443.2 pg/mL (range of SD, 219.4-896.5 pg/mL) and 121.6 pg/mL (range of SD, 69.5-212.7 pg/mL), respectively ($P = .002$). No such age-related changes were seen in the plasma levels of TARC and eotaxin among the control subjects.

Correlation of plasma TARC and MDC levels with other disease parameters in patients with AD

We next analyzed the correlation of the plasma chemokine level and SCORAD index in the patients with AD (n = 53; samples obtained from the same donors in different occasions were included). As shown in Fig 4, *A*, there were strong positive correlations between SCORAD scores and plasma levels of TARC ($r = 0.665$, $P < .0001$) and MDC ($r = 0.727$, $P < .0001$). There was, however, no significant correlation between SCORAD scores and

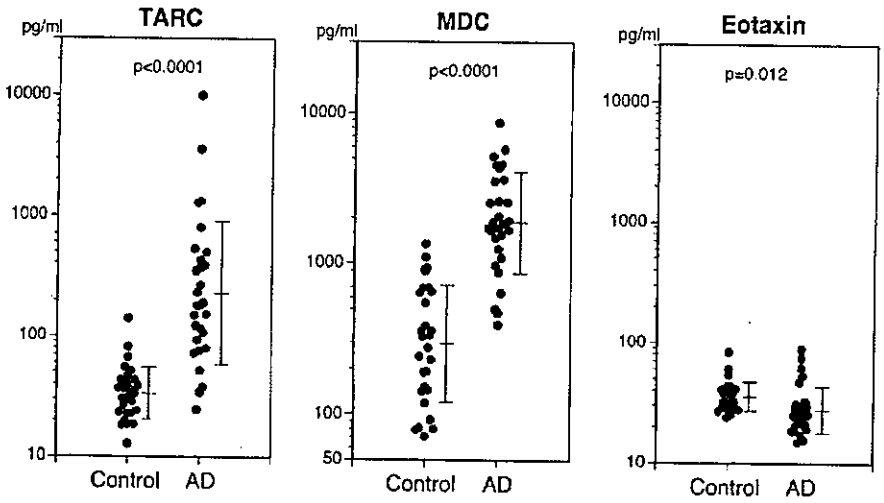

FIG 3. Elevated plasma levels of TARC and MDC in patients with AD. TARC, MDC, and eotaxin contents in plasma samples obtained from control subjects and patients with AD were measured with ELISA. All measurements were done in triplicate, and mean values were obtained. The mean values of control subjects and patients with AD were as follows: TARC, 33.4 pg/mL (range of SD, 20.2-55.0 pg/mL) and 228.6 pg/mL (range of SD, 58.2-897.2 pg/mL), respectively; MDC, 296.7 pg/mL (range of SD, 121.5-724.4 pg/mL) and 1891.2 pg/mL (range of SD, 869.9-4111.3 pg/mL), respectively; and eotaxin, 36.0 pg/mL (range of SD, 27.3-47.3 pg/mL) and 27.9 pg/mL (range of SD, 17.9-43.7 pg/mL), respectively.

plasma eotaxin levels (r = 0.104, P = .483). We also compared the plasma chemokine levels before and after treatment. Fifteen patients with severe disease (>70 SCORAD scores) were treated with a standard combination of skin hydration, topical steroids, and oral antihistamines. After 1 to 2 weeks, when their symptoms were improved, the second plasma samples were obtained. As shown in Fig 4, *B*, the mean SCORAD index was decreased from 81.7 to 39.3 (P = .0007). Accordingly, the plasma levels of TARC and MDC were significantly decreased (P = .0012 and P = .0007, respectively). On the other hand, no such significant change was seen in the plasma eotaxin levels (P = .3).

We further analyzed the correlation between the plasma chemokine levels and various clinicolaboratory parameters known to be increased in patients with AD. The results are summarized in Table I. Plasma TARC and MDC levels strongly correlated with serum LDH levels and weakly with blood eosinophil counts. Notably, plasma TARC and MDC levels also significantly correlated with blood platelet counts. Plasma TARC and MDC levels, however, showed no significant correlation with total serum IgE levels. Plasma eotaxin levels showed no significant correlation with any of these parameters.

DISCUSSION

Platelets play an important role not only in blood coagulation but also in inflammation.[19,20] Involvement of platelets in the pathogenesis of allergen-induced asthma has been reported.[21,22] Selective depletion of circulating platelets was shown to reduce the late-phase response, eosinophil infiltration, and bronchial hyperresponsiveness in animal models of asthma.[23,24] Platelets are also known to release various chemokines.[25-27] Release of RANTES from platelets on cross-linking of the high-affinity receptor for IgE has been suggested to promote allergic reactions by attracting CCR3-expressing cells, such as eosinophils.[18] Here we have demonstrated, for the first time, that platelets contain TARC (Fig 2) and release it on coagulation and thrombin stimulation. Furthermore, the contents of TARC, but not those of RANTES, in platelets are dramatically elevated in patients with AD (Fig 2). Plasma contents of TARC also strongly correlated with blood platelet counts in patients with AD (Table I). These results suggest that the T_H2-dominant conditions in AD enhance production of TARC, as well as that of platelets, by megakaryocytes in the bone marrow. Platelets are also known to express CCR4 and CXC4, and their ligands, TARC, MDC, and stromal cell–derived factor 1/CXCL12, have been shown to induce platelet aggregation through a pathway dependent on ADP and the P2Y(1)-type ADP receptor.[28-31] Thus release of TARC from activated platelets might further enhance their aggregation and release of TARC in an autocrine manner. Collectively, platelets in patients with AD, by releasing TARC within inflamed microvasculature, might play an important role in extravasation of T_H2 cells and CLA^+ skin-seeking memory T cells expressing CCR4.[4]

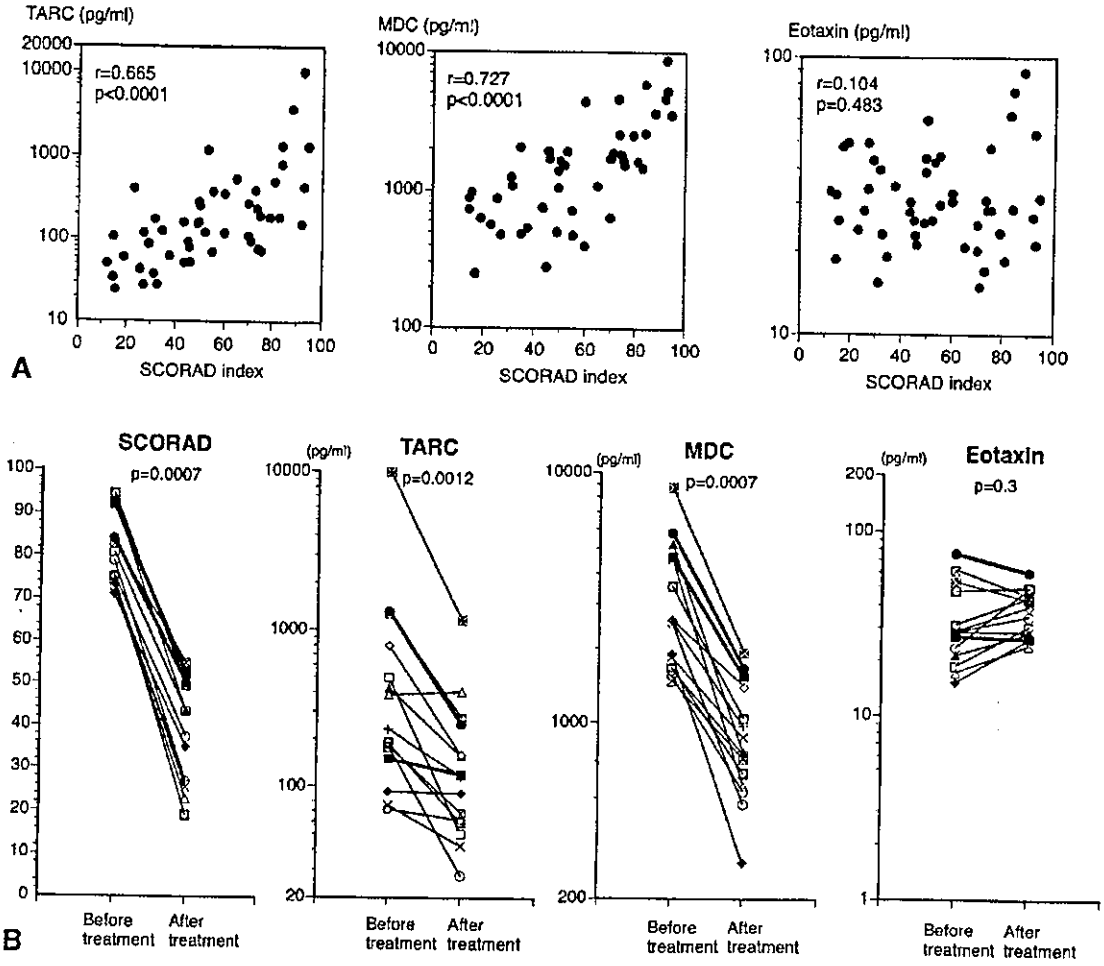

FIG 4. A, Correlation of plasma chemokine levels with disease activity of AD. Contents of TARC, MDC, and eotaxin in plasma samples obtained from patients with AD were measured with ELISA. All measurements were done in triplicate, and mean values were obtained. Disease severity was expressed by SCORAD index. B, Changes in SCORAD index and plasma chemokine levels before and after treatment. Mean values before and after treatment were as follows: SCORAD score, 81.7 and 39.3, respectively; TARC, 346.7 pg/mL (range of SD, 93.0-1292.9 pg/mL) and 120.5 pg/mL (range of SD, 45.5-319.0 pg/mL), respectively; MDC, 2876.3 pg/mL (range of SD, 1644.6-5030.6 pg/mL) and 836.8 pg/mL (range of SD, 494.0-1417.4 pg/mL), respectively; eotaxin, 30.3 pg/mL (range of SD, 18.6-49.5 pg/mL) and 36.1 pg/mL (range of SD, 27.0-48.3 pg/mL), respectively.

TABLE I. Spearmann correlation coefficients between plasma chemokine levels and other laboratory parameters in patients with AD

	LDH	Eosinophil count	Platelet count	IgE level
TARC	0.696 ($P < .0001$)	0.381 ($P = .007$)	0.562 ($P < .0001$)	0.004 ($P = .835$)
MDC	0.861 ($P < .0001$)	0.505 ($P = .0002$)	0.370 ($P = .01$)	0.008 ($P = .635$)
Eotaxin	0.003 ($P = .858$)	−0.260 ($P = .07$)	0.036 ($P = .819$)	0.008 ($P = .635$)

A total of 48 plasma samples were analyzed.

Previous reports demonstrated dramatic increases in serum TARC and MDC contents in patients with AD.[10,11] However, TARC contents in the serum are highly increased because of its release from platelets (Fig 1). Therefore we reevaluated the circulating contents of TARC, MDC, and eotaxin in control subjects and patients with AD by using plasma samples. We demonstrated that TARC and MDC levels in plasma were

increased in patients with AD and highly correlated with disease activity and the clinical course (Figs 3 and 4). This is the first observation that not only TARC but also MDC levels in circulation closely correlate with clinical status of AD. Correlation coefficients of plasma MDC levels with SCORAD scores, serum LDH levels, and eosinophil counts were even higher than those of plasma TARC levels (Fig 4 and Table I). Furthermore, there were only small differences between serum and plasma contents of MDC (Fig 1). Thus MDC might be more useful to monitor the disease course of AD than TARC. Notably, however, there are striking age-related differences in the plasma MDC levels among control subjects: Those younger than 16 years have much higher levels than those 16 years of age or older. Further studies are necessary to determine the age-related normal levels of MDC and a possible role of MDC during development.

In contrast, plasma levels of eotaxin were not significantly increased in patients with AD (Fig 3). Thus CCR3-directed chemokines different from eotaxin, such as RANTES, might play major roles in the accumulation of eosinophils in the lesional skin of patients with AD.[32,33] However, Hossny et al[34] recently reported that plasma eotaxin levels were significantly elevated in patients with AD. We do not know the cause of the discrepancy, but possible explanations might be different patient backgrounds, different timing of sampling, and different methods of assay. Furthermore, our observation does not exclude the possible involvement of eotaxin in the pathogenesis of AD. In fact, eotaxin expression was demonstrated to be highly upregulated in AD chronic skin lesions but not in acute skin lesion.[35]

In conclusion, TARC and MDC, the T_H2-type chemokines acting on CCR4, are likely to be closely involved in the pathogenesis of AD and possibly other T_H2-type diseases. Monitoring circulating levels of TARC and MDC might thus serve as useful markers for disease conditions in AD. Furthermore, platelets might play important roles in AD pathogenesis and other T_H2-type diseases by releasing TARC.

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Clin Exp Immunol 2002; 127:270–273

Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis

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(Accepted for publication 18 September 2001)

SUMMARY

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease characterized by the predominant infiltration of T cells, eosinophils and macrophages in lesional skin. Recently, macrophage-derived chemokine (MDC)/CCL22, a CC chemokine, was identified as a selective chemoattractant for CC chemokine receptor 4 (CCR4)-expressing cells, in addition to thymus and activation-regulated chemokine (TARC). We have previously reported that serum TARC levels correlate with the severity of AD. In this report, we investigated the participation of MDC in AD. First, we measured serum MDC levels in 45 patients with AD, 25 patients with psoriasis vulgaris and 25 healthy controls. Serum MDC levels in AD patients were significantly higher than those in healthy controls and psoriasis patients. Furthermore, the increases in serum MDC levels in AD patients were greater in the severely affected group than in the moderate or mild groups. We compared serum MDC levels in 11 AD patients, before and after treatment, and observed a significant decrease after treatment. Moreover, the serum MDC levels significantly correlated with the Scoring AD (SCORAD) index, serum soluble (s) E-selectin levels, serum soluble interleukin-2 receptor (sIL-2R) levels, serum TARC levels and eosinophil numbers in peripheral blood. Our study strongly suggests that serum MDC levels have a notable correlation with disease activity and that MDC, as well as the CC chemokine TARC, may be involved in the pathogenesis of AD.

Keywords MDC atopic dermatitis disease activity

INTRODUCTION

Atopic dermatitis (AD) is a chronic or chronically-relapsing skin disorder characterized by the infiltration of T cells, eosinophils, mast cells and macrophages in lesional skin [1,2]. Enhanced serum IgE levels, specific IgE environmental allergens such as house dust mites, and blood eosinophilia are also present in the majority of AD patients. It has been proposed that Th2-type cells play a key role in the pathogenesis of AD because of the increased expression of Th2-related cytokines, such as IL-4 and IL-5, in lesional skin [3] and the high responsiveness of peripheral blood mononuclear cells to IL-4, but not IL-2 [4]. Previously, it was shown that serum soluble (s) E-selectin and serum sIL-2 receptor (R) significantly correlate with the disease activity of AD [5–7].

Macrophage-derived chemokine (MDC), newly termed CCL22 [8], is a CC chemokine that potently serves as a chemoattractant for monocytes, monocyte-derived dendritic cells (DCs) and natural killer (NK) cells [9]. MDC is a ligand for CC chemokine receptor 4 (CCR4) [10], and is chemotactic for a fraction of CD4+CD45RO+T cells polarized to produce Th2-type cytokines [11]. We have previously shown that in NC/Nga mice, a mouse model for human AD, dermal DCs are immunoreactive for MDC, and that the immunoreactivity of dermal DCs for MDC was abolished by topical corticosteroid treatment [12]. This indicates that the DC is a main source of MDC in lesional skin of AD. Very recently, we reported that levels of thymus and activation-regulated chemokine (TARC), another ligand for CCR4, in AD sera significantly correlate with disease activity [13]. Moreover, it is reported that serum MDC levels in AD patients are higher than those in healthy controls [14], although the precise involvement of the high levels of MDC in AD has not yet been fully identified.

We measured serum MDC levels in a large number of patients with AD, and compared them with levels in psoriasis vulgaris patients and healthy controls. We also examined the correlation between serum MDC levels, disease severity and the change in serum MDC levels in AD patients, before and after treatment. In addition, we compared serum MDC levels with laboratory data for AD disease markers such as serum soluble (s) E-selectin,

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