# アトピー性皮膚炎のかゆみの解明と治療の標準化に関する研究

1）　本土・琉球クラスターにおけるアトピー関連遺伝子の探索に関する研究
2）　アトピー性皮膚炎の既存治療法のEBMによる評価と有用な治療法の普及

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研究要旨
1）アトピー性皮膚炎の疾患感受性遺伝子探索を目的とし、遺伝子背景の違いがわかってきている琉球クラスターと本土クラスターにおいて比較検討を進めながら、共通して変化し、臨床応用の可能性の高い候補遺伝子を策定する。すでに候補遺伝子としての同定がすんでいる遺伝子に関しては遺伝子改変マウスなどを用いて皮膚炎やかゆみへの貢献度を調べた。
2）前回のEBM評価（2003年まで）の2010年度アップデート版作成を目的とし、EBM評価に基づく治療法、合併症など、2009年9月までに発表された国内外の論文を検索・検討・評価し公表する。

## A. 研究目的

1）アトピー性皮膚炎の有病率は上昇しており、現在社会問題化している。本疾患の解明は未だ進んでおらず、分子生物学的アプローチを含めた、幅広い視点に立った病因の解明と新規治療薬の開発が切望されている。

最近、日本人がSNPタイピングにより大きくRyukyuとHondoクラスターの２つに分けられることが判明した。石垣島のコホート群における追跡調査、血液検査、遺伝子研究などで得られた成果 すなわち様々な臨床的アトピー関連因子や数々の候補遺伝子群やまた将来的にそれらを元にした病因の解明・新規治療などが日本人に広く応用可能であるかどうか、またはある特定の疾患サブグループや地域特異性（石垣島など）に認められる傾向にあるのかなどをより正確に検討するには、次なるステップとして同様の採血検査、遺伝子調査を本邦の他地域においても実施し、石垣島で得られた結果の有意性を確認する必要がある。そこで九州・山口地域一円からの人口流入地域である福岡と、全国からの人口流入地域である東京地域において得られる結果を、石垣スタディの結果と比較・検討したい。

２）これまで、平成14ー16年度（2002ー2004年度）の厚生労働省研究班（古江班）「アトピー性皮膚炎の既存治療法のEBMによる評価と有用な治療法の普及」のなかで、上記1. ～6. 課題について、前回のEBM評価で2003年までの国内外の発表論文を基に有効性と安全性を評価されている。そこで今回は、2010年度版EBM評価に基づく治療法、合併症などのアップデートを行う目的で最終的には2009年9月までに発表された国内外の論文を検索し、検討、評価するものであり、本稿は各項目の現在の進捗状況を示すものである。

## B. 研究方法

1）九州大学病院皮膚科、同総合診療部、慶応義塾大学病院皮膚科、東京大学にてアトピー性皮膚炎患者、健常者各1000人を目標に血液検体、アンケート調査票、ゲノムタイピングの結果を用いて解析する。

２）アトピー性皮膚炎（atopic dermatitis）と1. ～6. それぞれの関連キーワードを組み合わせ、2004ー2008年のPubMed（Medline）および医学中央雑誌（医中誌）による検索を行い、臨床効果・副作用等に関する論文を原則的にランダム化比較試験、システマティックレビュー、ケースコントロール試験、コホート研究を対象として内容を検討した。また、一部項目で、該当例がない、少ないものに関してはオープン試験やまとまった数の症例報告も参考として取り上げた。

（倫理面への配慮）

1）本研究は平成20年6月23日付けで九州大学ヒトゲ

ノム・遺伝子解析倫理審査専門委員会の承認を得ている。
2） 調査対象が既に公表された論文を検索、評価するものであるため倫理的問題は該当しないと考える。

C. 研究結果
1)本研究用に「医師調査票」と ISSAC13-14 と総合診療部調査票を元にした「患者アンケート票」を作成した。現在上記研究協力者の施設にて、倫理委員会での承認申請中、又は調査・血液サンプル取得中である。
2). 合併症（吉村、竹内）：ウイルス性疾患：(10 件)、細菌感染症：(12 件)、真菌感染症：(6 件)、白内障：(8 件)、漢方療法（江崎、竹内）：(清皮湯、消風散、黄皇経験方、複方苦参、桂枝加黄ろう湯・黄ろう解毒湯、補中溢気湯、温清散、当帰飲子、白虎加人参湯、桂枝茯苓丸ほか)：ランダム化比較試験が 2 件、10 件以上の症例集積研究が 9 件（それぞれ 30 例、94 例、10 例、65 例、23 例、14 例、36 例、34 例、158 例規模)、シクロスポリン内服療法（藤本）：ランダム化比較試験 2 件（タクロリムス外用との比較)、後ろ向きコホート 1 件（内服の有用性と安全性の検討)、症例集積研究（20 名以上）1 件、システマティックレビュー2 件、心身医学療法（羽白）：選択的セロトニン拮抗薬や新資料法・精神療法を中心に、RCT なし、メタアナリシス 2 件、比較研究 4 件、単群研究 7 件、症例報告 7 件、環境アレルゲン（秋山）：9 件が確認され、オックスフォード大 EBM センターのエビデンスレベル分類・評価に準拠している妥当性に関して考察した（幸野)。

D. 考察
現在までに九州大学病院皮膚科ではアトピー性皮膚炎患者 160 名の調査・サンプル採取が終了した。
2） 各研究分担者、研究協力者より提出された EBM は H22 年 10 月 1 日に HP にて公開された。

E. 結論
1)今後も継続的に積極的な患者への呼びかけとサンプル収集を行いたい。
2)公表後の年間アクセス数なども検討したい。

F. 健康危険情報
とくになし。

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H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
特になし。

2. 実用新案登録
特になし。

3.その他
特になし。

# アトピー性皮膚炎のかゆみの解明と治療の標準化に関する研究

## マウス皮膚炎モデルを用いた、皮膚炎・掻破行動・表皮内神経伸長に関連に関する研究

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研究要旨
かゆみと炎症の関連に関して、1)かゆみや掻破行動に与える免疫関連因子の探索とその影響を確認する。タクロリムスや副腎皮質ステロイド軟膏などの既存アトピー性皮膚炎の治療薬と比較して、我々が新規開発した新しい抗炎症薬であるMEK1/2シグナル阻害薬の皮膚炎や掻破行動に対する影響を慢性皮膚炎マウスモデルにて検証する。

A. 研究目的

アトピー性皮膚炎の慢性皮膚病変部においては、表皮内知覚神経侵入・伸長とその執拗な痒みとの関連が指摘されている。またマウスの慢性皮膚炎モデルにおいても、アトピー性皮膚炎治療外用薬タクロリムス(FK506)は表皮内神経伸長を抑制し、掻破行動も抑制すると報告され、関連が指摘されている。我々はこれまでに、ハプテン誘発性の慢性皮膚炎モデルにおいて、MAPK /ERK kinase1/2 (MEK1/2)阻害剤のひとつであるCX659Sが、FK506と同程度に表皮内神経伸長は抑制する一方、FK506 とは異なり掻破行動の抑制はしないことを確認した。そこで、CX695Sの掻破行動に対する効果を、NC/Ngaマウスなど別のアトピーモデルを用いてさらに検証したい。今回、ダニ精製蛋白抗原塗布を用いたアトピー性皮膚炎モデルをNC/Ngaマウスで作製し、皮膚炎・掻破の程度、表皮内神経伸長などを検討した。

B. 研究方法

NC/Ngaマウスの剃毛した背部と耳に、精製ダニ蛋白抗原（ビオスタAD®）を週に2回、4週間塗布して慢性皮膚炎を発症させた。外用薬として FK506、CX659S を用い、連日外用した。4週間後に、掻破行動、皮膚炎の状態、組織像等を比較検討した。

(倫理面への配慮)
本動物実験プロトコールは九州大学の動物実験に関する倫理委員会にて審議、認定されているプロトコールを用いて実験を行っている。

C. 研究結果

耳介腫脹反応」および「皮膚炎スコア」については、第2週から差が見られるようになり、FK外用群では陽性対照群と比較して有意に抑制されたものの、CX外用群では抑制されず、陽性対照群と同等の「耳介腫脹反応」が見られた。第4週目における「掻破行動」においても、FK外用群で陽性対照群と比較して有意に抑制されたものの、CX外用群では抑制される傾向がみられなかった。第4週後の血清総 IgE 値は FK 外用群でのみ有意に減少していたが、血清 TARC 値は群間で有意差はみられなかった。第4週後の背部皮膚炎惹起部の組織学的解析では、FK 外用群、CX 外用群ともに陽性対照群と比較して、有意に浸潤している「炎症細胞数」、「肥満細胞数」、および「IL-4陽性細胞数」は少なかった。「IL-31 陽性細胞数」「IFN-γ陽性細胞数」に関しては有意差は見られなかった。一方、「IL-17陽性細胞数」はFK 外用群でのみ有意に減少していた。「PGP9.5陽性神経線維」による表皮内神経伸長に関しては、FK 外用群、CX 外用群ともに陽性対照群と比較して、有意に神経伸長の程度が抑制されていた。

D. 考察

今回、精製ダニ蛋白抗原塗布を用いてアトピー性皮膚炎モデルの NC/Nga マウスに皮膚炎を発症させ、CX659Sの掻破行動に対する効果を検討した。組織学的な炎症細胞浸潤、および神経伸長に関してはハプテン塗布モデルと同様に FK外用群で有意な抑制がみられたが、掻破行動に関して CX 外用群では抑制が見られなかった。このことは、必ずしも炎症あるいは表皮内への神経伸長の抑制が掻破行動の抑制と直接関連しない、という可能性を示唆していると考えられた。浸潤細胞ではIL-17陽性細胞数でのみFK外用群と CX 外洋群で差が見られたため、Th17細胞と掻破行動・神経伸長との相関も検討する必要があると思われた。

E. 結論

表皮内への神経伸長や炎症細胞浸潤の抑制が必ずしも掻破行動の抑制に直接関連しないことが改めて示唆された。アトピー性皮膚炎治療

で臨床上問題となる痒みのメカニズム及びその治療を考える上で重要な知見と考える。

F. 健康危険情報
特になし。

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（予定を含む。）

1. 特許取得

特になし。

2. 実用新案登録

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Letter to the Editor

## Significant correlation of serum IL-22 levels with *CCL17* levels in atopic dermatitis

Atopic dermatitis (AD) is a common, chronic or chronically relapsing, severely pruritic eczematous skin disease, and Th1 and Th2 cytokines may differentially contribute to the pathogenesis of acute and/or chronic lesions of AD. The majority of allergen-specific T cells derived from skin lesions that had been provoked by the epicutaneous application of inhalant allergens were found to produce predominantly Th2 cytokines, which was initially considered to be a specific feature reflecting immune dysregulation in AD. Among various markers for AD including *CCL20* (macrophage inflammatory protein-3α) [1] and *CCL27* (cutaneous T cell-attracting chemokine) [2], serum CCL17 (thymus activation-regulated chemokine) level has been recognized to be a very sensitive and reliable parameter of disease severity of AD [2].

IL-22, a member of the IL-10 family, is known to be preferentially produced by Th17 cells [3,4]. IL-22 receptor is highly expressed on keratinocytes as well as other epithelial cells, inducing the production of antimicrobial proteins and keratinocyte proliferation [3]. Although IL-17 and IL-22 seem to be involved in the pathogenesis of psoriasis [3], attention has recently been drawn to a pathogenetic role of IL-22-producing T cells in AD [4,5]. There is a compelling evidence that IL-22-producing T cells, Th22 cells, distinct from Th17 and Th1 cells indeed exist and that the Th22 population is actually compartmentalized in lesional skin of AD with a reduction of Th17 cells [4,5]. It is intriguing that Th22 cells express the chemokine receptors CCR4, CCR6 and CCR10 targeted by *CCL17*, *CCL20* and *CCL27* [4]. In this study, we investigated the correlation of serum levels of these four chemo-cytokines in AD patients.

Sera were collected from 15 AD patients (mean ± SD age; 27.2 ± 6.1 years old) and 20 healthy volunteers (32.3 ± 6.5 years old) after written informed consent obtained. AD was diagnosed according to the Japanese Dermatological Association criteria [6]. The subjects received no systemic immunosuppressive drugs or corticosteroids. The experiment was approved by the Ethical Committee of Kyushu University. Quantification of the concentrations of IL-22, *CCL17*, *CCL20* and *CCL27* in sera was performed using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. Statistical analyses were performed using the 2-tailed Mann–Whitney *U* test for comparison between AD patients and normal controls and linear regression for ascertainment the correlation of serum levels of these markers. A *P*-value $< 0.05$ was considered significant.

The serum levels of IL-22 and *CCL17*, but not *CCL20*, were significantly higher in AD patients than those of normal controls (Fig. 1A). The serum *CCL27* levels tended to be elevated in AD, though they were not statistically significant compared with those in normal controls (Fig. 1A). As shown in correlation analysis, the serum levels of *CCL17* were significantly correlated with either those of IL-22 and *CCL27*, but not with *CCL20* (Fig. 1B). In addition, the serum IL-22 levels were significantly correlated with the serum *CCL27* levels ($r = 0.520$, $P = 0.039$). No significant association was observed either between the serum IL-22 and *CCL20* levels ($r = 0.446$, $P = 0.083$) or between the serum *CCL20* and *CCL27* levels ($r = 0.090$, $P = 0.748$).

In this study, serum IL-22 levels were elevated in AD patients compared with normal controls and were significantly associated with *CCL17* levels, suggesting that IL-22 could be considered as a activity marker for AD despite the narrow range of net values (10.2–53.4 pg/ml in this study) which was in sharp contrast to the wide range of net values (221.5–2144.0 pg/ml) of *CCL17* [2,5]. In the skin, IL-22 mediates keratinocyte proliferation and epidermal hyperplasia by downmodulating terminal differentiation genes, which highlighted a pathogenetic role of IL-22 in psoriasis [3]. Furthermore, patients with nickel contact dermatitis [7] and pityriasis rosea [8] showed elevated serum IL-22 levels. As for AD, the frequency of IL-22$^+$CD8$^+$ T cells in lesional skin of AD was correlated with the SCORing Atopic Dermatitis index [5], which supported our results. From the present and previous studies, IL-22 seems to play an important role in various inflammatory skin diseases including AD.

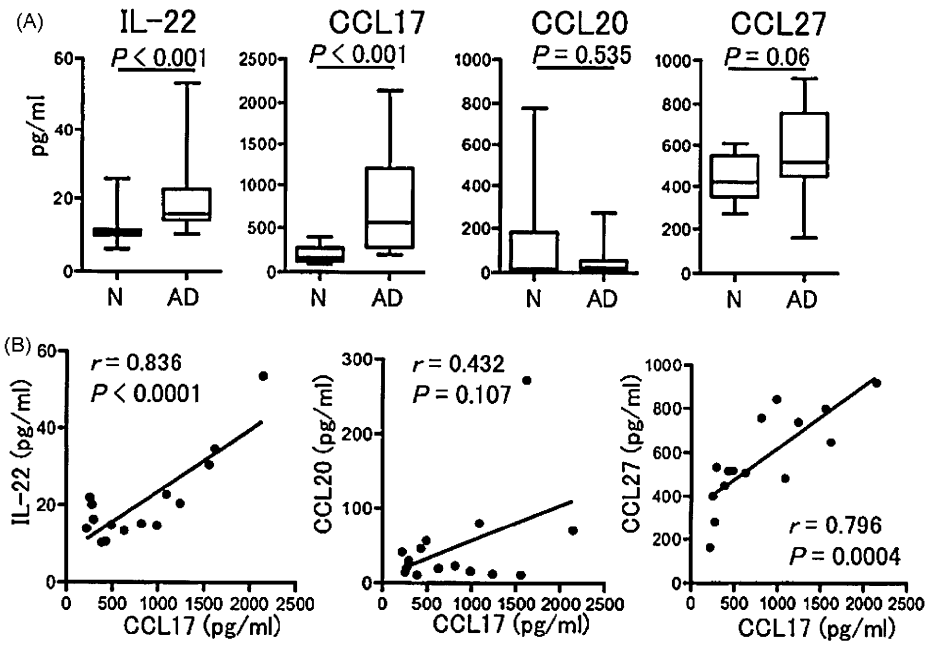


**Fig. 1.** (A) Serum concentrations of IL-22, *CCL17*, *CCL20* and *CCL27* in subjects using ELISA kits. The sera from normal controls (*N*; $n = 20$) and AD patients ($n = 15$) for measurement were collected. Boxes indicate 25–75% values, lines within boxes indicate medians, whiskers represent minimal to maximal of the data. *P*-values are determined by the Mann–Whitney *U* test. (B) Linear regression showing correlations of the serum levels of *CCL17* with those of IL-22, *CCL20* or *CCL27* in AD patients.

*CCL27* is a skin-associated chemokine that attracts skin-homing memory T cells produced mainly by activated keratinocytes in various skin diseases [2]. Similar to *CCL17*, *CCL27* preferentially attracts cutaneous lymphocyte antigen-positive Th2 cells from peripheral blood and the serum levels of *CCL27* have been shown to correlate with severity of AD [2]. In our study, the levels of *CCL27* in AD also significantly correlated with *CCL17* levels, although the elevation of serum *CCL27* did not reach to a statistical significance, which may be attributable to small sample size in our study. The significant correlation of IL-22 with *CCL27* levels found in the present study further supports the mutual positive interaction among *CCL17*, IL-22 and *CCL27* in the development of AD.

*CCL20* is a ligand for CCR6 that is constitutively expressed in normal skin and mucosa at low levels, but is strongly over-expressed in keratinocytes by proinflammatory cytokines and T cell-derived factors. Th17 cytokines including IL-17, IL-22 and TNF-α stimulate *CCL20* expression in keratinocytes in vivo and vitro [9]. Elevated serum levels and up-regulation of *CCL20* in lesional epidermis were shown in AD [1], however, Kim et al. demonstrated opposing evidence that AD skin was deficient in *CCL20* partly because of the overwhelming Th2-skewed milieu [10]. We also could not detect either the elevation of serum level of *CCL20* or its correlations with *CCL17*, IL-22 or *CCL27* level.

In conclusion, we have demonstrated for the first time that the elevated levels of IL-22 were significantly correlated with *CCL17* levels in AD. IL-22 could be a potential therapeutic target for the treatment of AD.

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## Letter to the Editor

### Histamine modulates the responsiveness of keratinocytes to IL-17 and TNF-α through the H1-receptor

Interleukin (IL)-17-producing $CD4^+$ helper T cells, Th17 cells, are involved in protection against bacterial pathogens and in the pathogenesis of various cutaneous inflammatory diseases, such as psoriasis and contact hypersensitivity [1]. In addition, a higher percentage of Th17 cells has been detected in the lesional skin and in the peripheral blood in the acute exacerbation phase of atopic dermatitis (AD), compared to normal controls [2,3]. It has been thought that IL-17 produced by Th17 cells infiltrating into the dermis acts on keratinocytes to produce inflammatory mediators, such as IL-8 and granulocyte macrophage colony-stimulating factor (GM-CSF) to chemoattract neutrophils and T cells and to activate Langerhans cells and endothelial cells, respectively [2,4], which initiates and enhances cutaneous inflammations.

Initiation of cutaneous inflammation correlates with rapid upregulation of pro-inflammatory mediators such as IL-1α, IL-1β, and tumor necrosis factor (TNF)-α. Recently, TNF-α has been paid special attention, since neutralizing anti-TNF-α therapy is effective in the treatment of a wide variety of diseases, including psoriasis. Other mediators, such as histamine, are thought to play important roles in AD and even in psoriasis, since mast cells are activated early in the developing psoriatic lesion and later increase in number in the upper dermis with concomitant expression of cytokines, histamine, and TNF-α [5]. However, the impact of mediators such as TNF-α and histamine on IL-17-induced inflammatory mediator production remains unclear. In this study,

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ORIGINAL ARTICLE

# Beneficial effect of a diet containing heat-killed *Lactobacillus paracasei* K71 on adult type atopic dermatitis

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## ABSTRACT

The purpose of this study was to investigate the clinical effect of a supplementary diet containing heat-killed lactic acid bacterium *Lactobacillus paracasei* K71 (LAB diet) on adult patients with atopic dermatitis (AD). A randomized, double-blind, placebo-controlled study was conducted in 34 adult type AD subjects who were treated with conventional topical corticosteroid and tacrolimus. LAB diet or placebo was added over 12 weeks. The primary end-point was the clinical severity of AD which was evaluated by a severity scoring system proposed by the guideline of the Japanese Dermatological Association. The effect was also secondarily evaluated by itch scores of visual analog scales (VAS), quality-of-life (QOL) impairment scores of Skindex 16 and consumption amounts of topical therapeutics. Data on these four assessment variables were collected at baseline and at week 4, 8 and 12. Within the study population, the skin severity scores were significantly decreased from baseline at week 8 ($P < 0.05$) and at week 12 ($P < 0.01$) in the LAB diet group but not in the placebo group. Influence of LAB diet on itch scores or QOL impairment scores was not evident. The consumption of topical therapeutics in the placebo group was 1.9-times greater in total amount compared with the corresponding value in the LAB diet group during the intervention period, although there was no significant difference. No LAB diet- or placebo-related adverse events were observed. We concluded that the LAB diet may have some benefits as a complementary therapy for adult AD patients who are managed with the conventional treatment.

**Key words:** atopic dermatitis, complementary therapy, heat-killed probiotic bacteria, *Lactobacillus paracasei*, randomized controlled trial.

## INTRODUCTION

Atopic dermatitis (AD) is a common, chronic and refractory skin disease manifesting as eczema and pruritus with repeated exacerbations and regressions.[1] Although AD was originally known to be mainly prevalent among infants and children, its incidence in adults has increased worldwide over the past decade.[2] The same trend is also observed in Japan, and according to a currently conducted nationwide epidemiological surveillance, the prevalence of AD in the Japanese populations in their 20s and 30s reached 10.2% and 9.0%, respectively.[3,4] As AD enormously interfere with individuals' daily life

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socially and psychologically, the adequate management of AD should be considered an important medical and health issue.

Because the exact pathogenesis of AD remains obscure, the current management of the disorder aims to relieve repeatedly-occurring dermal inflammation and prevent its flare-up. Topical corticosteroids and tacrolimus are widely accepted as the standard treatment options for AD.[1,5] Although these treatment options might control the symptoms, relapses are not infrequent. Moreover, extensive and prolonged use of corticosteroid implies a risk of local side-effects potentially causing skin atrophy and there are substantial numbers of AD patients who have corticosteroid phobia.[6] Therefore, novel approaches to the management of AD are urgently needed to enhance efficacy of the ordinary pharmacological treatment.

Of a number of complementary measures thus far studied to control AD and other allergic responses, probiotic supplementation seems to be the most promising.[7,8] A probiotic is defined by the World Health Organization and the Food and Agriculture Organization of the United Nations as "live microorganisms which, when administered in adequate amounts, confer a beneficial health effect on the host". Probiotic microorganisms most often belong to the genera *Bifidobacterium* and *Lactobacillus*.[9,10] Majamaa and Isolauri[11] first demonstrated the effectiveness of the probiotic lactobacilli to reduce the severity of eczema in children with AD. Also, most studies on the effects of probiotic bacteria on AD have been conducted in neonates and infants.[12–15] Recently, Michail *et al.*[16] performed a meta-analysis of 10 randomized controlled trials (RCT) that were conducted in children aged 2.5 months to 13 years to determine whether probiotics are efficacious in treating AD. The results showed a modest role for probiotics in these pediatric AD populations. In contrast, there are much fewer reports which dealt with the effect of probiotics in adult AD patients,[17,18] and none of these papers provided any convincing results.

The impact of probiotics on allergic diseases has been studied in several animal models. These experimental studies have found that probiotics exert strain-specific effects in the intestinal lumen on epithelial cells and immune cells with anti-allergic potential.[19,20] It has been also shown that inactivated (heat-killed) lactobacilli have similar beneficial immunomodulatory effects in experimental animals[21,22] and humans.[23] There is the possibility, therefore, that viability may not be an essential property for the probiotic activity of lactobacilli in the management of AD.

*Lactobacillus paracasei* K71 is an isolate from *sake* lees, a Japanese traditional fermented product made from polished rice. We recently found that a heat-killed *L. paracasei* K71 bacterium was effective in downregulating immunoglobulin (Ig)E synthesis *in vitro* and *in vivo* (Dr Takashi Hara *et al.*, pers. comm., 2008). A preliminary clinical study showed that a supplementary diet containing heat-killed bacteria of this *Lactobacillus* strain (LAB diet) was a safe agent that may have potential to exert a beneficial effect in adult type AD patients (Dr Nobuyuki Shimizu, unpubl. data, 2008). This tempted us to conduct a double-blind RCT aiming to investigate the clinical usefulness of the LAB diet in adult patients with AD. Because topical corticosteroid and tacrolimus is recommended as the standard treatment of AD in various guidelines, evaluation of efficacy of probiotics and any other complementary therapy should be performed in those AD patients who are managed with the guideline-based treatment. However, almost all such RCT have been conducted using untreated AD patients for the control arm. As far as we are aware, the only exception is an RCT reported by Kobayashi *et al.*[24] who evaluated efficacy and safety of a prescribed traditional herbal medicine in a special population of AD adults managed with the standard treatment.

Taking these situations into consideration, the present double-blind RCT was undertaken to investigate whether the supplementation with the LAB diet or placebo can affect the clinical course of adult type AD patients who are managed with the conventional treatment.

## METHODS

### Study diets

A supplementary diet containing a lactic acid bacterium used as the active diet was manufactured in powder form consisting of (in a daily dose of 500 mg) 100 mg ($\sim 2 \times 10^{11}$ bacteria) of heat-killed *L. paracasei* K71 and 400 mg of dextrin NSD300 (Nissi, Chita,

Japan). Dummy placebo contained (in a daily dose) 500 mg of dextrin NSD300 and 0.45 mg of carotene base to make the placebo indistinguishable from the LAB diet in color. Both LAB diet and placebo were given once a day after being dissolved in approximately 100 mL of water, coffee or tea.

**Subjects**

Male and female Japanese adult subjects, aged 20–65 years, with diagnosed mild or moderate AD were recruited. The diagnosis of AD was made according to criteria of the Japanese Dermatological Association.[1] Those subjects with a fairly stable symptomatic status who had received guideline-based standard treatment with topical corticosteroid and tacrolimus prior to the study and were expected to continue the same regimen after the start of the intervention participated in the study. Exclusion criteria were: routine use of health food(s) containing some constituent(s) with AD-affecting potential within 2 weeks before the study and expected to be continued; use of oral corticosteroid; potential allergy to the LAB diet; pregnant women, nursing mothers or women of child-bearing potential; and presence of any clinically significant medical condition judged by the Medical Investigator to preclude the subjects' inclusion in the study.

**Study design**

A prospective, randomized, double-blind, placebo-controlled, parallel-group comparative study took place from August to December 2008 and involved the participation of two clinical service organization centers in Japan. The study protocol was approved by the local ethics committee, and was conducted in accordance with the principles of the amended Declaration of Helsinki and Ethical Guideline for Epidemiological Research. Written informed consent was obtained from all participants prior to enrollment in the study. Thirty-four subjects eligible for the study were randomly assigned to receive LAB diet or placebo. All enrolled subjects were seen on day 0 (baseline) and weeks 4, 8 and 12 (four visits in all) to receive medical examinations and laboratory tests and to collect blood/serum samples. At each visit, the Medical Investigator examined usages of topical therapeutics (corticosteroid and tacrolimus), consumption of allotted study diets (LAB diet and placebo) and occurrence of adverse events based on the individual's study diary. All subjects were asked not to change the kinds of topical therapeutics as far as possible through the intervention period. The used amount of topical therapeutics was expressed as scores for the total equivalent amount (TEA) by multiplying potency equivalent factors according to Kobayashi *et al.*[24]

**Evaluation of outcome**

Intervention outcome of symptomatic changes was evaluated primarily by skin severity scores and secondarily by itch scores and quality of life (QOL) impairment scores. These outcome measures were collected at baseline and at weeks 4, 8 and 12 after the onset of intervention. Evaluations were performed at each time point.

Skin severity scores were measured using the criteria of the Japanese Dermatological Association.[1] In this scoring system, the disease severity is assessed and scored on the basis of eruption intensity and affected skin areas. The eruption intensity was scored from 0–4 (0 = no symptom, 1 = mild, 2 = moderate, 3 = severe, 4 = extremely severe). The sum of scores for each of five sections of the body surface (i.e. [i] head and neck; [ii] anterior trunk; [iii] posterior trunk; [iv] upper limbs; and [v] lower limbs) was used as the outcome measure for skin severity.

Itch scores of 100 mm-visual analog scales (VAS) were measured and assessed in the daytime and at night. VAS were scored from 0 (0 mm, the left end) to 100 (100 mm, the right end), where 0 indicates no itch and 100 indicates the worst itch imaginable.

The Japanese edition of Skindex 16 questionnaires,[25] a dermal disease-specific, self-administered questionnaire, were administered at each visit to evaluate the intensity of QOL impairment. The Skindex 16 comprises three subscales (physical symptom, feeling and daily functioning) and 16 component items, and each item is rated on an ordinal scale of 0–6, with higher scores indicating lower QOL. The sum of scores for all of the 16 items was used as the parameter for evaluation of QOL impairment.

**Evaluation of safety**

Tolerability and safety were evaluated on the basis of the incidence and severity of study diet-related adverse events experienced throughout the study in

comparison between the LAB diet group and placebo group. All adverse events occurring in the two groups were analyzed for their frequency, severity, relatedness with intervention and efficacy outcome.

### Statistics

Baseline characteristics were compared between the two groups with the two-sample Student's *t*-test. Changes in symptomatic scores over time were also compared with baseline by the paired Student's *t*-test. Intergroup comparison of the changes ($\Delta$-values) during intervention was made by the two-sample Student's *t*-test. $P < 0.05$ was regarded as statistically significant.

## RESULTS

### Baseline characteristics

Table 1 presents the baseline characteristics of 17 subjects in each of the LAB diet and placebo groups. The baseline variables included: demographic characteristics (age, men/women ratio); physical and physiological parameters (height, bodyweight, body mass index, systolic/diastolic blood pressures, pulse rate); subject distribution on the severity of AD; and symptomatic scores (skin severity scores, itch scores of VAS, QOL impairment scores of Skindex 16). There were no significant differences between the LAB diet group and placebo group in any of these variables.

Among a total of 17 subjects in the LAB diet group, one (a 30-year-old woman with mild AD) was aware of having been pregnant on day 53 after the start of intervention. She was immediately released from the study and excluded from all outcome evaluations. Adherence to the allotted study diet was: 100% and 90–99% for 13 and three subjects, respectively, in the LAB diet group and for 10 and seven subjects, respectively, in the placebo group. Thus, in both groups, there was no subject with a value below 80% that was considered a protocol violation. Based on these results, outcome evaluations based on symptomatic scores were performed with 16 subjects in the LAB diet group (10 with mild AD and six with

**Table 1.** Baseline characteristics of LAB diet and placebo groups

| Variables | LAB diet group ($n$ = 17) | Placebo group ($n$ = 17) |
|---|---|---|
| Age (years) | 29.4 ± 5.7 | 31.6 ± 10.1 |
| Sex (M : F) | 5:12 | 5:12 |
| Height (cm) | 162.45 ± 6.75 | 160.08 ± 7.92 |
| Bodyweight (kg) | 55.41 ± 8.44 | 55.41 ± 11.79 |
| AD severity distribution (subjects) | | |
| Mild | 11 | 12 |
| Moderate | 6 | 5 |
| Disease duration (years) | 17.4 ± 9.7 | 17.2 ± 10.2 |
| Skin severity scores | | |
| Head and neck | 0.82 ± 0.64 | 0.76 ± 0.66 |
| Anterior trunk | 0.65 ± 0.70 | 0.76 ± 0.66 |
| Posterior trunk | 0.53 ± 0.72 | 0.94 ± 0.56 |
| Upper limbs | 1.00 ± 0.50 | 1.06 ± 0.66 |
| Lower limbs | 0.71 ± 0.69 | 0.94 ± 0.75 |
| Total | 3.71 ± 1.76 | 4.47 ± 2.00 |
| Itch scores of VAS (mm) | | |
| In daytime | 37.38 ± 20.47 | 32.97 ± 16.97 |
| At night | 28.18 ± 24.63 | 24.28 ± 25.86 |
| Total | 65.56 ± 38.67 | 57.25 ± 38.90 |
| QOL impairment scores of Skindex 16 | | |
| Physical symptom | 35.05 ± 22.00 | 37.25 ± 25.62 |
| Feeling | 41.32 ± 29.12 | 53.46 ± 24.11 |
| Daily functioning | 11.96 ± 13.54 | 17.84 ± 20.38 |
| Total | 30.58 ± 19.82 | 38.36 ± 16.26 |

Values are the mean ± standard deviation except in sex and AD severity distribution. AD, atopic dermatitis; LAB diet, *Lactobacillus paracasei* K71; QOL, quality of life; VAS, visual analog scales.

**Table 2.** Usage of topical therapeutics during 12-week intervention period

| Group | Scores for TEA of topical therapeutics used | | | |
|---|---|---|---|---|
| | Weeks 0–4 | Weeks 5–8 | Weeks 9–12 | Total |
| LAB diet ($n$ = 16) | 31.58 ± 33.44 | 32.69 ± 42.99 | 41.88 ± 50.49 | 106.14 ± 116.60 |
| Placebo ($n$ = 17) | 57.86 ± 60.61 | 71.98 ± 96.55 | 70.08 ± 70.52 | 199.92 ± 206.72 |

Values are given as mean ± standard deviation. TEA, total equivalent amount; LAB diet, *Lactobacillus paracasei* K71.

moderate AD) and 17 subjects in the placebo group (12 with mild AD and five with moderate AD).

Table 2 shows the equivalent amounts of topical therapeutics measured every 4 weeks during the 12-week intervention period. In two groups, the amount for weeks 5–8 and 9–12 compared with the initial 4-week period appeared to be slightly increased. The extent of increase was approximately 33% (within weeks 9–12) in the LAB diet group and approximately 24% (within weeks 5–8) in the placebo group at the maximum, indicating that the dosage of topical therapeutics was kept almost constant in the two groups over the intervention period. By intergroup comparison, the consumption of topical therapeutics in the placebo group was 1.7–2.2-times greater at each measurement time point (1.9-times greater in total amount) compared with the corresponding value in the LAB diet group during the intervention period, although there was no significant difference.

### Effect on skin severity scores

Figure 1 shows the time-course changes in skin severity scores during the 12-week intervention period, along with the magnitude of changes from the baseline to each of three assessment time points (weeks 4, 8 and 12), in both groups. By within-group comparison, scores for the LAB diet group were decreased from baseline by 10.2%, 18.6% and 27.1% at weeks 4, 8 and 12, respectively, achieving statistical significance at the latter two time points ($P < 0.05$ and $<0.01$, respectively). In contrast, scores for the placebo group were decreased to a lesser extent without statistical significance (5.3% at week 8 and 15.8% at week 12). By intergroup comparison, although Δ-values of scores changed from baseline for the LAB diet group at week 8 (−0.69 ± 1.14) and at week 12 (−1.00 ± 1.21), they appeared to be greater than the corresponding values for the placebo group (−0.24 ± 2.08 and −0.71 ± 1.99, respectively), none of the differences reached a significant level.

### Effect on itch scores and QOL impairment scores

The efficacy to relieve itch and that to improve QOL were evaluated on the basis of itch scores of VAS and QOL impairment scores of Skindex 16, respectively. A trend toward reduction in itch scores was seen in both groups (Fig. 2). Compared with baseline, the values for the LAB diet group were reduced by 23.0% ($P = 0.032$) at week 4, 32.9% ($P = 0.064$) at week 8 and 17.8% ($P = 0.348$) at week 12. However, a similar trend of score reductions was also seen for the placebo group at week 4 (6.8%, $P = 0.538$), week 8 (27.5%, $P = 0.059$) and week 12 (39.2%, $P = 0.059$). Thus, both groups had the significant reduction or the likelihood of reduction in itch scores at two of the three assessment time points. No intergroup significant difference in Δ-values was observed at any time point.

As illustrated in Figure 3, both groups showed a trend toward the substantial reduction in QOL impairment scores over the intervention period. The extent of score reduction compared with baseline at weeks 4, 8 and 12 were 28.0%, 36.1% and 29.3%, respectively, in the LAB diet group and 28.3%, 42.5% and 41.9%, respectively, in the placebo group. All these changes in scores were statistically significant ($P < 0.01$ or $<0.05$), while there was no significant intergroup difference in Δ-values at any time point.

### Tolerability and safety

During the 12-week intervention period, one of 16 subjects in the LAB diet group experienced two adverse events (nausea and headache) and four of 17 subjects in the placebo group had six adverse events (headache, toothache, diarrhea, stomach ache, nausea and vomiting). None of these adverse events

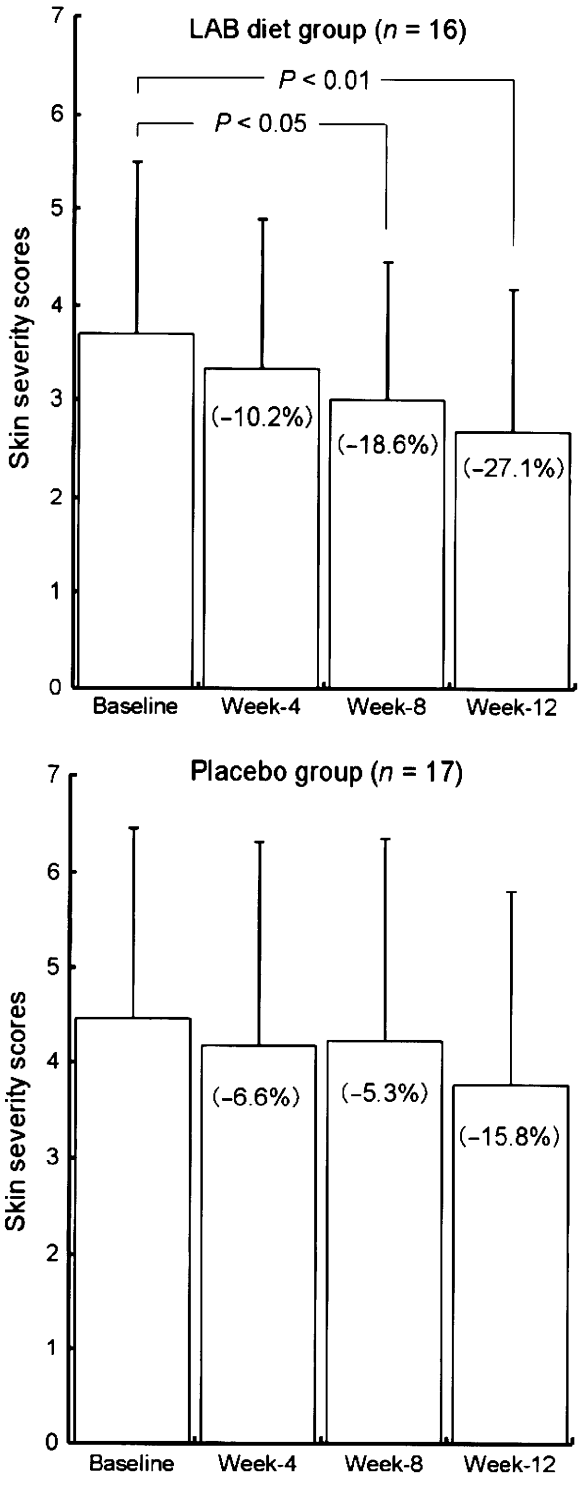


**Figure 1.** Time-course changes in skin severity scores during the 12-week intervention period. Numbers in parenthesis shown within the column are percent changes from baseline at each assessment time point. LAB diet, *Lactobacillus paracasei* K71.

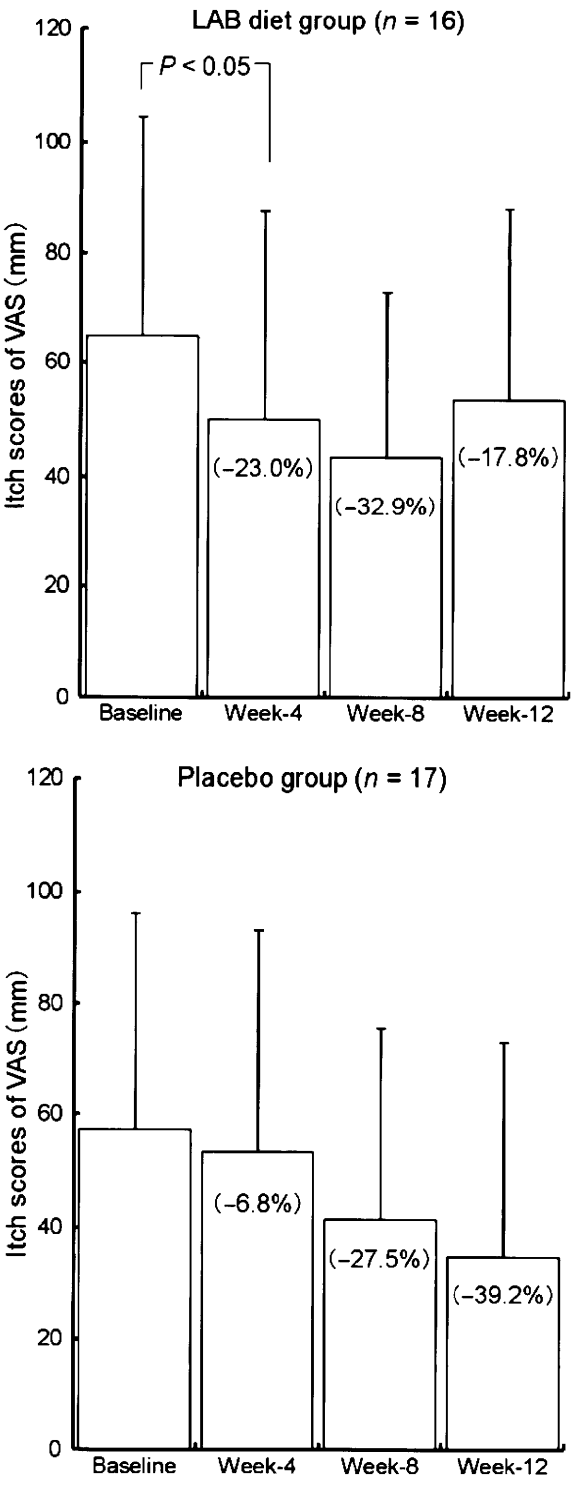


**Figure 2.** Time-course changes in itch scores of VAS during the 12-week intervention period. Numbers in parenthesis shown within the column are percent changes from baseline at each assessment time point. LAB diet, *Lactobacillus paracasei* K71; VAS, visual analog scales.

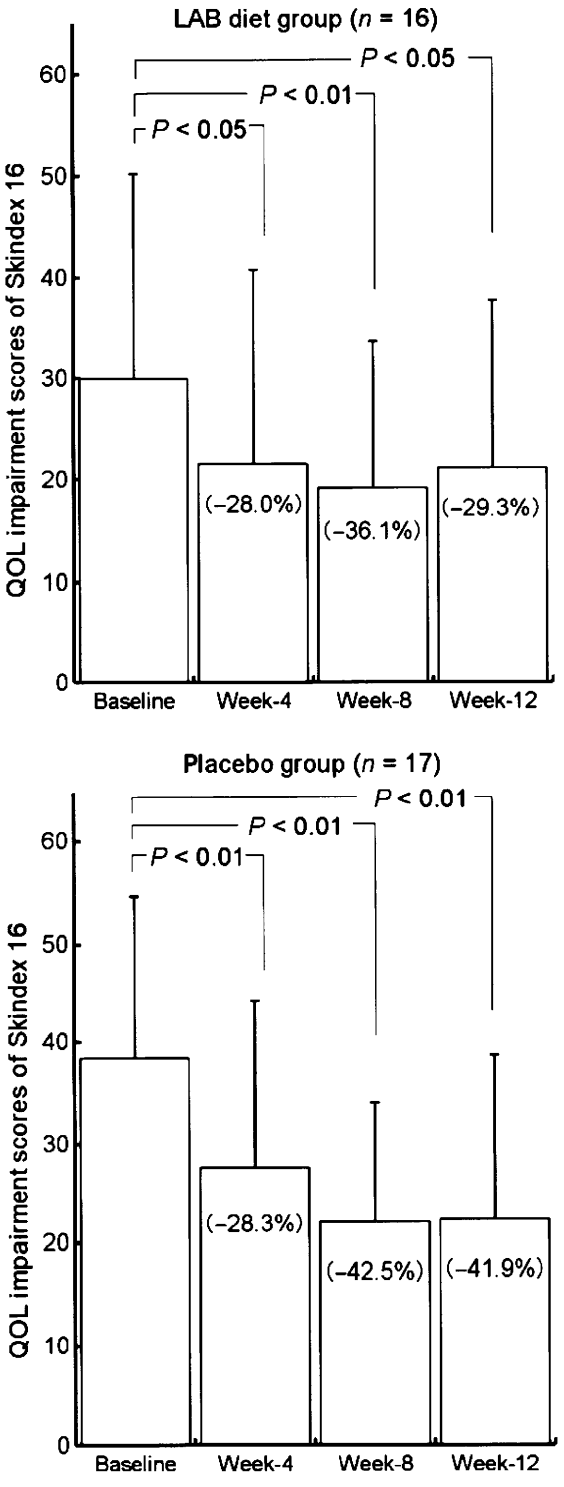


**Figure 3.** Time-course changes in QOL impairment scores of Skindex 16 during the 12-week intervention period. Numbers in parenthesis shown within the column are percent changes from baseline at each assessment time point. LAB diet, *Lactobacillus paracasei* K71; QOL, quality of life.

were severe and were unrelated to the study diet taken.

## DISCUSSION

There are a substantial number of reports which demonstrate the beneficial effect of probiotics, mostly live lactobacilli, in the management of pediatric AD.[13–15,26] In contrast, much fewer data are available on the effect of viable or non-viable probiotic bacteria in the prevention or treatment of AD in adult patients. With respect to probiotic effect of inactivated bacteria, several experimental studies with mice showed that p.o. administration of heat-killed bacteria of certain *Lactobacillus* strains are effective in protecting against intestinal inflammation,[27] reducing contact hypersensitivity reaction[28] and preventing spontaneous development of atopic skin lesions.[22] The use of inactivated instead of viable probiotic bacteria would have an advantage of lacking in infectivity and having technical merits in the form of longer shelf-life and reduced requirements for refrigerated storage. For these reasons, we have been very eager to investigate the clinical benefits of heat-killed probiotic bacteria, particularly those of *L. paracasei* K71, in the management of adult patients with AD.

In Japan, like almost all other developed countries, treatment with topical corticosteroid and tacrolimus has currently been recommended by the authorities as the standard treatment option for AD patients, although its therapeutic efficacy still has limitations.[1] Taking this medication status into consideration, the present clinical study was attempted to investigate potential usefulness of heat-killed probiotic lactobacilli administrated p.o. as a complementary therapy for adult AD patients who are managed with the conventional treatment.

In this double-blind RCT conducted in such patients, intake of a supplementary diet containing a heat-killed *L. paracasei* K71 bacterium (LAB diet) was shown to cause statistically significant decrease from baseline to weeks 8 and 12 in the mean skin severity scores, whereas no significant changes were observed after placebo intake. It appears, therefore, that the LAB diet may have some additive effect to improve the skin severity in adult AD subjects receiving standard treatment. However, the effect would be

considerably limited because there was no intergroup significant difference in Δ-values of skin severity scores after the start of intervention. Moreover, no substantial effect of LAB diet to improve itch or QOL impairment was demonstrated in this study. In interpretation of the results, it should be noted that the used amounts of topical therapeutics over the intervention period in the placebo group were 1.9-times greater than those in the LAB diet group. It suggests the possibility that the disease severity in the former group might be better controlled by the basal topical treatment than in the latter.

The patients with AD are known to have a decreased gut mucosal barrier function that permits the frequent transfer of exogenous antigens with resultant induction of symptomatic AD.[29–31] Matsumoto *et al.*[17] also reported that probiotic lactobacilli were useful in the management of adult patients with intractable AD through reversing increased intestinal permeability. These findings suggest that either viable or inactivated probiotic lactobacilli may exhibit beneficial activities in the management of symptomatic AD. Further studies on the clinical usefulness of a LAB diet as a complementary modality for AD patients with standard treatments are warranted to be continued in more detail.

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