

A PILOT STUDY EVALUATING THE CLINICAL AND IMMUNOMODULATORY EFFECTS OF AN ORALLY ADMINISTERED EXTRACT OF *DENDROBIUM HUOSHANENSE* IN CHILDREN WITH MODERATE TO SEVERE RECALCITRANT ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a common inflammatory skin disorder for which few safe and effective systemic treatments are available. To test the clinical and immunomodulatory effects of a crude polysaccharide fraction isolated from *Dendrobium huoshanense* for the treatment of AD, we conducted a pilot, uncontrolled case series study. Twenty-seven patients aged 4-18 years (mean±SD, 10.82±4.4) with AD that had not responded to topical therapy were treated with polysaccharide derived from *D. huoshanense* for 4 weeks and followed-up for another 4 weeks. Progression of AD was determined with the Lund-Browder chart for children, the Investigator's Global Atopic Dermatitis Assessment (IGADA), and the Scoring Atopic Dermatitis (SCORAD) at weeks 0, 2, 4, and 8. Serum levels of cytokines were evaluated. Safety was determined with laboratory and clinical tests. The lesion area, IGADA score, total SCORAD result, and score for sleeplessness decreased significantly from weeks 0 to 4, but did not change significantly between weeks 4 and 8. The scores for subjective symptoms and pruritus decreased significantly from week 0 to week 4 and increased significantly from week 4 to week 8. Serum levels of IL-5, IL-13, IFN- γ , and TGF- β 1 decreased significantly between weeks 0 and 4 and between weeks 0 and 8. No significant difference in the levels of IL-10 was found. The polysaccharide from *D. huoshanense* reduced the levels of some cytokines associated with AD and had beneficial effects on symptoms. No serious adverse effects occurred when it was administered orally for 4 weeks.

Atopic dermatitis (AD) is a common inflammatory skin disorder that afflicts from 10% to 20% of children in developed countries (1-2). It is a complex disease, but the presence of a strong immunomodulatory component has been generally recognized (3-4). Although most cases are mild and can be treated with topical agents (5), some patients develop a chronic, moderate to severe form of the disease that is recalcitrant to topical therapy

and requires systemic treatment. Recalcitrant AD is roughly defined as a disease that does not respond satisfactorily to conventional treatment including topical steroids, topical calcineurin inhibitors, or standard systemic therapies. Patients are considered to have recalcitrant AD if they are refractory to routine outpatient treatment, have had at least one episode of hospital-based in-patient treatment with intensive topical steroid therapy, or require short or

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longer courses of systemic corticosteroids. In these cases, the symptoms can cause a serious decrease in the quality of life for the patients and their families.

Several previous studies have evaluated alternative medicines for the treatment of recalcitrant AD. Consumption of oolong tea was found to be of benefit in an uncontrolled trial (6), but a controlled trial of Zemaphyte®, a standardized formulation containing ten Chinese herbs, found a trend toward clinical improvement in both the treatment and placebo groups, with no significant difference as a result of treatment (7). The current status of the search for suitable systemic therapies for AD has been recently reviewed (8), and currently, safe and effective therapies still need to be defined.

The leaves and stems from the *Dendrobium* species have long been used in traditional Chinese medicine as safe and efficacious treatments for a variety of disorders (9). The polysaccharide fraction from this plant has been licensed as a food product; however, it has also been shown to have immunomodulatory properties (10-12). The immunomodulatory effects of this medicinal plant and its relative safety encouraged us to explore its application in the treatment of AD.

In this report, we describe the first pilot, case series study evaluating the clinical efficacy and immunomodulatory effect of an orally administered 4-week course of a polysaccharide fraction isolated from *Dendrobium huoshanense* for the treatment of AD. Because this was a case series pilot study, we did not compare the response of the treatment group with that of patients receiving other medications or placebo.

MATERIALS AND METHODS

Study population

Twenty-seven patients from the pediatric allergy outpatient clinic of Taipei Veterans General Hospital, were enrolled in the study. To be eligible for participation in the study, all patients had to meet the clinical criteria of Hanifin and Rajka for diagnosis of AD (13). Most patients (25/27; 93%) had elevated serum levels of total IgE (mean: 2192 IU/mL, range: 205~5000 IU/mL). At the same time, sera from twenty-four patients (24/27; 88%) were positive (values of specific IgE antibodies > 0.35 IU/L) against at least one of the following six common allergens measured by a commercial Pharmacia CAP

system (Pharmacia Diagnosis AB, Uppsala, Sweden): house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), dog dander, cat dander, milk, and egg whites. In addition, the patients' lesions must have covered over 20% of the total body surface and not been significantly improved after treatment with topical ointment (corticosteroid and/or calcineurin inhibitors) with or without oral antihistamine. In other words, these patients suffered from recalcitrant AD. All showed various stages of acute and chronic forms of AD, including redness, exudation, dryness, excoriation, lichenification, desquamation, and scaling.

Patients were excluded from the study if they had any of the following: systemic opportunistic bacterial infection; treatment with oral or injected steroids, antibiotics, methoxsalen (psoralen) and ultraviolet A radiation (PUVA), or other immunosuppressants two weeks prior to entry into the study; markers of liver or kidney function that were elevated by 1.5-fold above normal; abnormal results of hematologic examination. All patients or their guardians provided written informed consent, and the protocol was approved by the Institutional Review Board of Taipei Veterans General Hospital.

Study design

The protocol for this case series study is shown in Fig. 1. One tablet (1000 mg/tablet) consisted of 135 mg *D. huoshanense* crude powder (Yuen Foong Yu Herba Dendrobii) (14) and 865 mg of excipient. The crude powder (Yuen Foong Yu Herba Dendrobii) was ground from the dried leaves and stems of *D. huoshanense* and not mixed with other ingredients. The crude powder consisted of 40 mg/g (4%) polysaccharide. For patients who weighed less than 30 kg, one tablet was administered orally twice per day (after breakfast and after the dinner) for a total daily dose of 2000 mg. For patients who weighed 30 kg or more, two tablets were administered orally twice per day (after breakfast and after dinner) for a total daily dose of 4000 mg. The treatment period was 4 weeks, with a subsequent additional 4 weeks of follow-up. Concomitant treatments, such as emollients or topical steroid therapy, were given as necessary during the research period.

Assessments

Clinical evaluations of AD: Patients were examined at baseline (visit 1, week 0), week 2, week 4, and week 8. At all visits, the area of the lesions was measured using the Lund-Browder chart for children and the severity was determined with the Investigator's Global Atopic Dermatitis Assessment (IGADA) (a 6-category scale in which lesions are graded as clear, almost clear, mild, moderate, severe, and very severe) and the Scoring Atopic

Dermatitis (SCORAD). The severity of AD was classified as mild (< 25), moderate (25-50), or severe (≥ 50) on the basis of the SCORAD.

Laboratory evaluations: Laboratory and clinical data collected at each visit included serum levels of IgE, creatinine and hemoglobin; total eosinophil, white blood cell, red blood cell, and platelet counts; serum glutamic oxaloacetic transaminase (SGOT) and alanine aminotransferase (SGPT) levels; blood pressure; and body weight.

Cytokine assays: Peripheral blood samples were drawn from all patients at baseline, 4 weeks, and 8 weeks after treatment. Sera were obtained after standard centrifugation and stored at -80°C until analysis. Measurement of the level of cytokines was performed with the commercial interleukin-5 (IL-5), interleukin-13 (IL-13), interleukin-10 (IL-10), transforming growth factor- $\beta 1$ (TGF- $\beta 1$), and interferon- γ (IFN- γ) ELISA kits (R&D systems, Minneapolis, MN, USA), according to the instructions of the manufacturer. The detection limits of these cytokines were greater than 5 pg/mL. All serum samples were measured in duplicate.

Statistical Analysis

Continuous data are presented as mean and standard deviation, and categorical data are presented as n (%). The effects of trends for repeated measurements over time were analyzed with a linear mixed model. Paired t tests were performed to assess the difference between each visit. However, non-parametric data (AD severity, IL-5, IL-13, IFN- γ , IL-10, and TGF- $\beta 1$) were compared with the Wilcoxon's signed-rank tests. Statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Data were analyzed with SAS 9.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

The demographic data for the 27 patients are summarized in Table I. Fifteen of the patients (55.56%) were male, and 12 (44.44%) were female. The mean age for all patients was 10.82 ± 4.4 years of age (range, 4-18 years), and the mean age at onset of AD was 4.69 ± 5.10 years. Allergic disorders were very prevalent among this population. Most patients had two or more allergies and a family history of allergy. More than half of the patients had a history of asthma (51.85%), allergic rhinitis (62.96%), or had allergic rhinitis in their family history (51.85%). A total of 24 diseases comprised the category of other allergic diseases, which affected 88.89% of the

patients.

The progression of AD in these patients during the 8-week study period is summarized in Table II. The extent (lesion area) and severity of the lesions, the IGADA score, and the total SCORAD decreased significantly ($P < 0.001$ for all). The extent and intensity criteria and each subcomponent of the SCORAD evaluation decreased significantly over the 8-week follow-up ($P < 0.001$ for all).

When the effectiveness was compared at each visit, it was revealed that the lesion area (Fig. 2a), IGADA score (Fig. 2b), total SCORAD (Fig. 2c), and the score for sleeplessness (Fig. 2f) decreased significantly from week 0 to week 4, but did not change significantly between weeks 4 and 8. However, the scores for the subjective symptom (Fig. 2d) and pruritus (Fig. 2e) components of SCORAD decreased significantly from week 0 to week 4 and increased significantly from week 4 to week 8. The distribution of AD severity was significantly altered between week 0 and week 2 and between week 2 and week 4. At baseline, none of the patients' conditions were classified as mild; but the number of patients with mild AD increased significantly during the treatment period (from week 0 to week 2 and week 4). No significant difference in the distribution of AD severity was found between week 4 and week 8 (the follow-up period) (Fig. 2g).

The effects of treatment on the levels of cytokines were analyzed with Wilcoxon's signed-rank test, as shown in Fig. 3. Serum levels of IL-5 (Fig. 3a), IL-13 (Fig. 3b), IFN- γ (Fig. 3c), and TGF- $\beta 1$ (Fig. 3e) decreased significantly between week 0 and 4 and between week 0 and 8. No significant difference in the levels of IL-10 between week 0 and 4 ($P = 0.532$) or between week 0 and 8 ($P = 0.810$) was found (Fig. 3d).

The values for the other laboratory tests and blood pressure remained within the normal range during the study period. The levels of total IgE and total eosinophil counts did not show significant changes throughout the study period (data not shown).

DISCUSSION

In this pilot, case series study, we found that systemic oral treatment with an extract of *D. huoshanense* caused a significant reduction of the

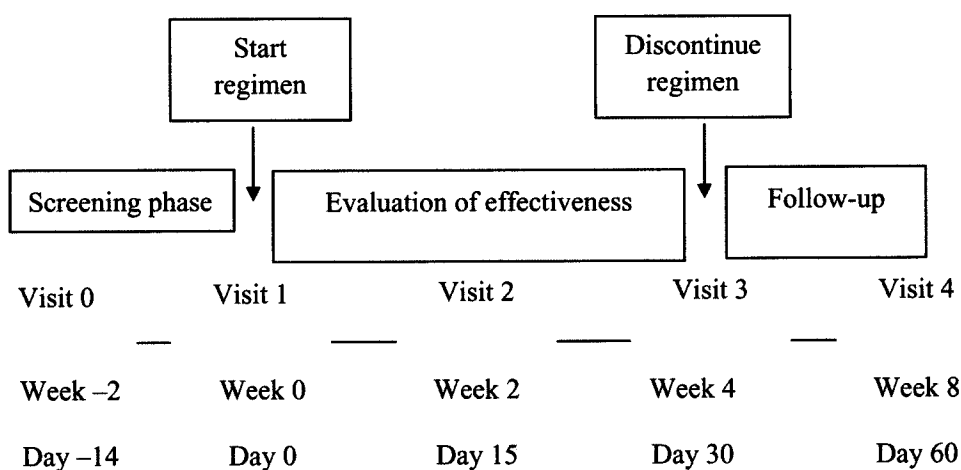


Fig. 1. Schematic diagram of protocol for case series study.

Table I. Patient demographic data ($n=27$).

	Summary
Sex	
Male	15 (55.56%)
Female	12 (44.4%)
Age (years)	10.82±4.4
Height (cm)	139.24±21.10
Weight (kg)	39.96±18.97
Age at onset (years)	4.69±5.10
Duration of illness (years)	6.71±4.60
Patients' history of allergy	
Other allergic disease	24 (88.89%)
Asthma	14 (51.85%)
Allergic rhinitis	17 (62.96%)
Urticaria	1 (3.70%)
Food allergy	7 (25.93%)
Medicine allergy	2 (7.41%)
Family history of allergy	
Atopic dermatitis	3 (11.11%)
Asthma	4 (14.81%)
Allergic rhinitis	14 (51.85%)
Urticaria	5 (18.52%)
Food allergy	4 (14.81%)
Medicine allergy	2 (7.41%)

Continuous data are presented as mean±SD

Categorical data are presented as n (%)

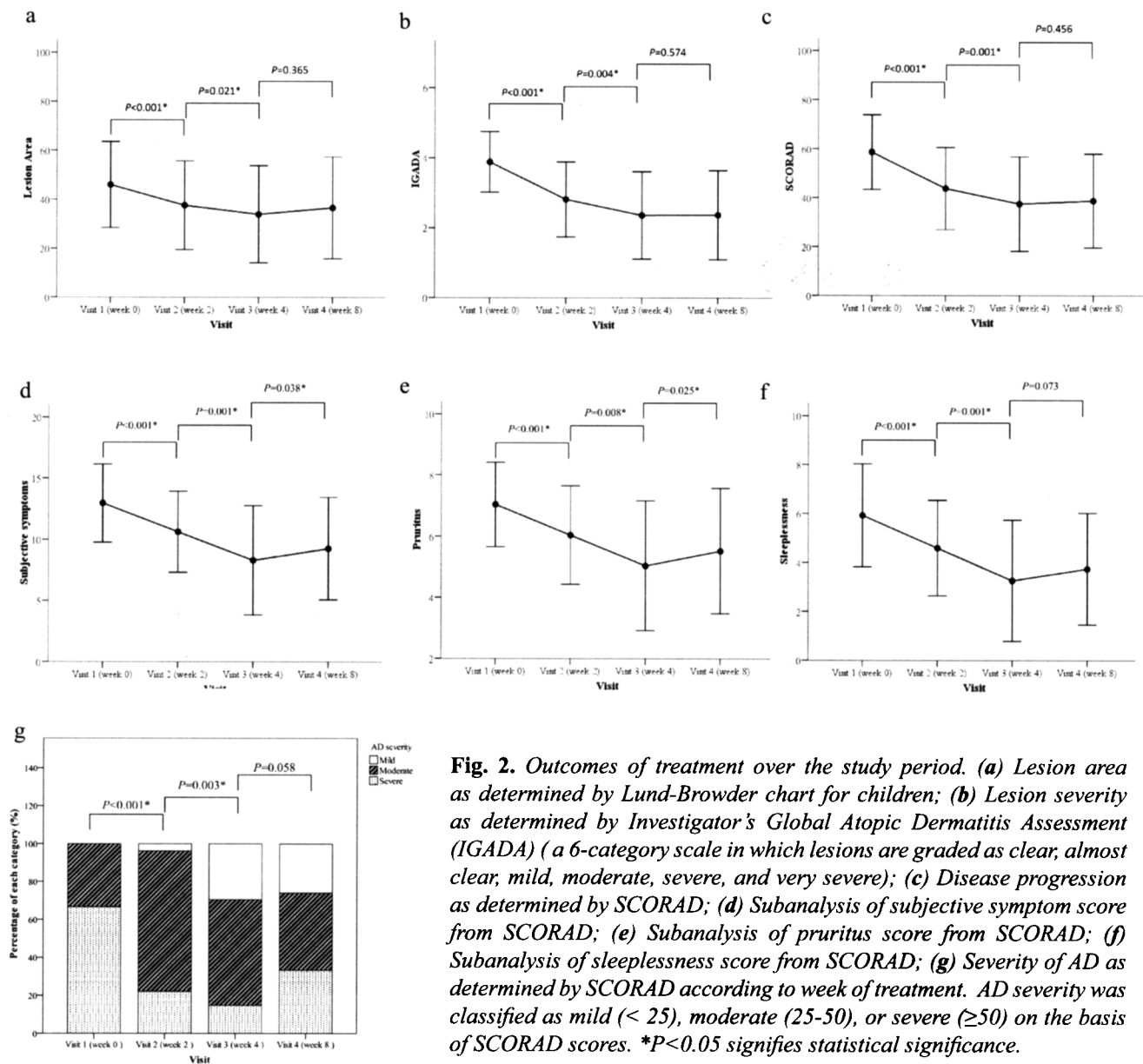


Fig. 2. Outcomes of treatment over the study period. (a) Lesion area as determined by Lund-Browder chart for children; (b) Lesion severity as determined by Investigator's Global Atopic Dermatitis Assessment (IGADA) (a 6-category scale in which lesions are graded as clear, almost clear, mild, moderate, severe, and very severe); (c) Disease progression as determined by SCORAD; (d) Subanalysis of subjective symptom score from SCORAD; (e) Subanalysis of pruritus score from SCORAD; (f) Subanalysis of sleeplessness score from SCORAD; (g) Severity of AD as determined by SCORAD according to week of treatment. AD severity was classified as mild (< 25), moderate (25-50), or severe (≥ 50) on the basis of SCORAD scores. * $P < 0.05$ signifies statistical significance.

signs and symptoms associated with AD compared to baseline. These beneficial effects were apparent after 2 weeks of therapy, and the improvement continued through weeks 2~4. After discontinuation of the regimen, the improvement persisted as measured by lesion area and the overall IGADA and SCORAD scores. However, several components of the SCORAD evaluation, such as, subjective symptoms and pruritus, had increased significantly by 4 weeks after the last treatment (8-week study visit). The patients also experienced a significant

decrease in the serum levels of IL-5, IL-13, IFN- γ , and TGF- β 1 but no significant change in the levels of IL-10.

First-line treatment of AD generally involves application of topical agents, such as, corticosteroids or calcineurin inhibitors (1, 2, 5, 15). Systemic treatments have, so far, been considered only for patients with severe, recalcitrant AD (8). Cyclosporin administered systemically has been shown to give 55% improvement in symptoms after 6~8 weeks, but most patients relapsed within 2~6 weeks after

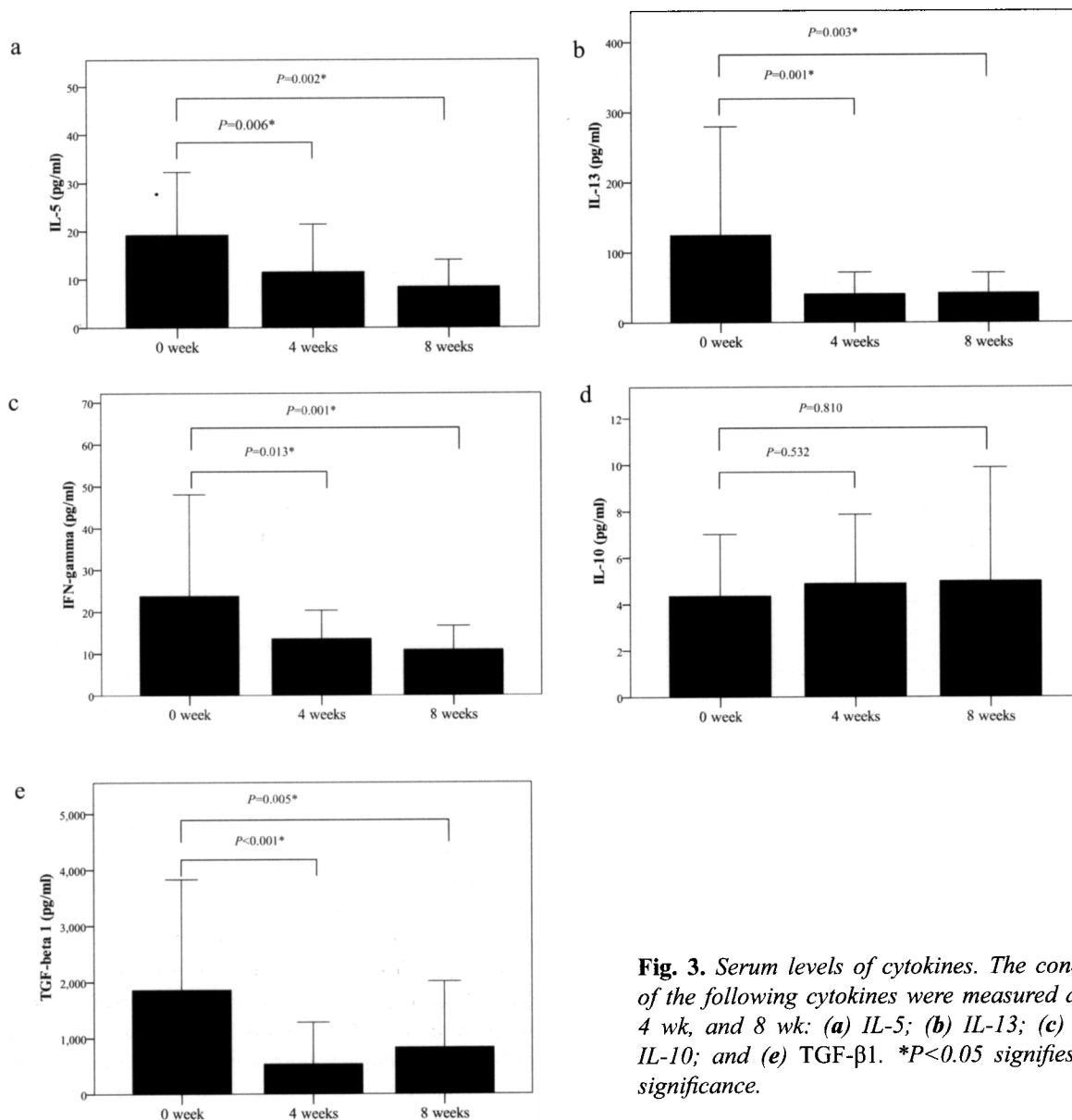


Fig. 3. Serum levels of cytokines. The concentrations of the following cytokines were measured at baseline, 4 wk, and 8 wk: (a) IL-5; (b) IL-13; (c) IFN- γ ; (d) IL-10; and (e) TGF- β 1. * $P<0.05$ signifies statistical significance.

discontinuation. Azathioprine, which affects purine metabolism, has been suggested as an appropriate option for short-term use in patients who fail therapy with cyclosporin (15). Mycophenolate mofetil has also shown some efficacy in treating severe AD in children (5). However, concerns about safety and tolerability of these agents have limited their clinical use. Recently, research efforts have focused on the application of more specific immunomodulatory treatments such as antibodies against IgE, CD-20, and CD-11 (16). The tendency of AD to recur after

cessation of treatment, however, suggests that there continues to be a need for safe agents that can be administered over long periods of time.

Dendrobium species have long been used in traditional Chinese medicine (9). Research is currently underway to isolate the individual components of several species of *Dendrobium* and examine their structure-activity relationships. Erianin, a low molecular-weight product isolated from *Dendrobium chrysotoxum*, has been shown to have anti-angiogenic activity *in vitro* and *in vivo*

Table II. Trends in severity of lesions over study period evaluated with a linear mixed model.

	Week 0	Week 2	Week 4	Week 8	P
Lesion Area ^a (%)	46.00±17.55	37.59±18.16	33.91±19.91	36.60±20.87	<0.001*
IGADA ^b	3.88±0.86	2.81±1.08	2.36±1.25	2.37±1.28	<0.001*
SCORAD ^c	58.63±15.23	43.74±16.77	37.44±19.31	38.69±19.18	<0.001*
Extent criteria	37.80±22.24	30.37±21.35	26.13±22.40	28.41±22.81	<0.001*
Intensity criteria	11.07±2.67	7.74±3.11	6.70±3.20	6.85±3.31	<0.001*
Erythema	2.04±0.71	1.37±0.63	1.33±0.55	1.67±0.48	<0.001*
Edema/Papules	1.96±0.71	1.30±0.67	1.11±0.64	1.33±0.73	<0.001*
Oozing/Crusts	1.30±0.72	1.07±0.68	0.85±0.66	0.78±0.75	<0.001*
Excoriations	1.48±0.80	0.81±0.79	0.67±0.83	0.52±0.70	<0.001*
Lichenification	1.89±0.75	1.33±0.73	0.96±0.76	0.96±0.90	<0.001*
Dryness	2.37±0.74	1.78±0.80	1.74±0.71	1.56±0.70	<0.001*
Subjective symptoms	12.96±3.19	10.63±3.31	8.30±4.46	9.26±4.18	<0.001*
Pruritus	7.04±1.38	6.04±1.60	5.04±2.12	5.52±2.05	<0.001*
Sleeplessness	5.93±2.10	4.59±1.95	3.26±2.47	3.74±2.28	<0.001*

Data are presented as mean±standard deviation. * $P < 0.05$ signifies statistical significance in the effects of trend for repeated measurements over time using linear mixed model

^a: Lesion area was calculated by the Lund-Browder chart for children.

^b: Investigator's global atopic dermatitis assessment

^c: Scoring atopic dermatitis

(17). Exploration of the effects of the polysaccharides derived from *Dendrobium* has also shown that they have immunomodulatory properties (10-12). The polysaccharides, especially the stem mucilage, significantly induced an increase in production of IFN- γ , IL-10, IL-6, and IL-1 α in murine splenocytes. Substantial amounts of hematopoietic growth factors, such as GM-CSF and G-CSF, were also induced (10). The immunomodulatory effects of this medicinal plant and its relative safety encouraged us to explore its application in the treatment of AD.

Although AD is a genetically complex disease and probably involves multiple subtypes (15), it is clear that modifications of the immune system play a prominent role in its etiology, and several reviews of the literature have noted the association of IgE and a variety of cytokines with AD (2, 3, 15). There is biphasic expression of cytokines in AD: acute inflammation strongly associated with a predominance of T_H2-produced cytokines, notably

IL-4 and IL-13, and a chronic form of the disease associated with increased T_H1-produced IFN- γ , IL-12, and IL-18 expression. The T_H2 cytokine IL-5, which is involved in eosinophil development and survival, also predominates in the chronic form of disease. The maintenance of chronic AD also involves production of the several remodeling-associated cytokines, such as, IL-11 and TGF- β 1, which are expressed preferentially in the chronic form of the disease (1). Specifically, increases in IL-4, IL-5, and IL-13 but decreases in the levels of IFN- γ have been reported in the peripheral blood of patients with AD. In our pilot study, the treatment reduced the serum levels of IL-5, IL-13, and IFN- γ . The suppressive effect was maintained during the 4 weeks after the last treatment. These results are consistent with the patients' significant improvement in lesion area, IGADA scores, and total SCORAD.

Our investigation showed that the levels of TGF- β 1 were also suppressed by the therapy, and

the levels remained lower throughout the study period. The role of TGF- β 1 in AD appears to be complex. TGF- β 1 is a potent fibrogenic cytokine that stimulates fibroblasts to produce extracellular matrix components (18). Toda et al. (19) found that TGF- β 1 expression was markedly enhanced in both the acute and, particularly, chronic lesions of AD and suggested that it was involved in the remodeling of the skin lesions in these patients. TGF- β 1 has also been shown to induce the down-regulation of TARC/CCL17 in HeCat cells that were co-stimulated with TNF- α and IFN- γ (20), and TARC/CCL17 has been shown to be elevated in the serum of patients with AD (21). Thus, the decrease in serum levels of TGF- β 1 among the patients in our study could account for the observed improvement in symptoms, or it could be an indirect effect of the alterations in other cytokines.

This is a pilot study evaluating the effects of a relatively crude fraction of *D. huoshanense* on a complex disorder. It has several limitations. No attempt was made in this study to categorize the patients' conditions into subtypes, such as, childhood vs adult forms of the disease. Nor did we evaluate the patients genotypically. Furthermore, we have not isolated or characterized the individual active compound(s) from the polysaccharide fraction. Finally, this was designed as a short-term case series rather than a randomized controlled clinical trial, therefore the data can only suggest improved symptoms and adequate safety relative to baseline conditions.

We conclude that this polysaccharide fraction from *Dendrobium huoshanense* had beneficial effects on the signs and symptoms of AD and produced no serious adverse effects when administered orally over a period of 4 weeks. This effect was accompanied by reduction of some cytokines associated with AD. These preliminary results suggest that future studies to determine the structures and activities of the individual components of this plant and their efficacy in treating AD with randomized controlled trials are warranted.

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