

IgE-mediated sensitivity to *Trichophyton rubrum* in a patient with chronic dermatophytosis and Cushing's syndrome

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Persistent Trichophyton rubrum infection has been observed previously in association with Cushing's syndrome. In this article, we demonstrate IgE-mediated sensitivity to Trichophyton in a patient with chronic dermatophytosis secondary to Cushing's syndrome. Cell-mediated immunity was absent, but Trichophyton extract produced an immediate skin response and also stimulated a concentration-dependent histamine release from basophils of the patient. After adrenalectomy, cell-mediated immunity to Trichophyton was restored, and infection was confined to the nails. Skin and leukocyte sensitivity to Trichophyton extract persisted and was even more pronounced than the sensitivity before adrenalectomy. RAST measurement confirmed the presence of Trichophyton-specific IgE after adrenalectomy. These results provide further evidence that IgE-mediated inflammatory events may be initiated by Trichophyton infection. (J ALLERGY CLIN IMMUNOL 1988;81:847-51.)

Chronic dermatophytosis caused by persistent *Trichophyton rubrum* infection has been observed in patients with Cushing's syndrome,¹⁻⁵ lymphoblastoma,⁶ and connective-tissue disease,⁷ as well as in patients without any predisposing abnormality.⁸⁻¹¹ Early articles in the literature have suggested an association between urticaria and *Trichophyton* infection.^{12, 13} More recently, several patients who had urticaria, recurrent angioedema, or asthma, and who also had superficial fungal infection, were found to have positive immediate skin reactions to intradermal injections of *Trichophyton* extract.^{14, 15} The presence of *Trichophyton*-specific IgE antibodies in sera from these patients subsequently was demonstrated by a RAST procedure.¹⁶

In this article, we present evidence for IgE-mediated activation of basophils and mast cells by *T. rubrum* in a patient with chronic dermatophytosis secondary to Cushing's syndrome.

Abbreviation used

EDTA: Ethylenediaminetetraacetic acid

MATERIAL AND METHODS

Patient

A 33-year-old white man was referred for evaluation of highly pruritic chronic *Trichophyton* infection that had been present for 4 years. There had been no improvement of pruritus or of the skin lesions after oral treatment with griseofulvin (750 mg/day) and ketoconazole (400 mg/day) and with topical application of clotrimazole for 3½ years. Past medical history revealed hypertension at age 25 years and recurrent episodes of nephrolithiasis with passage of calcium stones. There was no personal or family history of atopic disease. On physical examination, the patient was moderately obese, and the skin revealed diffuse erythematous, scaly plaques with lichenification on the trunk and extremities. Urticarial or ringworm-type lesions were not present. The nails of the fingers and toes were thickened, cracked, and friable with subungual debris. Frequent examination during the next 4 years demonstrated skin lesions that persisted despite continued treatment with combination topical and oral antifungal medication. Chlorpheniramine maleate (4 mg four times a day) and hydroxyzine (25 mg at bedtime) were effective in relieving the pruritus.

At age 37 years, the patient was admitted to the hospital for treatment of tenosynovitis of the left wrist. Physical examination at that time revealed persistence of the generalized tinea, central obesity, purple striae, moon facies,

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TABLE I. Influence of adrenalectomy on skin reactivity to *T. rubrum* and total serum IgE level

	Adrenalectomy	
	Before*	After†
Skin involvement	Generalized	Nails only
Cell-mediated skin reaction	Absent	Present
Immediate skin reaction	5 by 5 mm wheal	16 by 15 mm wheal
Total serum IgE	5050 IU/ml	2040 IU/ml

*Before adrenalectomy: 18 months before surgery.

†After adrenalectomy: 31 months after surgery.

and swelling and tenderness of the left wrist. Culture of the synovial fluid aspirated from the left wrist grew *Staphylococcus aureus*; he was treated with dicloxacillin and improved. Skin and nail cultures grew *T. rubrum*. Plasma cortisol levels were 30 µg/dl in the morning and 35 µg/dl in the evening (normal range, 7 to 25 µg/dl). Urinary-free cortisol was 282 µg/dl in 24 hours (normal range, 20 to 100 µg/dl). There was no suppression of the plasma cortisol level after administration of dexamethasone (2 mg/day for 2 days and 8 mg/day for another 2 days). Differential sampling of venous blood from the inferior petrosal sinus, jugular vein, superior vena cava, lower vena cava, and hepatic vein revealed similar plasma levels of adrenocorticotrophic hormone. Treatment with mitotane (2000 mg/day), an adrenal cytotoxic agent, was begun but had to be stopped because of side effects.

At age 39 years, bilateral adrenalectomy was performed; histology demonstrated bilateral, diffuse cortical hyperplasia. After adrenalectomy, the skin lesions gradually resolved, but the onychomycosis persisted. He was treated with corticoid replacement (cortisone acetate, 50 mg AM dose and 12.5 mg PM dose, plus fludrocortisone acetate, 0.1 mg three times a day). Eighteen months after adrenalectomy, the plasma adrenocorticotrophic hormone level increased to 120 pg/ml (normal range, 0 to 100 pg/ml). Radiographic examination of the chest indicated a mediastinal mass, surgery revealed an enlarged thymus, and histology demonstrated thymic hyperplasia. Subsequent examination 31 months after adrenalectomy revealed onychomycosis without evidence of skin lesions. Skin testing with extracts of major inhalant allergens produced weakly positive reactions to ragweed and house dust.

Cell-mediated immunity

Cell-mediated immunity was assessed by skin testing performed by the puncture technique with the Multitest CMI (Merieux Institute, Miami, Fla.). The panel consists of seven antigens: tetanus toxoid, diphtheria toxoid, and culture filtrates of group C *Streptococcus*, *Mycobacterium tuberculosis* and *M. bovis*, *Candida albicans*, *Trichophyton mentagrophytes*, *Proteus mirabilis*, and a glycerin negative control. A positive response to any antigen was defined as an induration ≥2 mm mean diameter 48 hours after application.

Leukocyte histamine release

Peripheral leukocytes were isolated by dextran sedimentation¹⁷ from venous blood of the patient and two control individuals. The plasma layer containing the leukocyte fraction was centrifuged for 10 minutes (200 g at 4° C), and the leukocyte pellet was washed twice at 4° C in buffer containing 25 mmol/L of piperazine-*N,N'*-bis-(2-ethane sulfonic acid) (Sigma Chemical Co., St. Louis, Mo.), 110 mmol/L of NaCl, 5 mmol/L of KCl, 40 mmol/L of NaOH, and 0.003% of human serum albumin (Calbiochem-Behring Corp., La Jolla, Calif.), pH 7.4. The leukocytes were suspended in this buffer containing 0.6 mmol/L of CaCl₂ and 1.0 mmol/L of MgCl₂, and aliquots were added to 12 by 75-mm plastic tubes (Falcon Labware, Oxnard, Calif.) to provide 2 × 10⁶ cells per tube. Serial dilutions of *T. rubrum* (Greer Laboratories, Lenoir, N.C.) or *C. albicans* (Hollister-Stier, Spokane, Wash.) extracts that had been dialyzed (molecular weight cutoff >1000) against saline for 24 hours were added, and the mixtures were incubated at 37° C for 30 minutes in an oscillating water bath. Final incubation volume was 1 ml. The reactions were terminated by centrifugation (1000 g for 2 minutes), and the histamine content of the cell-free supernatants was measured by the automated fluorometric procedure of Siraganian.¹⁸ Neither the *Trichophyton* extract nor the *Candida* extract produced any fluorescence at the dilutions tested. Spontaneous histamine release was determined with cells incubated in the absence of antigen and was always ≤5%. Total cellular histamine content was measured in supernatants of cells lysed with 2% perchloric acid, and release was calculated as the percent of total histamine content. Results are expressed as the mean net percent histamine release for duplicate determinations, which varied <5%.

Serum IgE measurement

Total serum IgE content was measured by PRIST (Pharmacia, Piscataway, N.J.). Measurement of IgE specific for *Trichophyton* was generously performed by Dr. T. A. E. Platts-Mills (University of Virginia) using a recently described RAST procedure.¹⁶

RESULTS

Anergy profile (cell-mediated immunity skin testing) at age 33 years revealed a positive delayed

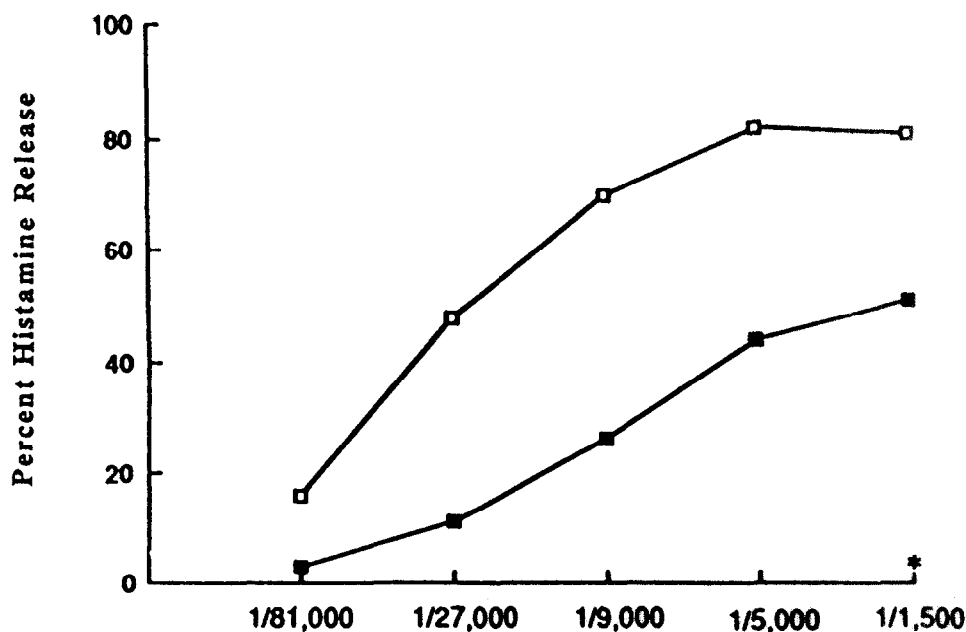


FIG. 1. Stimulation of leukocyte histamine release by *T. rubrum* extract. The patient's leukocytes were incubated with the indicated dilutions of *Trichophyton* extract for 30 minutes at 37° C, as described in the text. Results of two experiments, one performed 18 months before adrenalectomy (■) and one performed 31 months after adrenalectomy (□), are presented. The asterisk indicates the amounts of release obtained in the preadrenalectomy experiment with the 1/1500 dilution of the *Trichophyton* extract in the presence of 4 mmol/L of EDTA.

reaction to streptokinase-streptodornase. Four years later, at the time of diagnosis of Cushing's syndrome, none of the antigens in the cell-mediated immunity skin test panel, including the trichophytin, elicited a delayed response. However, a positive immediate skin reaction (5 by 5-mm wheal) to the trichophytin component of the test panel was observed (Table I). Measurement of total serum IgE yielded a value of 5050 IU/ml. Thirty-one months after adrenalectomy, repeat evaluation of cell-mediated immunity yielded positive delayed skin responses to the trichophytin, diphtheria, and streptococcal antigens. An immediate reaction to the trichophytin component of the skin test panel was observed once again. Moreover, the response was even more strongly positive, with the appearance of a 16 by 15-mm wheal. The total serum IgE was 2040 IU/ml.

To assess further the presence of *Trichophyton*-specific IgE antibody, a basophil histamine release assay was performed at the time the Cushing's syndrome was diagnosed. Incubation of the patient's leukocytes with serial dilutions of a commercial *Trichophyton* extract for 30 minutes at 37° C produced concentration-dependent histamine release (Fig. 1). A maximum histamine release of 51% was obtained at the highest concentration (1/1500 dilution) tested;

the release was inhibited by addition of 4 mmol/L of EDTA to the incubation mixture. *Candida* extract did not stimulate any histamine release in the same experiment (results not presented). Thirty-one months after adrenalectomy, incubation of the patient's leukocytes with *Trichophyton* extract once again produced a concentration-dependent histamine release, with a maximum release of 82% (Fig. 1). As noted for the immediate skin test reaction, the amount of histamine release exceeded that obtained before adrenalectomy. No histamine release was detected when leukocytes from two control donors, one atopic and one nonatopic, were incubated with the same concentrations of *Trichophyton* extract (results not presented).

The development of a RAST procedure specific for measurement of IgE antibody to *Trichophyton*¹⁶ made corroborative testing possible at the postadrenalectomy evaluation. RAST measurement yielded a serum *Trichophyton*-specific IgE level of 1400 U/ml.

DISCUSSION

This patient presented with a chronic, generalized *T. rubrum* infection and subsequently was determined to have Cushing's syndrome. Previous studies¹⁻⁵ have identified an infrequent association between Cushing's

syndrome and chronic *Trichophyton* infection. As in the previously reported cases,¹⁻⁵ the infection in this patient was resistant to antifungal therapy and was associated with an absence of cell-mediated immunity, as measured by the delayed skin response. Resolution of the skin infection and restoration of cell-mediated immunity was achieved only by adrenalectomy. The development of chronic *Trichophyton* infection in Cushing's syndrome likely reflects suppression of the cell-mediated immune response by the endogenous corticosteroids.¹⁹ In contrast, specific defects in the immune response to *Trichophyton* have been proposed to explain the development of chronic dermatophytosis in patients without Cushing's syndrome.⁸⁻¹¹

Early evidence of an association between urticaria and *Trichophyton*,^{12,13} as well as studies indicating that atopic individuals may be predisposed to chronic dermatophytosis,^{11,20} have suggested a pathogenic role of IgE in the inflammatory events initiated by *Trichophyton* infection. Recent studies have further documented the occurrence of immediate skin reactions to *Trichophyton* extract in atopic patients with superficial fungal infection^{11, 12} and have demonstrated the presence of *Trichophyton*-specific IgE antibody in the sera of these patients.¹⁶

Involvement of *Trichophyton*-specific IgE in the present case initially was suggested by the clinical symptom of pruritus and by the occurrence of an immediate skin reaction to trichophytin antigen. The production of significant basophil histamine release during incubation of the patient's leukocytes with commercial *Trichophyton* extract, abrogation of the histamine release by addition of EDTA, and the failure of the *Trichophyton* extract to stimulate histamine release from leukocytes of two control subjects subsequently provided support for an IgE-mediated, non-cytolytic sensitization of basophils to the *T. rubrum*. The skin mast cell and basophil sensitivity to *Trichophyton* extract persisted 31 months after adrenalectomy and was even more marked than the sensitivity observed at the time the infection was most pronounced. Although the use of different lots of extracts on the two occasions might provide a trivial explanation for this finding, the ability of corticosteroids to inhibit antigen-stimulated mediator release from basophils in vitro²¹ and to deplete skin mast cells in vivo²² is consistent with the hypothesis that the high cortisol levels present before adrenalectomy had a suppressive effect on basophil and mast cell reactivity. Significantly, RAST testing confirmed the presence of *Trichophyton*-specific IgE in the patient's serum, and the level markedly exceeded previously reported values.¹⁶ Although the markedly elevated IgE level and association with infection is suggestive of the

hyper-IgE syndrome, the absence of recurrent staphylococcal abscesses and systemic infection is inconsistent with the hyper-IgE syndrome.²³ The high RAST value, together with an extremely elevated total serum IgE level in the presence of high concentrations of circulating endogenous corticosteroids, suggest that the *T. rubrum* were responsible for much of the total IgE. Consistent with this conclusion, the diminution in *Trichophyton* infection after adrenalectomy was associated with a 60% decrease in total serum IgE.

In summary, this case confirms that impairment of cell-mediated immunity facilitates skin colonization by *Trichophyton*. Importantly, however, this case also indicates that IgE-mediated sensitization of basophils and mast cells may contribute to the symptoms associated with the skin lesions in patients with Cushing's syndrome complicated by chronic dermatophytosis. As such, these findings provide further evidence for a possible pathogenic role of IgE-mediated events in urticaria, angioedema, asthma, or endocrine dysfunction associated with *Trichophyton* infection.

REFERENCES

1. Pillsbury DM, Jacobson C. Cushing's syndrome complicated by generalized *Trichophyton rubrum* infection. *AMA Arch Derm Syph* 1953;67:436.
2. Nelson LM, McNiece KJ. Recurrent Cushing's syndrome with *Trichophyton rubrum* infection. *AMA Arch Derm Syph* 1959;80:700.
3. Canizares O, Shatin S, Kellert AJ. Cushing's syndrome and dermatophytosis. *AMA Arch Derm Syph* 1959;80:705.
4. Litvin Y, Weinrauch L, Rubinstein N. Reversible Cushing-induced generalized fungal skin infection. *Cutis* 1982;30:643.
5. Lewis JE. Reversible, Cushing-induced generalized dermatophyte infection. *Cutis* 1983;31:644.
6. Lewis GM, Hooper ME, Scott MJ. Generalized *Trichophyton rubrum* infection associated with systemic lymphoblastoma: report of 3 cases. *AMA Arch Derm Syph* 1953;67:247.
7. Hay RJ. Failure of treatment in chronic dermatophytic infections. *Postgrad Med J* 1979;55:608.
8. Svejgaard E. Humoral antibody responses in the immunopathogenesis of dermatophytosis. *Acta Derm Venereol (Stockh)* 1981;121(S):85.
9. Hay RJ, Brostoff J. Immune responses in patients with chronic *Trichophyton rubrum* infections. *Clin Exp Dermatol* 1977; 2:373.
10. Jones HE, Reinhardt HJ, Rinaldi MG. Immunologic susceptibility to chronic dermatophytosis. *Arch Dermatol* 1974;110: 213.
11. Svejgaard E, Christiansen AH, Stahl D, Thomsen K. Clinical and immunological studies in chronic dermatophytosis caused by *Trichophyton rubrum*. *Acta Derm Venereol (Stockh)* 1984; 64:443.
12. Sulzberger MB, Wise F. Urticaria and hay fever due to *Trichophyton*. *JAMA* 1930;99:1504.
13. Weary PE, Guerrant JL. Chronic urticaria in association with dermatophytosis: response to the administration of griseofulvin. *Arch Dermatol* 1967;95:400.
14. Fiocco FP, Wilkins SR, Hayden ML, Guerrant JL, Platts-Mills TAE. Anaphylaxis and severe angioedema associated with im-

- mediate hypersensitivity to *Trichophyton* infection [Abstract]. *J ALLERGY CLIN IMMUNOL* 1986;77:189.
15. Platts-Mills TAE, Fiocco GP, Pollart S, Hayden ML, Jackson S, Wilkins SR. *Trichophyton* allergy in a 24-year-old man with intrinsic asthma. *Ann Allergy* 1986;56:454.
 16. Platts-Mills TAE, Fiocco GP, Hayden ML, Guerrant JL, Pollart SM, Wilkins SR. Serum antibodies to *Trichophyton* in patients with urticaria, angioedema, asthma, and rhinitis: development of a radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* 1987;79:40.
 17. Lichtenstein LM, Osler AG. Studies on the mechanism of hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen. *J Exp Med* 1964;120:507.
 18. Siraganian RP. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:383.
 19. Fauci AS, Dale DC, Balow JE. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. *Ann Intern Med* 1976;84:304.
 20. Hay RJ, Shennan G. Antibody responses in *Tinea imbricata*: the role of immunoglobulin E. *Trans R Soc Med Hyg* 1984;78:653.
 21. Schleimer RP, McGlashan DW Jr, Gilliespie E, Lichtenstein LM. Inhibition of basophil histamine release by anti-inflammatory steroids. II. Study of the mechanism of action. *J Immunol* 1982;129:1632.
 22. Lavker RM, Schechter NM. Cutaneous mast cell depletion results from topical corticosteroid usage. *J Immunol* 1987;135:2368.
 23. Donabedian H, Gallin JJ. The hyperimmunoglobulin E recurrent-infection (Job's) syndrome: a review of the NIH experience and the literature. *Medicine* 1983;62:195.

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