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Trichophyton tonsurans ALLERGEN I

Characterization of a Protein That Causes Immediate but Not Delayed Hypersensitivity[1]

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Fungal infections of skin or nails are extremely common and often caused by dermatophyte fungi of the genus *Trichophyton*. These fungi are unusual in that they can give rise to delayed hypersensitivity (DH) or immediate hypersensitivity (IH) responses. Recently, IH to *Trichophyton tonsurans* has been demonstrated in patients by skin tests, serum IgE antibody test (RAST), and positive nasal and bronchial challenges. To further investigate the immunology of *Trichophyton*, a 30-kDa *T. tonsurans* allergen was isolated by gel filtration and hydrophobic interaction chromatography. This protein, *Tri t* I, gave a single band on SDS-PAGE, and the 30 amino-terminal amino acids have been determined. Among patients with positive IH skin tests, 34 of 48 (71%) had IgG antibody and 26 of 48 (54%) had IgE antibody to *Tri t* I. Among those who had positive responses to both skin tests and RAST, 22 of 30 (73%) had IgE antibodies to *Tri t* I; thus, this protein represents a major allergen. Twelve clones of murine IgG mAb antibodies were produced. Two clones, 2F2-F7 and 6B11-C2, were found to define separate epitopes on *Tri t* I and were used to develop an immunometric assay for the quantitation of *Tri t* I.

Twenty-three of 38 volunteers with a history of athlete's foot were found to have either IH and/or DH to *Trichophyton* mix and underwent further testing with purified *Tri t* I. Of the nine found to have IH to the mix, eight were sensitive to *Tri t* I. Seven of these eight had IgG and IgE antibodies to *Tri t* I, by Ag-binding RIA, and all were RAST positive to the unpurified extract. An additional 14 had either DH alone ($n = 7$) or a wheal and flare response followed by DH at 48 h ($n = 7$). Of these 14 who had DH responses to *Trichophyton* mix, only one showed DH to an equivalent quantity of purified *Tri t* I; among this group, none showed IH or serum IgE antibodies and only one had detectable IgG antibody to *Tri t* I. The results suggest that the majority of subjects with DH to *Trichophyton* are responding to a protein other than *Tri t* I and that the wheal that precedes DH reactions is some patients is not associated with IgE antibodies.

The genus *Trichophyton* includes several dermatophyte fungi that commonly cause infection of human skin or nails. DH[3] to this fungus is extremely common; indeed, *Trichophyton* extract is one of the three or four extracts used in an anergy panel to assess the competence of DH. Since the 1930s there have also been reports of IH skin test responses to dermatophytes, particularly among patients presenting with asthma or rhinosinusitis (1–3). Recently, we have demonstrated that the upper and lower respiratory tracts of these patients are specifically sensitized to *Trichophyton* (4, 5). The hypothesis is that, among those patients that have IH, persistent absorption of dermatophyte Ag can give rise to inflammation of bronchi and/or nasal passages (4, 5). IH or wheal and flare skin responses to *Trichophyton* have been shown to correlate well with serum IgE antibodies measured by RAST (6). Most of the clinic patients who had IH did not demonstrate DH at 24 or 48 h. It is assumed that this dichotomy between IgE antibody and DH is, at least in part, due to differences in T cell responses and subsequent lymphokine production. Although there is evidence in the literature that human T cells will proliferate in vitro if cultured with *Trichophyton* extracts, analysis of these responses has been difficult because of the lack of purified Ag (7–9). Previous attempts at purification have generally resulted in preparations with several different proteins, and it has not been possible to define the Ag accurately (10–13).

To further investigate the immune response to *Trichophyton*, purification of an Ag reacting with IgE antibodies was combined with development of IgG mAb. A 30-kDa hydrophobic allergen was purified from *Trichophyton tonsurans*, whose 30 amino-terminal amino acids have been determined and which has been designated *Tri t* I. Although *Tri t* I gives rise to IH and IgE antibody production, it does not give DH responses, even in patients with marked DH against the crude extract.

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[3] Abbreviations used in this paper: DH, delayed hypersensitivity; SAS, saturated ammonium sulfate; BBS, borate-buffered saline, pH 8.0, 0.15 M; PBS-T, PBS, pH 7.4, containing 0.05% Tween 20; IH, immediate hypersensitivity; RAST, radioallergosorbent test; *Tri t* I, *T. tonsurans* allergen I.

MATERIALS AND METHODS

Purification of Tri t I

One hundred grams of dried *T. tonsurans* mycelia (lot HSA 7369) (kindly provided by Dr. Shirley Williamson, Hollister-Stier Inc., Spokane WA) was ground into a fine powder, by using a mortar and pestle, and then extracted in 1000 ml of BBS, pH 8.0, with stirring in the cold room overnight. The solids were removed via filtering through cheesecloth and were centrifuged at 13,000 rpm for 20 min. The extract was precipitated with SAS, and the 60 to 90% precipitate was centrifuged at 13,000 rpm for 20 min, dissolved in BBS, dialyzed against four changes of BBS, freeze dried, and redissolved in 10 ml of BBS. The 60 to 90% SAS fraction contained approximately 10 mg/ml protein, as determined by Bradford assay. In a similar manner, 60 to 90% SAS fractions were also prepared from extracts of *Trichophyton rubrum* and *Trichophyton mentagraphytes* mycelia.

Sixty milligrams of the 60 to 90% SAS fraction were applied to a 2.5- × 100-cm Sephacryl S-200 gel filtration column (Pharmacia, Uppsala, Sweden) and eluted with BBS. The eluted samples were pooled into four fractions, A to D, based on their molecular weights (A, >80 kDa; B, 40 to 80 kDa; C, 12 to 50 kDa; and D, <12 kDa). They were dialyzed against BBS, freeze dried, and redissolved at 4 mg/ml in BBS.

Approximately 10 mg of each Sephacryl fraction were applied to a 19-ml phenyl-Sepharose (Pharmacia) column and eluted with decreasing molarities of NaCl (4 M, 2 M, 1 M, and 0.15 M) and then distilled water. Fractions were pooled, dialyzed, freeze dried, and redissolved in 200 μl of BBS. Each fraction was analyzed by SDS-PAGE. The distilled water fraction derived from fraction C gave a single band at approximately 30 kDa and is referred to as *Tri t* I.

Iodination of Proteins

Iodination of mAb and *Tri t* I was carried out by using the chloramine-T technique (14). In each case, 20 μg of protein were radiolabeled with 0.5 μCi of ^{125}I to a specific activity of 15 to 20 μCi/μg of protein. Before radiolabeling, mAb 2F2-F7 and 6B11-C2 were purified by preparative IEF, by using the technique described previously (15).

Human Subjects

Sera were obtained from 48 patients with asthma, rhinitis, or urticaria who had positive IH skin tests to intradermal testing with 0.03 ml of Hollister-Stier *Trichophyton* mix (1/200, w/v), none of whom reported a DH skin response. An additional 16 sera were obtained from patients who had negative IH skin tests to *Trichophyton*. Five of these control patients had DH skin responses to *Trichophyton*. An additional 38 volunteers, who had a history of athlete's foot and who responded to an advertisement, were skin tested with Hollister-Stier *Trichophyton* mix. The 23 individuals in this group who had either DH or IH skin responses were skin tested with purified allergen.

Skin Testing

After filtering through a 0.45 μm Gelman Acrodisc sterile disposable filter, serial 10-fold dilutions of *Tri t* I (4 through 0.004 μg/ml) were prepared in 0.05% human serum albumin in phenol/saline solution. Skin testing was done by the prick test or the end point titration method, by using 0.03 ml intradermally. Increasing concentrations were used, up to that which gave an 8 × 8-mm wheal. Test sites were examined 15 min after scratch or intradermal injection. Skin testing of subjects was approved by the Human Investigation Committee of the University of Virginia.

mAb

Methods for the production of murine mAb have been reported previously (15, 16). Five BALB/c mice were given three injections of 60 μg of fraction C in CFA, over a period of 4 weeks. Sera from these mice were screened for IgG antibody to *Tri t* I by Ag-binding RIA. The two mice with the highest titers were given an intrasplenic boost with fraction C (15 μg), and an additional 60 μg was given i.p. Four days later, spleen cells from one mouse were fused with Sp2/0 myeloma cells. After 10 days, 258 hybrids were screened for IgG antibodies to *Tri t* I, and 10 hybrids were cloned by limiting dilution. Twelve clones were obtained that produced IgG anti-*Tri t* I antibodies, and these clones (2 × 10^6 cells) were injected i.p. into pristane-primed mice for the production of ascites.

Selected mAb (clones 2F2-F7 and 6B11-C2) were purified from 50% ammonium sulfate fractions of ascites by preparative IEF. These mAb were shown to be of the IgG1 isotype, by using immunodiffusion.

RAST

The 60 to 90% SAS fraction of crude *T. tonsurans* was coupled to cyanogen bromide-activated filter paper discs (Whatman no. 541). The discs were incubated overnight at room temperature in 100 μl of serum samples diluted 1/2 and 1/10 in neat horse serum. After extensive washing in RAST buffer (PBS-T), the discs were incubated overnight at room temperature with 100 μl of ^{125}I-labeled goat anti-human IgE, again diluted in horse serum. After extensive washing in PBS-T, the remaining radioactivity was determined with a γ-counter (Micromedic 4/200; Horsham, PA).

A control curve using duplicate doubling dilutions from 1/2 to 1/4096 was constructed by using the serum of a highly *Trichophyton*-sensitive patient (J. C.). A 1/2 dilution of the standard serum was arbitrarily defined as 700 RAST U. Sera from skin test-negative patients were used as negative controls. A positive result was defined as 2 SD above the mean of four skin test-negative controls run in parallel with test sera.

Ag-Binding RIA

Assays for measuring murine IgG and human IgG and IgE antibodies to other allergens have been described previously (17–21). Briefly, diluted serum or ascites was incubated with 2 ng of ^{125}I-*Tri t* I for 4 h and then was immunoprecipitated with the appropriate monospecific antiserum, i.e., goat anti-mouse IgG (Chemicon, El Segundo, CA), sheep anti-human IgG (The Binding Site, La Jolla, CA), or goat anti-human IgE. After 16 h at 4°C, the precipitates were washed three times in BBS and counted on a γ-counter (Micromedic 4/200).

Epitope Analysis Using mAb

The epitope specificity of murine mAb was compared by cross-inhibition RIA using ^{125}I-labeled mAb in a solid phase assay, in a modification of previously described techniques (16, 17). Plastic microtiter wells (Immulon II Removawells; Alexandria, VA) were coated with 10 μg/well mAb 2F2-F7 or 6B11-C2, in 0.1 M $NaHCO_3$ buffer, pH 9.6, overnight at 4°C, and residual binding sites were blocked with 0.1 ml of 1% BSA in PBS-T. Each coated well was incubated with 2 ng of ^{125}I-labeled *Tri t* I plus 50 μl of a 1/100 dilution of the ascites to be tested for 4 h. After further washing, the plate was dried and wells were counted in a gamma counter. Uninhibited binding of ^{125}I *Tri t* I was calculated from the mean cpm of eight wells in which diluent was added in place of the test ascites.

Immunoassays To Quantitate Tri t I

Inhibition RIA. A 1/50 dilution of serum from a *Trichophyton*-allergic patient (W. L.) was incubated with 100 μl of serial dilutions of *Trichophyton* extracts or test samples, at room temperature, for 2 h. One hundred microliters of ^{125}I-*Tri t* I were added for 2 h, and the Ag-antibody complexes were precipitated with goat anti-human IgG, overnight, at 4°C. The precipitates were washed three times and counted in a γ-counter.

Two-site mAb assay. Immulon II Removawells were coated with 10 μg/well 6B11-C2 in 0.1 M $NaHCO_3$ buffer, pH 9.6, overnight, at 4°C. Each well was incubated with 100 μl of diluted samples containing *Trichophyton* Ag, for 2 h. After five washes with PBS-T, 100 μl (2 ng) of iodinated 2F2-F7 (specific activity, 20 μCi/μg) were added for 2 h, and wells were washed 10 times and counted in the γ-counter. A control curve was established using serial twofold dilutions of the *T. tonsurans* 60 to 90% SAS fraction, from 1/4,000 to 1/4,096,000. This extract was arbitrarily assigned a *Tri t* I concentration of 40,000 U/ml. Preliminary data suggest that 1 U of *Tri t* I equals approximately 16 ng of protein.

Amino-Terminal Sequence Analysis

A total of 10 μl of *Tri t* I (~4 μg) was sequenced on an Applied Biosystems 420-A protein sequenator, and phenylthiohydantoin amino acids were identified on an Applied Biosystems 120-A liquid chromatograph (Applied Biosystems, Foster City, CA).

Statistical Analysis

Contingency tables were analyzed using Fisher's exact test.

RESULTS

Purification of Tri t I. Initial experiments were designed to identify an allergen from *T. tonsurans* that would react

with IgE antibodies from the majority of allergic individuals. Purification procedures were monitored by RAST and by quantitative intradermal skin tests for IH. The 60 to 90% SAS fraction of *T. tonsurans* extract was separated by using gel filtration over Sephacryl S200. The fractions from the S200 column showed maximum RAST reactivity in a broad peak of 12 to 180 kDa and were separated into four fractions, A to D (Fig. 1). These fractions were further purified by hydrophobic interaction chromatography, by using phenyl-Sepharose. Elution of fraction C (12–50 kDa) with distilled water yielded a protein that gave a single strongly stained band on SDS-PAGE (Fig. 2). This protein is hydrophobic and is 30 kDa, and its purity was further demonstrated by determination of the 30 amino-terminal amino acids (Fig. 3).

Measurement of serum antibodies to Tri t I. The ^{125}I-labeled *Tri t* I was used in an isotype-specific Ag-binding assay to measure the levels of IgG and IgE antibodies in the sera of patients with asthma, rhinitis, or urticaria who had positive IH skin tests to *Trichophyton*. Of these, 34 of 48 (71%) had IgG antibody to *Tri t* I and 26 of 48 (54%) had IgE antibody to *Tri t* I. IgE antibody to the 60 to 90% SAS *T. tonsurans* extract was also detected by RAST in 30 of 48 (63%) of these patients. Presumably, the five patients who had positive RAST responses but did not have detectable IgE antibody to *Tri t* I have formed IgE antibody to other Ag in the 60 to 90% fraction. This could not be confirmed by RAST testing with *Tri t* I, because of insufficient quantity of purified Ag. The majority of patients who had both skin test and RAST positive responses to the 60 to 90% SAS fraction of *Trichophyton*, i.e., 22 of 30 (73%), had detectable IgE antibody to *Tri t* I, suggesting that this protein represents a major *Trichophyton* allergen. Skin test-negative controls from the clinic (n = 16) had no detectable IgG or IgE antibody to *Tri t* I (Table I).

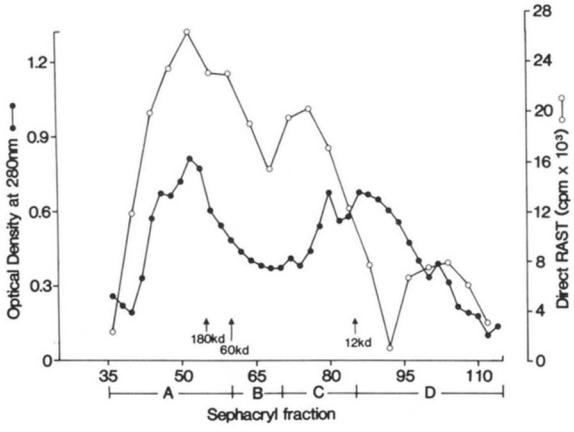

Figure 1. Eluted fractions of crude *T. tonsurans* from Sephacryl S-200 chromatography, showing OD at 280 nm (●) and RAST results (○). Column was calibrated with blue dextran (m.w., >1 × 10^6), human IgG (m.w., 1.5 × 10^5), BSA (m.w., 6 × 10^4), and cytocrome *C* (m.w., 1.2 × 10^4). Four fractions, A through D, were pooled as indicated.

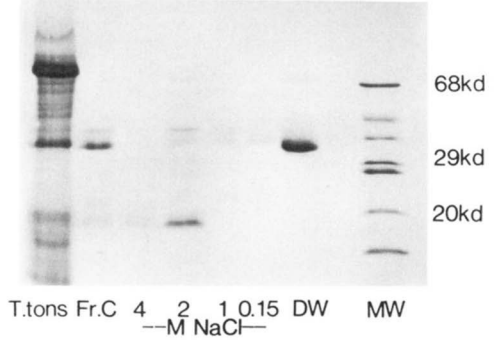

Figure 2. SDS-PAGE analysis showing, from *left* to *right*, crude *T. tonsurans* (*T. tons*), fraction C from Sephacryl S200 (*Fr.C*), phenyl-Sepharose fractions eluted with 4, 2, 1, and 0.15 M NaCl and distilled water (*DW*), and m.w. markers (*MW*).

1				5					10					15
Asp	-Asp	-Met	-**Ala**	-Phe	-Ser	-Leu	-Gly	-**Val**	-Lys	-Gly	-Pro	-Asp	-**Gly**
N	N	M	**(A)**	F	S	L	G	**V**	K	G	P	D	**G**
16				20					25					30
Ser	-Val	-Lys	-Gln	-**Leu**	-Val	-Asp	-Phe	-Glu	-**Gln**	-Asp	-Phe	-Val	-Ala	-**Leu**
S	V	K	Q	**L**	V	D	F	E	**Q**	D	F	V	A	**L**

Figure 3. Amino-terminal amino acid sequence analysis of *Tri t* I.

Thirty-eight subjects with a history of athlete's foot were skin tested with *Trichophyton* mix (Table II). Of these, nine gave a wheal and flare response, with no response at 48 h, and had a positive RAST response to the 60 to 90% SAS fraction. Among these nine, eight showed an IH skin response to *Tri t* I, whereas seven of eight had both IgG and IgE antibodies specific for *Tri t* I in Ag-binding RIA. Fourteen individuals had an indurated erythematous response to the mix at 48 h. When skin tested with *Tri t* I, none of this group showed immediate wheals, and only 1 of 14 showed a response at 48 h ($p <$ 0.001). In addition, these patients had no IgE antibody to *Tri t* I and negative RAST response. There was no difference in the serum response to *Tri t* I between those individuals who had DH alone and those who had DH preceded by a wheal at 15 min. There are two striking

TABLE I

Prevalence of serum antibodies to T. tonsurans in patients with positive IH skin tests[a]

Patient group	n	IgE antibody to *T. tonsurans* (RAST⁺)	Specific antibody to *Tri t* I		IgE antibody to *Tri t* I (RAST⁺)
			IgG	IgE	
Asthma	32	21/32	22/32	16/32	14/22
Rhinitis	7	4/7	5/7	4/7	3/4
Urticaria	9	5/9	7/9	6/9	5/5
Total[b]	48	30/48	34/48	26/48	22/30
Controls[c]	16	0/16	0/16	0/16	0/16

[a] All patients had 6- × 8-mm wheals on intradermal skin testing at 20 min.

[b] Of the 30 RAST-positive patients, 22 had IgE antibody to *Tri t* I and 25 had IgG antibody to *Tri t* I. All 26 individuals who had IgE antibody to *Tri t* I also had IgG antibody to *Tri t* I.

[c] Controls had negative IH skin tests to *T. tonsurans*.

TABLE II

Skin test responses to Trichophyton mix and to purified Tri t I in volunteers

Response to *Trichophyton* mix[a]	n	Skin response to *Tri t* I		Serum antibodies to *Tri t* I		IgE antibody to *Trichophyton* (RAST)
		IH	DH	IgG	IgE	
IH alone	9	8/9[b]	(0/9)[c]	7/8	7/8	8/8
DH alone[d]	7	0/7	0/7[e]	0/7	0/7	0/7
DH plus IH	7	0/7	1/7	1/7	0/7	0/7
Negative	15	ND	ND	0/8	0/8	0/8

[a] Skin tests to *Trichophyton* mix were carried out with 0.03 ml (1/200, w/v) containing 200 U/ml *Tri t* I.

[b] IH responses (≥6-mm wheal) were observed with 10^{-4} to 10^{-6} dilutions (~25 to 0.25 U/ml *Tri t* I).

[c] DH responses to *Tri t* I in this group are in parentheses because the concentration of *Tri t* I used for skin testing was limited by IH response.

[d] DH responses are defined as erythema ≥7 mm diameter at 48 h.

[e] Patients were tested with serial dilutions of *Tri t* I up to 1/100 (~250 U/ml).

features of these results, first, that *Tri t* I is not related to DH skin responses and, second, that the wheal preceding a DH response is not associated with measurable IgE antibodies.

mAb production and epitope analysis. Twelve clones producing IgG antibodies specific for *Tri t* I were detected by Ag-binding RIA. The ascites produced from these clones was also screened for IgG antibodies to *Tri t* I. In this assay, polyclonal mouse serum bound 31%, and two clones, 2F2-F7 and 6B11-C2, bound 20.6% and 9.6% of the radiolabeled allergen, respectively. Selected clones were used in an inhibition assay for binding of *Tri t* I, to compare the epitope specificity. These experiments demonstrated that mAb 2F2 could inhibit binding to itself by 72% but inhibited binding to 6B11 by <20%. Furthermore, 6B11 inhibited binding to itself by 94% but did not inhibit binding to 2F2. Thus, it was clear that these two mAb recognized separate epitopes on *Tri t* I (Table III).

Immunoassays for Tri t I. Initially, an inhibition RIA was developed to measure *Tri t* I by using human IgG antibodies. The assay was inhibited by commercial extracts of *Trichophyton* species, up to 85%, and by extracts made from cultures of *T. rubrum* grown from the skin of patients. Extracts of *T. mentagraphytes* and *T. rubrum* also inhibited this assay by 75%, thus demonstrating cross-reactivity among the three *Trichophyton* species (Fig. 4*A*). We subsequently developed a two-site radioimmunometric assay for the detection of *Tri t* I, using the mAb (Fig. 4*B*). A 60 to 90% SAS fraction of *T. tonsurans* was arbitrarily assigned 40,000 U/ml, and a control curve was constructed by using serial twofold dilutions of this fraction. In the mAb assay, serial dilutions of the 60 to 90% SAS extracts of *T. rubrum* and *T. mentagrophytes* produced parallel curves, indicating that the mAb recognized epitopes on *Tri t* I that are cross-reactive between the three species. The concentration of the protein equivalent to *Tri t* I in the *T. rubrum* and *T. mentagrophytes* extracts was 3200 and 7300 U/ml, respectively (Fig. 4*B*). The commercial *Trichophyton* mix (1/10, w/v) used for intradermal skin tests contained approximately 2200 U/ml, whereas purified *Tri t* I contained 25,000 U/ml. In addition, we assayed 39 extracts of house dust. Of these, the majority, 31 of 39, had less than 1 U/ml *Tri t* I; however, eight had levels of between 2 and 20 U/ml. By using the two-site mAb assay for detection of *Tri t* I in 16 commonly used fungal skin test extracts, we found only one extract (*Candida albicans*) that contained >1 U/ml material cross-reacting with *Tri t* I.

TABLE III

Fluid-phase binding of ^{125}I-Tri t I by murine mAb: inhibition of two epitopes

Clone	^{125}I-*Tri t* I bound (%)[a]	Inhibition (%) of binding of ^{125}I-*Tri t* I to[b]	
		6B11-C2	2F2-F7
2F2-H2	19.4	16.0	69.7
2F2-F7	20.6	17.0	72.9
4B2-A3	4.7	<1.0	46.7
6B11-C2	9.6	94.1	<1.0
6B11-D2	8.6	85.5	<1.0
7E1-F1	3.2	58.9	<1.0
1B11-F3	2.5	72.1	<1.0
Polyclonal mouse IgG anti-*T. tonsurans* antibody	31.0	48.8	32.0
Normal mouse serum	0.5	1.0	<1.0
Anti-mite mAb	0.4	3.5	3.8

[a] Binding of *Tri t* I when precipitated with goat anti-mouse IgG.

[b] Inhibition of binding of *Tri t* I (~100,000 cpm/100 μl) to mAb adherent to microtiter wells by 100 μl of a 1/100 dilution of ascites added to each well.

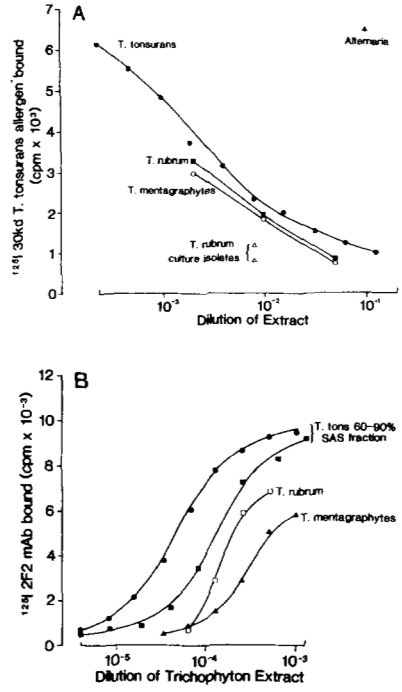

Figure 4. A. Inhibition RIA to measure *Tri t* I using human IgG, showing inhibition by three trichophyton species, *T. tonsurans* (●), *T. rubrum* (■), and *T. mentagraphytes* (○), and by an extract of *T. rubrum* (△) made from a culture from a patient's foot. No inhibition was seen with an *Alternaria* extract (▲). Results are expressed as the quantity of ^{125}I-*Tri t* I precipitated in the presence of the inhibitors (see *Materials and Methods*). *B,* two-site mAb immunometric RIA for detection of *Tri t* in three trichophyton species, *T. tonsurans* (●, ■), *T. rubrum* (□), and *T. mentagraphytes* (▲). Results are expressed as the quantity of second mAb bound, in cpm × 10^3.

DISCUSSION

Jones, Hay, and co-workers (22–26) have reported an increased prevalence of IH to *Trichophyton* among individuals with severe chronic fungal infections of the skin or nails. They observed patients who had wheal responses followed by DH and those with wheal responses alone. They further suggested that DH to dermatophytes played a protective role against chronic infection. The view that IH was not protective was also supported by a case report of an individual who was experimentally infected with *T. mentagrophytes,* who initially developed DH skin response but when retested had developed an IH wheal and flare response, coinciding with rapid spread of the fungal infection (27). Our objective was to identify and purify a protein from *T. tonsurans* that would produce IH responses. We expected that this same protein would give DH responses in those individuals who had DH. However, the protein purified, which appears to be an important Ag for IgE responses, did not give rise to any skin responses in the majority (13 of 14) individuals who have strong DH to *Trichophyton* extract. Furthermore, this 30-kDa allergen, *Tri t* I, does not induce the production of IgG antibodies in patients with DH.

Although partial purifications of dermatophyte Ag have been reported in the past, the definition of these proteins is unclear. The allergen reported here is defined by its physical properties, by the sequence of its 30 amino-

terminal amino acids, and by murine mAb. The development of mAb was made possible by a screening assay using radiolabeled Ag. This was necessary because initial experiments demonstrated that the Ag did not bind effectively to a microtiter plate. The initial yield of *Tri t* I from Sephacryl gel filtration followed by hydrophobic interaction chromatography was very low, i.e., approximately 200 μg from 100 g of crude mycelia. The mAb have been used for affinity purification, and *Tri t* I has been eluted with either distilled water or ethylene glycol. We are currently refining this technique to improve the yield of allergen. In addition, the mAb assay has been used to define two epitopes on *Tri t* I and to develop an immunometric assay for detection of this allergen. It is now possible to make estimates of the quantities of *Tri t* I in the skin test reagents, at various stages of the purification procedure, and in the environment.

It has become apparent through skin testing of a large number of patients in our outpatient clinic that some patients produce wheal and flare responses to *Trichophyton* but not to any other fungi. This suggests that in these patients the antibody response is specific to *Trichophyton*. In addition to this, preliminary data from absorption experiments suggest that *Trichophyton* IgE antibodies are not absorbed by using *Aspergillus* and *Alternaria* immunosorbents. Our results do not support the widely held notion that antibodies to fungi are cross-reactive among genera (28). Furthermore, by using the mAb assay we were unable to detect molecules cross-reacting with *Tri t* I in the majority of fungal skin test extracts tested. In contrast, cross-reactivity among *Trichophyton* species is extensive, as judged either by skin tests or by using assays based on human antibodies or mAb.

DH responses in humans are commonly observed to only a select group of Ag, e.g., tuberculin, mumps, *Candida*, and *Trichophyton*. In contrast, most of the Ag that are associated with IH never, or only very rarely, give rise to DH skin responses. This is true both in the patients who give wheal and flare responses and in those who give no immediate response. *Trichophyton* is, thus, unusual in that it gives DH responses in some individuals and IgE antibody responses in others. It is generally assumed that these different immune responses reflect differences in the dosage, route of exposure, and the effects of adjuvants. Typically, IgE antibody responses in mice require multiple small doses (≤0.1 μg), without the use of adjuvants such as CFA (29). In keeping with this, inhaled allergens such as pollen, animal danders, and dust mites induce IgE antibodies, if there is any response. At one time it was thought that the Ag (or atopens) that gave rise to IgE responses must have chemical properties different from those of other Ag. However, detailed analyses of many of these proteins over the last 10 years, including sequencing, have not revealed any characteristic features. In fact, the results have led to the conclusion that the nature of the immune response is dictated largely by the immunization regime and hardly at all by the nature of the protein. For this reason, we initially assumed that different immune responses to *Trichophyton* would be directed against the same dominant proteins. Our results now suggest very strongly that at least one of the major allergens (*Tri t* I) does not give rise to DH and does not induce IgG (or IgE) antibodies in individuals with DH.

The seven patients who were identified in this study as having a wheal and flare response, preceding DH, to the *Trichophyton* mix but who had no serum antibodies or skin reactivity to *Tri t* I represent an interesting group for further investigation. Although these patients had negative RAST response to the 60 to 90% SAS fraction, the wheals could reflect low levels of IgE antibody to an Ag other than *Tri t* I. Alternatively, they could reflect an early response that forms part of the DH reaction mediated by non-Ig T cell-derived factors (30). It is obviously interesting to ask how these subjects would respond to skin testing with a DH Ag from *Trichophyton* or how T cells from these patients would respond in vitro, in comparison with those from patients with IH or DH alone. The present results do not resolve why certain individuals develop different responses. We are now aware of several individuals who have IH to *Trichophyton* but have no particular difficulty with fungal infection. Furthermore, there is no simple relationship between IgE responses to other allergens and IgE responses to *Trichophyton*. Some "highly allergic" individuals have delayed responses to *Trichophyton*, whereas other individuals give wheal and flare responses to no allergens except *Trichophyton*. At present, it seems equally likely that prolonged infections predispose to IgE antibody responses as that IgE antibody responses block T cell responses and thus lead to more resistant infections. A major objective of our future studies is to purify an Ag that gives rise to DH, in order to test whether the individuals who have IgE antibodies to *Tri t* I have any evidence of DH against other proteins. Preliminary evidence using Sephacryl fractions suggests that DH responses are directed against a higher m.w. fraction of ~70 kDa (fractions A and B from S200; Fig. 2). However, it seems unlikely that high m.w. alone would explain the difference.

The demonstration of bronchial and nasal responses, as well as skin responses, to *Trichophyton* among individuals with asthma and rhinitis demonstrates generalized sensitization and supports the view that this Ag may play a role in hypersensitivity disease (5). The route by which sensitization occurs has not been resolved; however, we assume that absorption through the skin is the primary route. *Trichophyton* species are dependent on the use of keratin for growth and are, therefore, restricted to hair, skin, and nails; certainly, our own efforts to grow this fungus from nasal or lung secretions have been uniformly negative (G. Karlsson, G. W. Ward, B. Deuell, and T. A. E. Platts-Mills, unpublished results). It is possible (indeed likely) that inhaled *Trichophyton* gives rise to the sensitization that has been reported among podiatrists (31). Our present results from an immunoassay suggest that the quantities of *Tri t* I in house dust are generally very low, which argues against an inhaled route for sensitization of individuals other than podiatrists. Taking all the evidence together, we consider that absorption of Ag through the skin is the most likely route by which sensitization to *Trichophyton* species occurs. If so, it remains to be resolved why some individuals develop IgE antibodies to proteins including *Tri t* I, whereas others develop DH responses to proteins other than *Tri t* I.

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