

The Immune Response to *Trichophyton tonsurans*

Distinct T Cell Cytokine Profiles to a Single Protein Among Subjects with Immediate and Delayed Hypersensitivity¹

Jeffrey B. Slunt,* Ernesto A. Taketomi,*[†] Judith A. Woodfolk,* Mary L. Hayden,* and Thomas A. E. Platts-Mills^{2*}

Skin testing with an extract from the dermatophyte fungus *Trichophyton tonsurans* can result in either immediate (IH) or delayed hypersensitivity (DH). These experiments were designed to examine in vitro T cell cytokine production in response to purified proteins from *T. tonsurans* in subjects with different skin test reactivities. Peripheral blood mononuclear cells were obtained from subjects with immediate, delayed, or negative skin tests, and cellular proliferation was studied. Subjects with either IH or DH had positive proliferative responses to crude extracts and two purified proteins, protein IV (83 kDa) and *Tr* t 1 (30 kDa). Nine cell lines were established from 5 IH subjects, which produced a cytokine profile characteristic of Th2/Th0 cells, i.e., ratio of IFN- γ to IL-4 or IL-5 <2:1. By contrast 8 of 10 cell lines from DH subjects had a Th1 profile, i.e., IFN- γ to IL-4 or IL-5 >20:1. Lymphocytes from subjects with negative skin tests show very poor proliferative responses; however, 6 cell lines derived from these individuals showed a cytokine profile characteristic of Th1 cells. Levels of IL-5 were significantly different when comparing the IH group with the DH group ($p < 0.001$). The results demonstrate that a single defined protein from *T. tonsurans* can produce distinct T cell cytokine profiles that correspond to in vivo skin test reactivities and serum Ab levels. *The Journal of Immunology*, 1996, 157: 5192–5197.

Dermatophytes require keratin as a nutrient source, and infection of human skin or nails with these fungi is common (1–3). The main defense against these fungi is cell-mediated immunity, which can be demonstrated by an indurated skin test reaction appearing 24 to 48 h after s.c. injection of Ag, i.e., delayed hypersensitivity (DH)³ (1, 4–7). Experimental studies indicate that a positive DH reaction develops 10 days after fungal infection, and an additional 10 days is generally required before the fungus is cleared (7–9). However, in some individuals, the DH reaction is superseded by a wheal and flare skin test reaction, i.e., immediate hypersensitivity (IH) (7, 10–13). It has been postulated that this is the result of a chronic fungal infection (1, 6). Immediate skin test reactions to dermatophyte fungi have been associated with serum IgG4, and IgE Ab, as well as clinical conditions, including urticaria and late onset asthma (1, 7, 10–13). Distinct responses of this kind to a single Ag are unusual; DH reactions to

common allergens are very rare, as are IH reactions to most DH Ag (12, 13).

The immune system has been characterized as having at least two distinct types of T helper cells, Th1 and Th2 (14–16). T cells of the Th1 phenotype are primarily responsible for immunity to intracellular bacteria, protozoa, and viruses and produce IFN- γ , IL-2, and TNF- β (17–21). Typically an immune response including specific Th1 T cells is characterized by DH skin test responses and IgG Ab (17–21). T cells of the Th2 phenotype are associated with immune responses to helminths and allergens. These T cells secrete IL-4, IL-10, and IL-13 and help B cells to produce IgG4 and IgE Ab (17, 19–21). In addition, Th2 cells secrete IL-5, a cytokine that promotes the differentiation and proliferation of eosinophils (16, 22, 23). Eosinophils are believed to play a direct role in causing inflammation of the airways and asthma (24, 25). Th1 and Th2 cells negatively interact with each other; IFN- γ inhibits the development of Th2 cells, while IL-4, at high doses, suppresses the development of Th1 cells (15, 26). A third subclass of T helper cells, Th0, has been described that produces cytokines typical of both Th1 and Th2 cells. It is believed that these are precursors of Th1 and Th2 cells (17, 19).

The murine model of leishmaniasis has been extensively used to study the development of Th1 and Th2 cells. Most mouse strains produce a Th1 response to this parasite, which is associated with rapid resolution of infection (27, 28). However, BALB/c mice produce Th2 responses, produce high levels of Ab, develop visceral disease, and do not resolve the infection (27–29). The strongest influence on the types of cells that respond appears to be the cytokines that are present during the initial presentation of Ag to the naive T cell. The cytokines IFN- γ and IL-12, or Ab to IL-4, are able to reverse the disease outcome in BALB/c mice (30, 31). A similar reciprocal immune response has been postulated for parasite infections in humans. Cutaneous leishmaniasis and tuberculoid leprosy heal spontaneously and have been associated with DH skin

*Asthma and Allergic Diseases Center, Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, VA 22908; and [†]Immunology Division, Department of Pathology, Federal University of Uberlandia, Uberlandia, Minas Geras, Brazil

Received for publication March 15, 1996. Accepted for publication September 5, 1996.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This research was supported by National Institutes of Health Grants AI-20565, AI-30840, AI-34607, RR-00847, and Brazil's National Council of Scientific and Technological Development Grant 200155/93.

² Address correspondence and reprint requests to Dr. Thomas Platts-Mills, Asthma and Allergic Diseases Center, Box 225, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

³ Abbreviations used in this paper: DH, delayed hypersensitivity; IH, immediate hypersensitivity; *T. tons.* extract, 60 to 90% saturated ammonium sulfate fraction of a *T. tonsurans* mycelial extract; Dpt, dust mite *Dermatophagoides pteronissinus*; SI, stimulation index.

reactions, while visceral leishmaniasis and lepromatous leprosy tend to be chronic and have been linked to increased levels of circulating IL-4 (29, 32–34).

Previously our laboratory reported the purification of *Tri t 1* (30 kDa), the first major allergen purified from a dermatophyte fungus (12). A second Ag has recently been purified from *Trichophyton tonsurans* and has been designated protein IV (83 kDa); this is the first protein that can consistently produce IH or DH skin reactions in different individuals (13). The present studies were designed to characterize the cytokine profile of circulating T cells from individuals with different skin responses using purified *T. tonsurans* proteins. The results suggest that the immune response to *Trichophyton* is an excellent model for studying human Th1 and Th2 development.

Materials and Methods

Skin testing and assays for serum Ab

Individuals were skin tested with 0.02 ml of Hollister-Steir Trichophyton mix (1/200, w/v), which contains *T. tonsurans*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* (Hollister-Steir, Miles Pharmaceutical Division, Indianapolis, IN) (12, 13). Test sites were examined 15 min after injection and at 24 and 48 h. A positive IH skin test was defined by a wheal $\geq 6 \times 6$ mm at 15 min. A positive DH reaction was distinguished by an induration ≥ 6 mm in diameter at 24 or 48 h. IgG Ab to protein IV was measured by Ag-binding RIA using methods described previously (13, 35). A radioallergosorbent assay was used to measure IgE Ab to a 60 to 90% saturated ammonium sulfate fraction of a *T. tonsurans* mycelial extract (hereafter referred to as *T. tons.* extract) and samples were read from a standard curve (12, 13). Specific IgE and IgG4 Ab were measured by mAb-based ELISA using a method previously described (13).

Purification of Ag

Protein IV and *Tri t 1* were purified by electroelution from a 12% SDS-polyacrylamide gel using a micro-electroelutator (Amicon, Inc., Beverly, MA) as described previously (13, 36). Briefly, *T. tons.* extract was applied to a gel, and appropriately sized bands were excised and then electroeluted for 2 h. Purity was assessed by SDS-PAGE analysis and Ag-specific ELISA (12, 13). An extract of the house dust mite *Dermatophagoides pteronyssinus* (Dpt) was prepared from dried material kindly provided by Dr. Larry Arlian (Wright State University, Dayton, Ohio) and contained 285 μ g/ml Der p 1 and 121 μ g/ml Der p 2. Human serum albumin (Sigma Chemical Co., St. Louis, MO) was eluted from 12% polyacrylamide gels run in parallel experiments.

PBMC proliferation assays

Blood (50–100 ml) was drawn in syringes containing preservative-free heparin (Sigma Chemical Co.) (10 U/ml blood) and diluted 1/2 in RPMI 1640 (Bio-Whittaker, Walkersville, MD). Venipuncture was approved by the Human Investigations Committee at the University of Virginia. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). Cells (3×10^5 /well) were cultured at 37°C in triplicate in a 96-well plate (Sarstedt, Newton, NC) with Ag for 7 days in complete media. Complete media consisted of 10% pooled human AB serum (Sigma Chemical Co.), 100 U/ml penicillin, 100 μ g/ml streptomycin, MEM amino acids solution (1 $\times$), MEM nonessential amino acids solution (0.1 mM), sodium pyruvate (1.0 mM), HEPES buffer (15 mM) (Life Technologies, Gaithersburg, MD), and 50 μ g/ml gentamicin solution (Sigma Chemical Co.) in RPMI 1640. The cells were incubated in the presence of [^{3} H]TdR (ICN, Irvine, CA) for the final 16 h of culture before being harvested onto a glass fiber filter and counted with a Packard (Meriden, CT) Matrix 9600 direct beta counter. Results were expressed as a stimulation index (SI), calculated by dividing the mean of triplicate wells with Ag by the mean of triplicate wells cultured with albumin or without Ag. A SI ≥ 5 was considered positive.

Generation of T cell lines

Freshly isolated PBMC were cultured with crude extracts or purified proteins in a 24-well plate (2×10^6 cells/well). On day 6 of culture, 30 U/ml recombinant IL-2 (Hoffmann-La Roche, Nutley, NJ) was added. At day 14 of culture, the cells were harvested and the Ag specificity was determined using a proliferation assay. The phenotypic profile of the cell lines was examined using a FACScan (Becton Dickinson, San Jose, CA) and FITC-

Table 1. Skin test response and serum Ab titers to purified Ag (U/ml)

Subject	Skin Test	RAST ^a	Protein IV			<i>Tri t 1</i>
			IgG	IgG4	IgE	IgE
HL	IH	200	1050	<40	62	202
EP	IH	800	1000	<40	<40	90
CG	IH	ND	153	<40	<40	ND
MH	IH	<40	40	<40	164	118
JN	IH	1493	319	525	4000	4000
JL	IH	<40	129	338	2720	2400
JF	IH	867	1250	50	1044	1376
MB	IH	<40	510	<40	<40	<40
RH	IH	<40	319	<40	68	<40
KA	DH	<40	191	<40	<40	<40
TP	DH	155	94	<40	<40	<40
AM	DH	ND	ND	ND	ND	ND
GW	DH	<40	475	<40	<40	<40
ET	DH	<40	163	<40	<40	<40
AA	DH	ND	ND	ND	ND	ND
SS	DH	ND	ND	<40	<40	<40
GR	neg.	<40	65	20	158	104
SC	neg.	<40	1250	<40	<40	<40
PH	neg.	<40	43	<40	<40	<40
PW	neg.	<40	174	<40	<40	<40
HK	neg.	ND	ND	<40	<40	<40
KM	neg.	<40	<40	<40	<40	<40
MP	neg.	ND	ND	ND	ND	ND

^a RAST, radioallergosorbent assay. Measures IgE Ab to *T. tons.* extract.

conjugated mAb directed to CD3, CD4, CD8, and CD20 (Biosource Inc., Camarillo, CA). Cells (2×10^5 /well) were then cultured with 1×10^6 irradiated autologous PBMC, Ag, and rIL-2 for an additional 2 wk. The cell lines were considered Ag specific if the SI was greater than five times the control, or if the SI was greater than twice the control in those cases where the control was less than the mean of all the controls plus one standard deviation.

Cytokine assays

Cell culture supernatants were collected at various times from separate wells, and cytokines present were assayed using a standard ELISA with paired mAb (IL-4 and IL-5, PharMingen, San Diego, CA) (IFN- γ , Endogen, Cambridge, MA). Results were expressed in ng/ml as determined from a standard curve using recombinant cytokines. The lower level of cytokine detection ranged from 0.02 to 0.10 ng/ml for the IFN- γ assay, 0.06 to 0.23 ng/ml for IL-4, and 0.01 to 0.05 ng/ml for IL-5.

Statistical analysis

Statistical significance was demonstrated using an unpaired Student *t* test.

Results

Twenty individuals with a history of athlete's foot disease and/or onychomycosis and three individuals without a history of fungal infection (KM, HK, MP) were studied. Nine subjects had a positive immediate skin test reaction (Table 1). Seven subjects had delayed hypersensitivity, while an additional seven subjects, including the three individuals without a history of infection, were skin test negative. IH subjects had higher levels of Ag-specific IgE and IgG4 Ab when compared with the DH or skin test-negative subjects. Allergen-specific IgG was found in all skin test groups.

Peripheral blood mononuclear cells were separated from whole blood and used in a standard proliferation assay. Cells were cultured with *T. tons.* extracts, as well as purified protein IV and *Tri t 1*. As controls, cells were cultured with medium only, electroeluted human serum albumin, or phytohemagglutinin (PHA). Subjects with an IH or DH skin test had stronger responses to the crude extracts as compared with the skin test-negative subjects (Table

Table II. PBMC proliferative responses at day 7 to purified *Trichophyton* proteins

Subject	Skin Test	Stimulation Indices				
		0-60% ^a	<i>T. tons.</i>	Prot. IV	<i>Tri t 1</i>	PHA
HL	IH	20	44	82 ^b	62 ^b	99 ^b
EP	IH	18	7	15	10	121 ^b
CG	IH	27	7	35	15	59 ^b
MH	IH	21	7	4	<1	46 ^b
JN	IH	3	6	19	11	84
JL	IH	6	2	7	3	93
JF	IH	13	7	9	18	18
MB	IH	20	10	7 ^b	2	82 ^b
RH	IH	39	35	90	58	169 ^b
KA	DH	52	32	39	29	48
TP	DH	150	116	49	32	85
AM	DH	14 ^b	8 ^b	1 ^b	3 ^b	26 ^b
GW	DH	53	32	2	3	18
ET	DH	16	19	8	8	38
AA	DH	6	5	3	4	36 ^b
SS	DH	97	128	28	35	49
GR	neg.	7	4	<1	8	8
SC	neg.	8	5	2	2	19
PH	neg.	<1	4	1	<1	11
PW	neg.	2	2	2	2	94
HK	neg.	2	3	2	<1	36
KM	neg.	4	3	3	3	42
MP	neg.	<1	4	2	1	19 ^b

^a The 0-60% saturated ammonium sulfate fraction of *T. tonsurans* mycelial extract.

^b Proliferation measured on day 5.

II). Eight of nine IH subjects had a positive proliferative response to either protein IV or *Tri t 1*, as did four of the seven DH subjects. Five of the seven skin test-negative subjects did not show a proliferative response to any of the Ag tested. The two skin test-negative subjects whose T cells proliferated to purified Ag (GR and SC, had serum Ab to protein IV (Table I). None of the subjects had a positive proliferative response to human serum albumin purified by the same techniques used to purify the *Trichophyton* proteins (data not shown).

Two cell lines were established in parallel from an individual (SS) who had an IH skin test to dust mite (Dpt) extract and a DH skin test reaction to *Trichophyton*. During the culture period following a second stimulation with allergen, distinct cytokine profiles were seen. The reaction to Dpt was characterized by the production of large amounts (>1 ng/ml) of IL-4, IL-5, and IFN- γ (Fig. 1A), while the *T. tons.*-specific cell line released only IFN- γ (Fig. 1B). Both cell lines were shown to be Ag specific by proliferation assay (data not shown).

Nine cell lines were established from 5 subjects who had IH skin tests to *Trichophyton* mix. These cell lines, specific to either protein IV, *Tri t 1*, or *T. tons.* extract, showed 2 patterns of cytokine production. Two cell lines specific to protein IV produced >1 ng/ml of IFN- γ , IL-4, and IL-5, a result typical of Th0 cell lines (Fig. 2A). Seven cell lines from the subjects EP, HL, and MH produced >6 ng/ml IL-5 but <1.2 ng/ml of IL-4 or IFN- γ . This cytokine pattern is suggestive of a Th2 phenotype (Fig. 2B). A total of 10 cell lines specific to protein IV, *Tri t 1*, or the *T. tons.* extract were established from 6 subjects who had DH skin reactions (Fig. 2C), and 6 cell lines specific to the *T. tons.* extract were established from skin test-negative subjects. Cells from both subject groups released high concentrations of IFN- γ (>2 ng/ml), with little to no IL-4 or IL-5. There was a clear difference in the concentrations of IL-4 and IL-5 between the IH subject group and the DH and skin test-negative group. Looking at the level of cy-

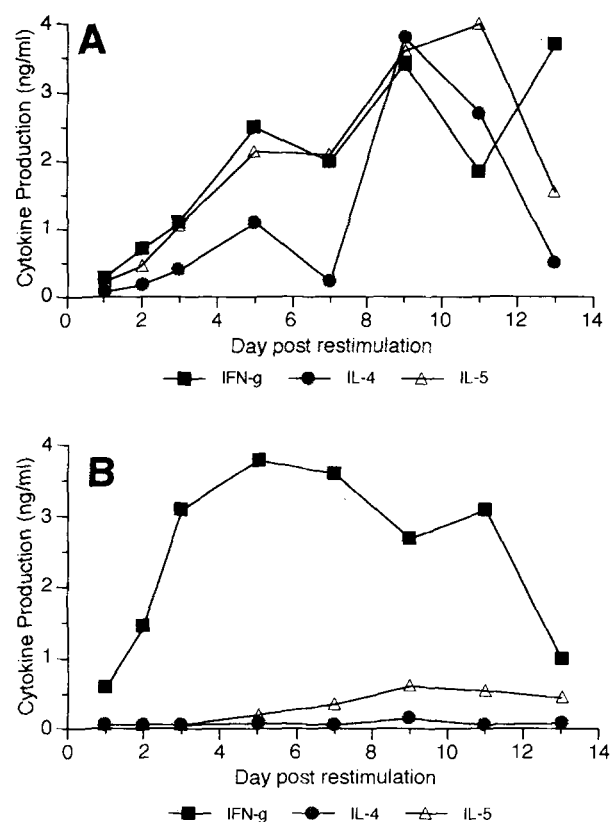


FIGURE 1. T cell cytokine (IFN- γ , IL-4, and IL-5) production by two Ag-specific T cell lines from subject SS. Supernatants were harvested from separate wells, and accumulated cytokine levels are given in ng/ml at various days after restimulation of the cell line. A, T cell line specific to Dpt extract, to which the subject had an IH skin test response. B, T cell line specific to *T. tons.* extract, to which the subject had a DH skin response.

tokine production at day 7 after restimulation (Fig. 3), there was a geometric mean level of 0.51 ng/ml IFN- γ , 0.11 ng/ml IL-4, and 2.8 ng/ml IL-5 among the 9 cell lines from the IH subject group. The geometric mean level of cytokine production among the DH subject cell lines showed a higher level of IFN- γ (1.4 ng/ml) and much lower levels of IL-4 (0.10 ng/ml) and IL-5 (0.10 ng/ml). Similar values were seen with cell lines from skin test-negative subjects with 1.2 ng/ml IFN- γ , 0.07 ng/ml IL-4, and 0.30 ng/ml IL-5. The level of IL-5 was significantly increased among the IH subjects as compared with the cell lines from the DH subjects at days 3, 5 ($p < 0.01$), 7, and 9 ($p < 0.001$). FACS analysis of the cell lines showed a range of CD3, CD4, and CD8 positivity but did not demonstrate consistent differences among cell lines associated with IH, DH, or negative skin tests (Table III). All cell lines were shown to be Ag specific by proliferation assay (Table IV).

Discussion

There are many factors that may play a role in determining the immune response to a foreign protein. The first is the nature of the Ag source, i.e., bacterial, fungal, viral, or proteins derived from airborne particles, such as pollen grains. The next important factor is the route of exposure. Inhalation may give rise to strikingly different T cell responses compared with absorption of Ag through the skin or gastrointestinal tract (37, 38). Indeed, in the murine model of leishmaniasis, C57BL/6 mice, which normally clear the parasites, die when given a live parasite challenge by intravenous

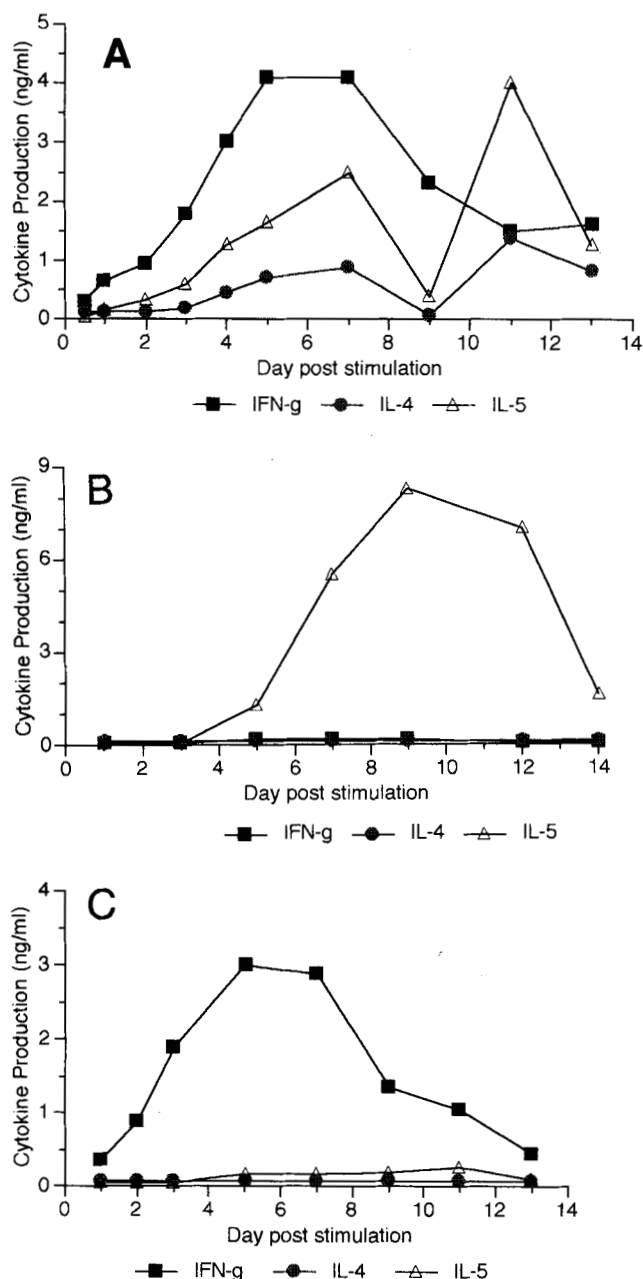


FIGURE 2. T cell cytokine (IFN- γ , IL-4, and IL-5) production by three protein IV-specific T cell lines derived from subjects with immediate hypersensitivity (2A, 2B) or delayed hypersensitivity (2C). Supernatants were harvested from separate wells, and accumulated cytokine levels are given in ng/ml at various days after restimulation of the cell line. A, T cell line from subject JL (IH). B, T cell line from subject EP (IH). C, T cell line from subject KA (DH).

injection (39, 40). In large part, it is assumed that this is because the Ag is presented by a different APC (38). Different APC are characterized by the presence or absence of costimulatory molecules and by their production of cytokines (17, 40, 41). These factors can alter the development of T helper cells. In fact, in vitro cloning studies have demonstrated that the presence of the cytokine IL-4 during Ag presentation drives cells toward the Th2 phenotype, while the presence of IFN- γ and IL-12, with suboptimal concentrations of IL-4, will induce cells of the Th1 phenotype (17, 40–43). As stated earlier, this appears to be the most important

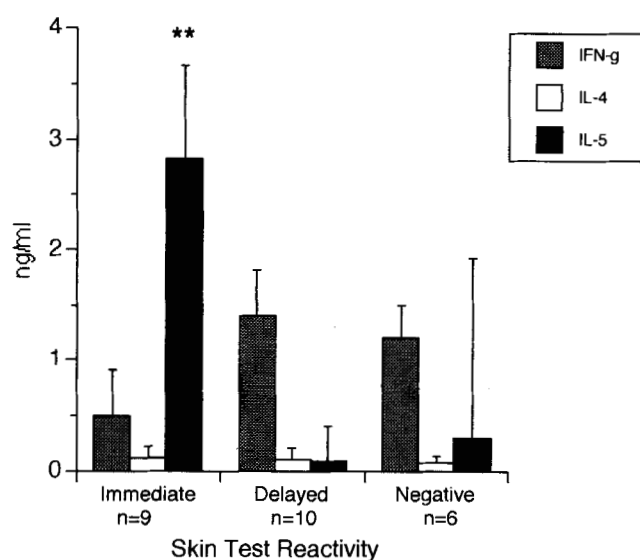


FIGURE 3. Geometric mean level of cytokine (IFN- γ , IL-4, and IL-5) production and the standard error of the mean at day seven after restimulation among all Ag specific T cell lines from the different subject groups. Cytokine levels for the IH subject group were 0.51 ± 0.41 ng/ml IFN- γ , 0.11 ± 0.09 ng/ml IL-4, 2.8 ± 0.84 ng/ml IL-5. The DH subject group had a mean level of 1.4 ± 0.41 ng/ml IFN- γ , 0.10 ± 0.03 ng/ml IL-4, and 0.10 ± 0.31 ng/ml IL-5, while the skin test-negative subjects had 1.2 ± 0.28 ng/ml IFN- γ , 0.07 ± 0.01 ng/ml IL-4, and 1.60 ± 1.60 ng/ml IL-5. ** Levels of IL-5 were significantly increased among the IH group as compared with the DH group ($p < 0.001$).

Table III. Cell surface phenotype of T cell lines

Subject	Skin Test	Cell Line Specific to	% Cells Positive			
			CD3	CD4	CD8	CD20
HL	IH	<i>T. tons.</i>	82.6	77.0	12.4	6.1
HL	IH	Prot. IV	83.3	74.6	15.2	6.4
HL	IH	<i>Tri t 1</i>	85.7	73.7	16.0	7.0
EP	IH	<i>T. tons.</i>	82.7	52.9	35.8	5.9
EP	IH	Prot. IV	86.4	51.9	35.8	ND
EP	IH	<i>Tri t 1</i>	84.4	51.8	35.2	ND
MH	IH	<i>T. tons.</i>	91.2	80.9	14.0	2.5
JN	IH	Prot. IV	74.7	78.2	3.1	3.6
JL	IH	Prot. IV	91.2	76.0	9.2	3.6
KA	DH	<i>T. tons.</i>	97.9	33.8	35.7	0.7
KA	DH	Prot. IV	79.3	56.7	11.6	5.2
KA	DH	<i>Tri t 1</i>	81.0	58.9	10.6	5.1
TP	DH	<i>T. tons.</i>	63.0	34.1	11.9	0.1
TP	DH	Prot. IV	66.1	30.3	14.1	0.1
TP	DH	<i>Tri t 1</i>	81.4	46.2	10.9	0.2
AM	DH	<i>T. tons.</i>	ND	ND	ND	ND
ET	DH	<i>T. tons.</i>	82.7	38.4	34.9	0.7
AA	DH	<i>T. tons.</i>	85.6	66.9	16.8	14.1
SS	DH	<i>T. tons.</i>	86.3	57.3	34.8	2.6
GR	neg.	<i>T. tons.</i>	78.5	70.1	22.5	2.2
SC	neg.	<i>T. tons.</i>	80.7	77.4	9.8	3.2
PH	neg.	<i>T. tons.</i>	92.8	71.2	22.4	8.9
PW	neg.	<i>T. tons.</i>	96.7	80.2	9.1	ND
HK	neg.	<i>T. tons.</i>	ND	ND	ND	ND
MP	neg.	<i>T. tons.</i>	94.7	83.0	14.3	3.8

influence in determining the outcome of leishmaniasis in the mouse.

In the immune response to *T. tonsurans*, the determining factor in the development of responding Th1 or Th2 cells is not the source of the Ag; both IH and DH reactions have been demonstrated to the same organism (2, 12, 13). In addition, the difference

Table IV. Cell line proliferation results

Subject	Skin Test	Cell Line Specific to	Stimulation Indices		
			PHA	Trich. Ag	Dpt
HL	IH	<i>T. tons.</i>	459	30.9	2.2
HL	IH	Prot. IV	411	43.7	3.8
HL	IH	<i>Tri t 1</i>	352	52.4	1.8
EP	IH	<i>T. tons.</i>	110	7.8	1.2
EP	IH	Prot. IV	699	23.7	3.9
EP	IH	<i>Tri t 1</i>	761	28.1	1.4
MH	IH	<i>T. tons.</i>	935	17.8	<1
JN	IH	Prot. IV	331	3.6	1.6
JL	IH	Prot. IV	41	7.1	<1
KA	DH	<i>T. tons.</i>	106	7.4	<1
KA	DH	Prot. IV	73	5.9	1.1
KA	DH	<i>Tri t 1</i>	48	3.6	1.1
TP	DH	<i>T. tons.</i>	480	7.0	2.6
TP	DH	Prot. IV	363	27	<1
TP	DH	<i>Tri t 1</i>	1153	4.0	<1
AM	DH	<i>T. tons.</i>	160	6.7	<1
ET	DH	<i>T. tons.</i>	206	55.6	<1
AA	DH	<i>T. tons.</i>	451	15.3	1.8
SS	DH	<i>T. tons.</i>	640	47.5	<1
GR	neg.	<i>T. tons.</i>	202	3.8	<1
SC	neg.	<i>T. tons.</i>	121	16.3	1.4
PH	neg.	<i>T. tons.</i>	558	95.9	1.4
PW	neg.	<i>T. tons.</i>	158	4.6	1.5
HK	neg.	<i>T. tons.</i>	230	3.1	<1
MP	neg.	<i>T. tons.</i>	878	3.4	<1

is not likely to be the nature of the Ag, since the same protein can cause two types of skin test responses (13), and the present results demonstrate that the same protein can induce the release of different cytokines in vitro. It has been reported that inhalation of *Trichophyton* proteins by podiatrists can induce IH skin tests and bronchial responses (12, 44). Inhalation may be introducing the Ag to the immune system by a different route, thereby contacting different APC, resulting in a Th2 immune response. However, studies in our laboratory have not been able to find significant levels of *Tri t 1* or protein IV in house dust, including dust from subjects with high levels of *T. tonsurans*-specific IgE (13). Therefore, we theorize that absorption of fungal Ag through the skin can elicit Th1 or Th2 T cell responses. Current studies in our laboratory are focusing on the role of the APC in the different immune responses to *T. tonsurans*.

The presence of IFN- γ in our cultures from IH subjects may be considered surprising since IFN- γ can down-regulate the development of Th2 cells. However, these results are consistent with findings from our laboratory when T cells were cultured from dust mite-allergic individuals and with results published from other laboratories (18, 45, 46). Depletion of CD8⁺ cells at the initiation of culture of Dpt-specific T cell lines did not reduce levels of IFN- γ as compared with cultures established with CD8 cells present. This suggests that polyclonal T cell lines among allergic individuals are likely to be Th0/Th2, with CD4⁺ cells capable of producing IFN- γ , and not the clear Th2 phenotype that has been demonstrated in T cell clones. Even with this IFN- γ production in vitro, immediate hypersensitivity to *Trichophyton* proteins can persist and has been documented in patients undergoing long term treatment with antifungal agents. In addition we have previously shown that IH skin tests to *Trichophyton* are not necessarily correlated with IH skin tests to other allergens (47).

In this study, two skin test-negative subjects had a positive proliferative response to at least one of the purified Ag. Both of these subjects had allergen-specific IgG in their serum, confirming that

they had made an immune response to *T. tonsurans*. Other skin test-negative individuals had little to no proliferative response, and we did not anticipate that we would be able to culture cell lines from these subjects. However, after repeated stimulation with *T. tons.* extract, Ag-specific T cell lines were recovered. In each case these cells produced IFN- γ with little to no IL-4 or IL-5. This cytokine response was similar to that seen among the DH subjects. It is not clear what differences there are between these groups; however, the lower level of proliferation among the skin test-negative subjects suggests a lower frequency of Ag-specific T cells. Similar results have been found in our laboratory and others when culturing T cells from dust mite skin test-negative subjects with a Dpt extract (15, 48, 49). Despite having Ag-specific T cells that produce IFN- γ , subjects who are not allergic to dust mites do not have demonstrable DH skin reactions.

These results establish for the first time that proteins derived from a single source giving rise to delayed or immediate hypersensitivity are associated with characteristic T cell responses. In addition, it is clear that these T cells are related to different serum Ab. The individuals whose T cells selectively produce IFN- γ in vitro have IgG Ab and DH skin responses. Skin test-negative individuals had a cytokine profile and Ab levels similar to the DH subject but had a much lower level of Ag-specific in vitro proliferation at day 7. The Th2/Th0 cytokine profile is related to IgG, IgG4, and IgE Ab and immediate skin test reactivities. Finally, the results establish that the IH response to *Trichophyton*, which has been associated with late onset asthma and eosinophilia, is characterized by T cells which produce IL-5 in vitro.

T. tonsurans infection is comparable to infection by *Leishmania major* in mice and humans and *Mycobacterium leprae* in humans in that it can give rise to two distinct immune responses. The results presented in this article pose the question why different individuals respond dissimilarly to the same protein. Future studies will address this issue by looking at the effect of different APCs and cytokines on the in vitro response to *Trichophyton* Ag. This work will be aided by the fact that the distinct cytokine responses to *Trichophyton* correlate with different skin test reactivities. The dichotomy between immediate and delayed hypersensitivity responses to dermatophyte infections is important in relation to both allergic disease and skin infection. Investigating the immune response to fully characterized *Trichophyton* Ag can provide basic information about this dichotomy in the human immune response.

Acknowledgments

We thank Hollister-Steir, Miles Pharmaceutical Division, for supplying the *T. tonsurans* culture material. We also thank Dr. George Ward for supplying sera and clinical data and Nancy Malone for help preparing the manuscript. Dr. Taketomi and Dr. Woodfolk are especially grateful to Allergy Control Products (Ridgefield, CT) for educational grants.

References

- Dahl, M. V., and S. A. Grando. 1994. Chronic dermatophytosis: what is special about *Trichophyton rubrum*? *Adv. Dermatol.* 9:97.
- Sohnle, P. G. 1993. Dermatophytosis. In *Fungal Infections and Immune Responses*. J. W. Murphy, H. Friedman, and M. Bendinelli, eds. Plenum Publishing Corp., New York, p. 27.
- Dahl, M. V. 1993. Suppression of immunity and inflammation by products produced by dermatophytes. *J. Am. Acad. Dermatol.* 28:S19.
- Sohnle, P. G. 1989. Dermatophytosis. In *Immunology of the Fungal Diseases*. R. A. Cox, ed. CRC Press, Inc., Boca Raton, FL, p. 1.
- Blake, J. S., M. V. Dahl, M. J. Herron, and R. D. Nelson. 1991. An immunoinhibitory cell wall glycoprotein (mannan) from *Trichophyton rubrum*. *J. Invest. Dermatol.* 96:657.
- McGregor, J. M., A. J. Hamilton, and R. J. Hay. 1992. Possible mechanisms of immune modulation in chronic dermatophytosis: an in vitro study. *Br. J. Dermatol.* 127:233.
- Jones, H. E. 1993. Immune response and host resistance of humans to dermatophyte infection. *J. Am. Acad. Dermatol.* 28:S12.

8. Jones, H. E., J. H. Reinhardt, and M. G. Rinaldi. 1974. Model dermatophytosis in naturally infected subjects. *Arch. Dermatol.* 110:369.
9. Jones H. E., J. H. Reinhardt, and M. G. Rinaldi. 1974. Acquired immunity to dermatophytes. *Arch. Dermatol.* 109:840.
10. Ward, G. E., G. A. Karlsson, G. Rose, and T. A. E. Platts-Mills. 1989. *Trichophyton* asthma: sensitization of bronchi and upper airways to dermatophyte antigen. *Lancet* 8643:859.
11. Kivity, S., Y. Schwarz, and E. Fireman. 1992. The association of perennial rhinitis with *Trichophyton* infection. *Clin. Exp. Allergy* 22:498.
12. Deuell, B., L. K. Arruda, M. L. Hayden, M. D. Chapman, and T. A. E. Platts-Mills. 1991. *Trichophyton tonsurans* allergen I: characterization of a protein that causes immediate but not delayed hypersensitivity. *J. Immunol.* 147:96.
13. Woodfolk, J. A., J. B. Slunt, B. Deuell, M. L. Hayden, and T. A. E. Platts-Mills. 1996. Definition of a *Trichophyton* protein associated with delayed hypersensitivity in humans: evidence for immediate (IgE and IgG4) and delayed hypersensitivity to a single protein. *J. Immunol.* 156:1695.
14. Mosmann, T. R., H. Cerwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136:2348.
15. Wierenga, E. A., M. Snoek, H. M. Jansen, J. A. Bos, R. W. A. van Lier, and M. L. Kapsenberg. 1991. Human atopen-specific types 1 and 2 T helper cell clones. *J. Immunol.* 147:2942.
16. Romagnani, S. 1991. Type 1 T helper and type 2 T helper cells: functions, regulation, and role in protection and disease. *Int. J. Clin. Lab. Res.* 21:152.
17. Seder, R. A. 1994. Acquisition of lymphokine-producing phenotype by CD4⁺ T cells. *J. Allergy Clin. Immunol.* 94:1195.
18. Jutel, M., T. Wyss-Coray, J. M. Carballido, K. Blaser, U. R. Muller, and W. J. Pichler. 1995. Selective restimulation of antigen or allergen preactivated T cells using OKT3 F(ab')₂ results in the secretion of TH-1 or TH-2-like cytokine patterns. *Clin. Exp. Allergy* 25:1108.
19. Kamogawa, Y., L. E. Minasi, S. R. Carding, K. Bottomly, and R. A. Flavell. 1993. The relationship of IL-4 and IFN- γ producing T cells studied by lineage ablation of IL-4 producing cells. *Cell* 75:985.
20. Romagnani, S. 1994. Regulation of the development of type 2 T-helper cells in allergy. *Curr. Opin. Immunol.* 6:838.
21. Marshall, J. D., Y. Wen, J. S. Abrams, and D. T. Umetsu. 1993. In vitro synthesis of IL-4 by human CD4⁺ T cells requires repeated antigenic stimulation. *Cell. Immunol.* 152:18.
22. Campbell, H. D., W. Q. J. Tucker, Y. Hort, M. E. Martinson, G. Mayo, E. J. Clutterbuck, C. J. Sanderson, and I. G. Young. 1987. Molecular cloning and expression of the gene encoding human eosinophil differentiation factor (IL-5). *Proc. Natl. Acad. Sci. USA* 84:6629.
23. Wierenga, E. A., B. Backx, M. Snoek, L. Koenderman, and M. L. Kapsenberg. 1993. Relative contributions of human types 1 and 2 helper cell-derived eosinophilotropic cytokines to development of eosinophilia. *Blood* 82:1471.
24. Gleich, G. J. 1990. The eosinophil and bronchial asthma: current understanding. *J. Allergy Clin. Immunol.* 85:422.
25. Corrigan, C. J. 1992. T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today* 13:501.
26. Seder, R. A., W. E. Paul, M. M. Davis, and B. F. de St. Groth. 1992. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4⁺ T cells from T cell receptor transgenic mice. *J. Exp. Med.* 176:1091.
27. Scharton-Kersten, T., L. C. C. Afonso, M. Wysocka, G. Trinchieri, and P. Scott. 1995. IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development in experimental leishmaniasis. *J. Immunol.* 154:5320.
28. Afonso, L. C. C., T. M. Scharton, L. Q. Vieira, M. Wysocka, G. Trinchieri, and P. Scott. 1994. The adjuvant effect of interleukin-12 in a vaccine against *Leishmania major*. *Science* 263:235.
29. Liew, F. Y. 1992. Induction, regulation and function of T-cell subsets in leishmaniasis. In *Regulation and Functional Significance of T-Cell Subsets*. R. L. Coffman, ed. Chemical Immunology Series, Vol. 54. S. Karger, Basel, p. 117.
30. Reiner, S. L., Z.-E. Wang, F. Hatam, P. Scott, and R. M. Locksley. 1993. Th1 and Th2 cell antigen receptors in experimental leishmaniasis. *Science* 259:1457.
31. Bliss, J., V. Van Cleave, K. Murray, A. Wiencis, M. Ketchum, R. Maylor, T. Haire, C. Resmini, A. K. Abbas, and S. F. Wolf. 1996. IL-12 as an adjuvant promotes a T helper 1 cell, but does not suppress a T helper 2 recall response. *J. Immunol.* 156:887.
32. Salgame, P., M. Yamamura, B. R. Bloom, and R. L. Modlin. 1992. Evidence for functional subsets of CD4⁺ and CD8⁺ T cells in human disease: lymphokine patterns in leprosy. In *Regulation and Functional Significance of T-Cell Subsets*. R. L. Coffman, ed. Chemical Immunology Series, Vol. 54. S. Karger, Basel, p. 44.
33. Waldorf, G. S., J. N. Sheagren, J. R. Trautman, and J. B. Block. 1966. Impaired delayed type hypersensitivity in patients with lepromatous leprosy. *Lancet* ii:733.
34. Kemp, M., T. G. Theander, and A. Kharazmi. 1996. The contrasting roles of CD4⁺ T cells in intracellular infections in humans: Leishmaniasis as an example. *Immunol. Today* 17:13.
35. Chapman, M. D., S. Rowntree, E. B. Mitchell, M. C. Di Prisco de Fuenmajior, and T. A. E. Platts-Mills. 1983. Quantitative assessments of IgG and IgE antibodies to inhalant allergens in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* 72:27.
36. LeGendre, N. M., M. Mansfield, A. Weiss, and P. Matsudaira. 1993. Purification of proteins and peptides by SDS-PAGE. In *A Practical Guide to Protein and Peptide Purification for Microsequencing*, 2nd Ed. P. Matsudaira, ed. Academic Press, Inc., San Diego, p. 71.
37. Jutel, M., W. J. Pichler, D. Skrbic, A. Urwyler, C. Dahinden, and U. R. Muller. 1995. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen-stimulated T cell cultures. *J. Immunol.* 154:4187.
38. Secrist, H., R. H. DeKruyff, and D. T. Umetsu. 1995. Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by Ag concentration and antigen-presenting cell type. *J. Exp. Med.* 181:1081.
39. Scott, P. A. 1996. Th cell development and regulation in experimental cutaneous leishmaniasis. In *Th1 and Th2 Cells in Health and Disease*. S. Romagnani, ed. Chemical Immunology Series, Vol. 63. S. Karger, Basel, p. 98.
40. Duncan, D. D., and S. L. Swain. 1994. Role of Ag-presenting cells in the polarized development of helper T cell subsets: evidence for differential cytokine production by Th0 cells in response to Ag presentation by B cells and macrophages. *Eur. J. Immunol.* 24:2506.
41. Reiner, S. L., and R. A. Seder. 1995. T helper cell differentiation in immune response. *Curr. Opin. Immunol.* 7:360.
42. Scott, P. A., and J. P. Farrell. 1982. Experimental cutaneous leishmaniasis: disseminated leishmaniasis in genetically susceptible and resistant mice. *Am. J. Trop. Med. Hyg.* 31:230.
43. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251.
44. Davies, R. R., M. A. Ganderton, and M. A. Savage. 1983. Human nail dust and precipitating Abs to *Trichophyton rubrum* in chiropodists. *Clin. Allergy* 13:309.
45. Bryon, K. A., R. M. O'Brien, G. A. Varigos, and A. M. Wootton. 1994. *Dermatophagoides pteronyssinus* II-induced IL-4 and IFN- γ expression by freshly isolated lymphocytes of atopic individuals. *Clin. Exp. Allergy* 24:878.
46. Looney, R. J., D. Pudiak, and S. I. Rosenfeld. 1994. Cytokine production by mite-specific T cells from donors with mild atopic disease. *J. Allergy Clin. Immunol.* 93:476.
47. Platts-Mills, T. A. E., G. P. Fiocco, M. L. Hayden, J. L. Guerrant, S. M. Pollart, and S. R. Wilkens. 1987. Serum IgE Abs to *Trichophyton* in patients with urticaria, angioedema, asthma, and rhinitis: development of a radioallergosorbent test. *J. Allergy Clin. Immunol.* 79:40.
48. O'Hehir, R. E., V. Bal, D. Quint, R. Moqbel, A. B. Kay, E. D. Zanders, and J. R. Lamb. 1989. An in vitro model of allergen-dependent IgE synthesis by human B lymphocytes: comparison of the response of an atopic and a non-atopic individual to *Dermatophagoides* spp. (house dust mite). *Immunology* 66:499.
49. Wierenga, E. A., M. Snoek, C. De Groot, I. Chretien, J. D. Bos, H. M. Jansen, and M. L. Kapsenberg. 1990. Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic patients. *J. Immunol.* 144:4651.