

Diversity of the Human Allergen-Specific T Cell Repertoire Associated with Distinct Skin Test Reactions: Delayed-Type Hypersensitivity-Associated Major Epitopes Induce Th1- and Th2-Dominated Responses¹

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Distinct immune responses in humans to the *Trichophyton rubrum* Ag, Tri r 2, are associated with different patterns of T cell epitope recognition based on in vitro proliferation to peptides derived from this 29-kDa protein. Specifically, the amino-terminal immunodominant epitope, peptide 5 (P5), stimulates strong T cell proliferative responses in subjects with delayed (DTH), but not immediate (IH) hypersensitivity skin tests. Evidence of a role for cytokines or changes in epitope recognition over time was examined in responses to *Trichophyton* using primary PBMC cultures established from seven IH and seven DTH subjects. Responses stimulated by Tri r 2 were dominated by the Th1 cytokine IFN- γ (IFN- γ :IL-5 $\geq$ 4:1) in five DTH subjects, even in the presence of Th2-dominated responses (IFN- γ :IL-5 $\leq$ 3:1) to a subset of major epitopes. Paradoxically, P5 induced IL-5 and IL-10 production in DTH, but not IH subjects ($p = 0.003$ (IL-5), $p = 0.024$ (IL-10)), with no significant difference in IFN- γ levels between the two groups. In cultures from IH responders, no IL-5 was measurable after stimulation with P6 and P7 (as well as P5); this region of the molecule was shown previously to stimulate markedly reduced T cell proliferation in these individuals. Repeat proliferation assays confirmed no change in the pattern of peptide recognition after ≥ 20 mo in IH or DTH subjects. We conclude that T cell repertoires associated with distinct immune responses to Tri r 2 can be distinguished based on Th2 cytokine induction by DTH-associated major epitopes localizing to the amino-terminal region of the molecule. *The Journal of Immunology*, 2001, 167: 5412–5419.

Characterization of distinct CD4⁺ Th lymphocyte subsets by Mosmann et al. (1) in the 1980s opened the door to studies aimed at defining the role of T lymphocytes in the immune response. Ags derived from a variety of pathogens can elicit distinct immune responses, and studies in mice and humans have provided strong evidence for involvement of different CD4⁺ T lymphocytes in these responses (2–6). The dermatophyte fungus *Trichophyton* elicits distinct skin test reactions in different individuals. Immediate hypersensitivity (IH)³ responses are associated with chronic recurrent infections and the presence of serum IgG, and IgG4 Ab, in addition to IgE Ab, specific for *Trichophyton* Ags (7–10). Furthermore, improvement of asthma symptoms in a subset of IH responders with late-onset asthma after treatment with the systemic antifungal fluconazole has provided convincing evidence for the role of Ags derived from *Trichophyton* in allergic disease (11). Delayed-type hypersensitivity (DTH) responses to *Trichophyton* are common among the general population (12). However, the association of DTH skin tests with acute infections that clear spontaneously has led to the view that these responses, which do not include IgE Ab, are protective (9, 13–15).

There is strong evidence for the role of Th2 responses to inhalant allergens in humans, as judged by the presence of serum IgE and IgG4 Ab and isolation of allergen-specific Th2 clones (16–19). IH and DTH responses to Tri r 2 may reflect differential involvement of T lymphocyte subsets with a Th2 and Th1 phenotype, respectively. Indeed, we previously showed that short-term T cell lines established from IH and DTH responders had a Th2/Th0 and Th1 phenotype, respectively (20). However, studying cytokine production induced by whole Ag in T cell clones or lines does not provide insight into the diversity of T cell populations that constitute allergen-specific T cell repertoires. Cloning of Ags derived from *Trichophyton rubrum* has provided molecular tools for examining the T cell repertoire associated with distinct immune responses to *Trichophyton* (21). We recently reported major differences between IH and DTH responders in T cell repertoires specific for a 29-kDa allergen derived from *T. rubrum*, Tri r 2, which is a serine proteinase homologue (22). Those studies identified an amino-terminal immunodominant epitope (peptide 5 (P5)) specifically associated with DTH. The goal of our present study was to examine whether differences in T cell proliferative responses to Tri r 2 peptides in IH and DTH responders reflected differences in production of cytokines associated with distinct Th subsets or differences in stability of the T cell repertoire. Our results show that diminished T cell proliferation to the major epitope P5 in IH responders cannot be explained by decreased production of IFN- γ , and that this epitope is associated with production of the Th2 cytokine IL-5 in DTH, but not IH responders.

Materials and Methods

Patients

T cell responses in peptide-stimulated PBMC cultures were analyzed in seven individuals with IH and seven with DTH skin tests to recombinant Tri r 2 (r-Tri r 2³). Clinical and serologic characteristics of these skin test

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³ Abbreviations used in this paper: IH, immediate hypersensitivity; DTH, delayed-type hypersensitivity; P, peptide; r-Tri r 2, recombinant Tri r 2.

groups are described in detail elsewhere (22). Briefly, patients were classified as IH responders based on a positive immediate skin test to *Trichophyton* extract and to purified r-Tri r 2, and the presence of serum anti-Tri r 2 IgE Ab. DTH responders had a DTH skin test response to *Trichophyton* extract and r-Tri r 2, and no measurable anti-Tri r 2 IgE Ab. Skin testing of human subjects and drawing of blood samples for T cell studies were approved by the University of Virginia human investigation committee.

Preparation of Ags and peptides

r-Tri r 2 (29 kDa) was produced in *Escherichia coli* strain BL21 as a GST fusion protein using the pGEX-4T-3 expression vector (21). rGST produced by the same method was used as a negative control in skin test and in vitro lymphocyte cultures. r-Tri r 2 and rGST were absorbed with poly-myxin B agarose (Sigma, St. Louis, MO) before use in cell cultures. A set of synthetic peptides, including twenty-seven 20 mers (designated P1 through P27) and one 19 mer (P28), which were designed for mapping T cell antigenic determinants of Tri r 2, was used to study cytokine responses and T cell proliferative responses in primary PBMC cultures. Peptides were designed with a 10-aa overlap spanning the full-length primary amino acid sequence of Tri r 2 (22).

PBMC cultures

PBMC were isolated from 100 ml blood by density gradient centrifugation over Ficoll-Paque, according to methods previously described (22). Conditions for induction of cytokines in peptide-stimulated PBMC cultures were as follows: cells were cultured in 24-well plates at 4×10^6 cells/well in the presence of Tri r 2 peptides (7.5 μ M). Complete medium contained RPMI 1640 (Life Technologies, Gaithersburg, MD) with L-glutamine, 10% heat-inactivated autologous human serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin. Culture supernatants were harvested on day 5 and stored at -70°C before assay. Positive control wells included r-Tri r 2 and PHA, while unstimulated, GST-stimulated, and medium-only wells served as negative controls. Repeat proliferation assays were conducted on one IH and one DTH responder using methods described previously (22). Briefly, PBMC (3×10^5 /well in 96-well plates) were stimulated with 7.5 μ M peptide for 5 days in complete medium containing 10% autologous serum (non-heat-inactivated). Each assay incorporated 12 replicate cultures for each of 28 peptides and 60 unstimulated wells as negative controls. Each of four 96-well plates consisted of 84 wells containing 7 peptides and 12 unstimulated wells. Plate 5 contained an additional 12 control wells and quadruplicate cultures of GST, r-Tri r 2, tetanus toxoid, and PHA. There was no significant difference in the interval between T cell proliferation studies conducted previously and cytokine studies for DTH (mean, 17 mo (range 9–24 mo)) and IH (mean, 18 mo (range 13–23 mo)) responders.

Chemiluminescent ELISA for IFN- γ , IL-4, IL-5, and IL-10

Cytokines were measured using matched mAb pairs (Pierce-Endogen, Woburn, MA). The assay was conducted at room temperature. Briefly, primary Ab (1 μ g/well in 100 μ l) diluted in PBS, pH 7.4, was added to 96-well opaque plates (ISC BioExpress, Kaysville, UT) and incubated overnight at room temperature. Plates were washed five times with PBS containing 0.1% Tween 20 between each subsequent step. After washing, plates were blocked for 1 h with PBS, pH 7.4, containing 1% BSA. Culture supernatants were added to the plate in duplicate (100 μ l/well) and incubated for 2 h. Plates were then incubated for 1 h with secondary Ab at 10 ng/ml (100 μ l/well). After a further 30-min incubation with HRP-streptavidin conjugate (1/320,000 dilution, 100 μ l/well), the assay was developed by adding 100 μ l/well ELISA Super Signal Pico (Pierce-Endogen) and counted in a Topcount NXT Microplate Luminescent Counter (Packard, Meriden, CT). Values were extrapolated from standard curves established in complete medium on the same assay plate. Sensitivity was 2 pg/ml for IL-4, IL-5, and IFN- γ , and 4 pg/ml for IL-10.

Statistical analysis

Linear regression analysis was used to determine relationships between cytokine levels, serum Ab titers, and T cell proliferation (stimulation index (r-Tri r 2) or standardized index (peptides)). The standardized index was derived as described previously (22). Briefly, data for 28 peptides (12 replicate wells each) were log transformed. The standardized index value was calculated by subtracting the mean proliferation value for 12 unstimulated wells from the mean proliferation value for each peptide contained on the same assay plate (22). Cytokine levels in peptide-stimulated cultures were compared between the two skin test groups using two-sample Student's *t* test for IFN- γ (assuming normal distribution of values) and the nonparametric Mann-Whitney *U* test for independent variables for IL-5 and IL-10. Statistical tests were conducted using the SPSS (Chicago, IL) 10.1 software package.

Results

Induction of cytokines by r-Tri r 2 and antigenic peptides in primary PBMC cultures

Cytokines induced by r-Tri r 2. Measuring T cell proliferative responses to peptides derived from Tri r 2, we previously demonstrated differences in the recognition of T cell epitopes between subjects with IH and DTH skin tests (22). Seven IH (mean age, 59 years) and seven DTH (mean age, 38 years) subjects were available for further study (Table I). Initial studies examined kinetics of

Table I. Cytokine production in PBMC cultures stimulated with r-Tri r 2^a

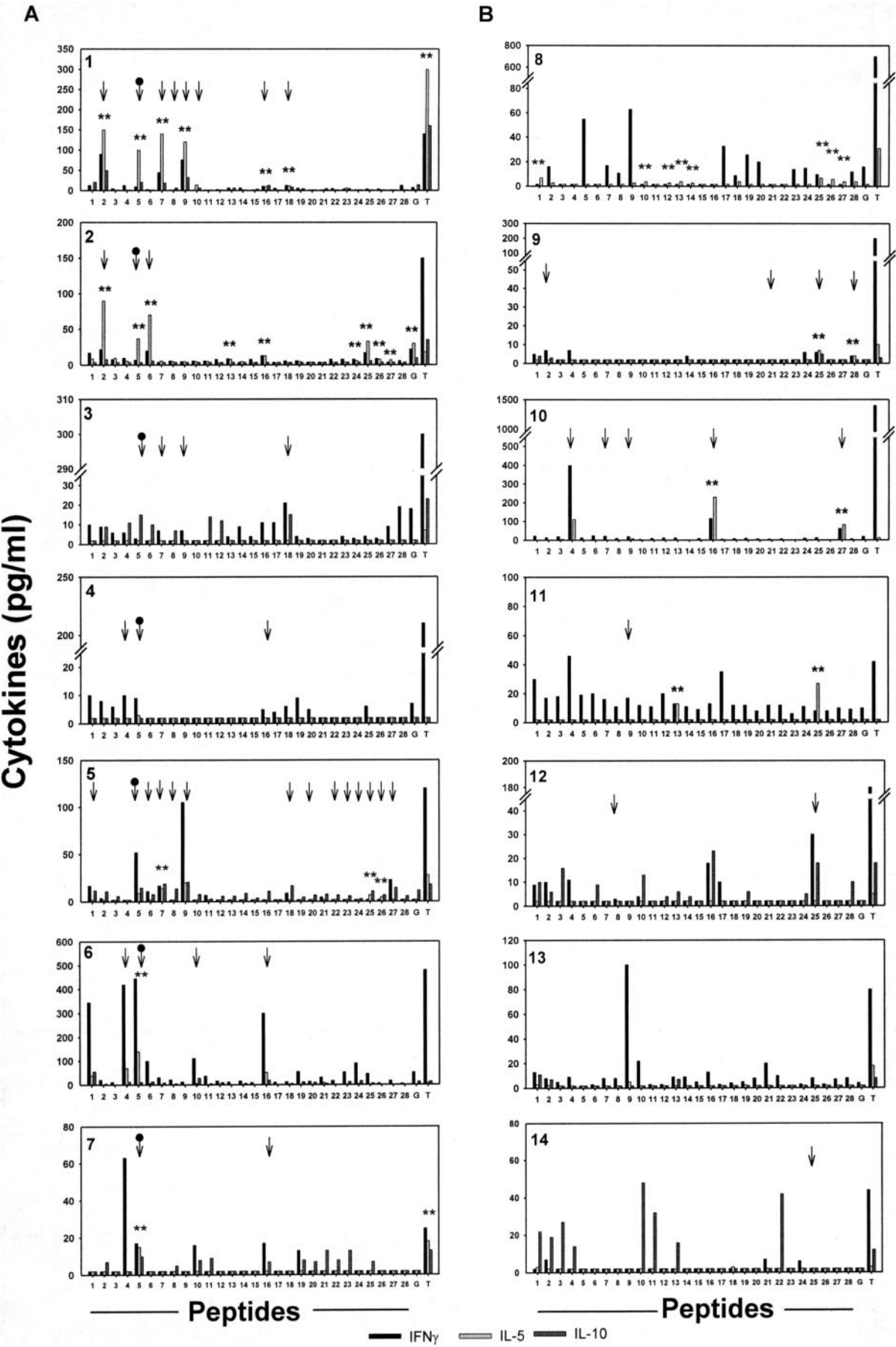
Subject	Age (years)	Sex	Race	Allergic Disease	Anti-Tri r 2 Ab ^b		Response to Tri r 2 in PBMC Cultures				
					IgG (U/ml)	IgE (cpm)	SI	IFN- γ^c	IL-5 ^c	IL-10 ^c	IFN- γ :IL-5
DTH responders											
1	30	M	W	SR	35	<400	130	140	250	160	0.56
2	39	F	W	A	340	<400	7	150	18	35	8.3
3	31	F	W		510	<400	39	300	7	23	43
4	59	M	W		90	<400	23	210	<2	<4	105
5	28	M	W		<20	<400	28	120	28	18	4.3
6	41	F	W		840	<400	157	480	8	14	60
7	35	M	B	SR	125	<400	40	25	18	13	1.4
Mean	38				142 ^d	<400	40 ^d	152 ^d	16 ^d	21 ^d	
IH responders											
8	56	F	W	A	1,900	49,000	36	700	31	ND	23
9	76	M	W	S	2,200	3,357	58	200	10	32	20
10	75	F	W	A	1,150	713	46	1,400	13	<4	108
11	69	M	W	U	950	6,314	8	42	<2	<4	21
12	48	M	B	PR	850	451	9	180	5	18	36
13	62	M	W	PR	4,720	2,293	53	80	18	8	4.4
14	26	M	W	PR	4,400	2,465	5	44	3	12	15
Mean	59				1,872 ^d	2,938 ^d	21 ^d	176 ^d	8 ^d	10 ^d	

^a M, male; F, female; W, white; B, black; SR, seasonal rhinitis; A, asthma; S, sinusitis; U, urticaria; PR, perennial rhinitis; SI, stimulation index.

^b Measured by Ag binding radioimmunoassay.

^c Values in picograms per milliliter measured by ELISA.

^d Geometric mean.



IL-5 and IFN- γ production in response to r-Tri r 2 and a subset of Tri r 2 peptides (P5, P9, and P25). Stimulation with whole Ag or peptides induced dose-dependent increases in IL-5 and IFN- γ ; maximal levels were achieved for r-Tri r 2 and peptides after 4 and 6 days, respectively. Previous studies showed that optimal patterns of T cell proliferation using peptides were achieved after 5 days of culture, and this time point was selected for cytokine studies. Tri r 2 stimulated production of IFN- γ , IL-5, and IL-10 in both IH and DTH responders, with no significant difference in mean cytokine levels or in the ratio of Th1 (IFN- γ) and Th2 (IL-5) cytokines between the two skin test groups (Table I). IL-4 was not measurable in any subjects. Cytokine levels were not significantly correlated with stimulation index in r-Tri r 2-stimulated cultures. Production of IFN- γ was positively correlated with serum anti-Tri r 2 IgG Ab levels in the DTH group ($r = 0.877$, $p = 0.01$), but was inversely related in the IH group ($r = -0.401$, $p = 0.372$) (Table I).

Cytokines induced by Tri r 2 peptides. Production of IFN- γ , IL-5, and IL-10 was measured in response to 28 peptides derived from Tri r 2. A high cell density (4×10^6 /well) was used to allow for the low expected frequency of Ag-specific T cells in peripheral blood and to optimize conditions for detection of cytokines in early (i.e., day 5) cultures. Each subject showed a unique pattern of cytokine production (Fig. 1). Cytokine levels were generally lower in peptide- compared with r-Tri r 2-stimulated cultures; however, some peptides induced IFN- γ at levels comparable with whole Ag (DTH responders, P4, P5, and P9; IH responders, P4 and P9) (Fig. 1). Although r-Tri r 2 stimulated Th1-dominated responses (IFN- γ :IL-5 $\geq 4:1$) in the majority of DTH ($n = 5$) and IH ($n = 7$) subjects, Th2-dominated responses (IFN- γ :IL-5 $\leq 3:1$) were induced by a subset of peptides in a number of these individuals (three DTH and four IH subjects) (Fig. 1). Two DTH responders gave Th2-dominated responses in cultures stimulated with whole Ag and peptides. Surprisingly, the DTH-associated major epitopes induced Th2-dominated peptide responses (denoted by arrows in Fig. 1). This response localized to the amino-terminal region of Tri r 2 in DTH, but not IH responders. Furthermore, Th2-dominated responses to peptides were observed in all three DTH subjects with a history of allergic disease (subjects 1, 2, and 7) (Table I, Fig. 1). Production of IL-10 was generally low in peptide-stimulated cultures, with the exception of two IH responders who gave IL-10-dominated responses (>20 pg/ml) to a subset of peptides (subjects 12 and 14) (Fig. 1).

Previous studies showed that the amino-terminal peptide, P5, induced significantly higher T cell proliferation in DTH responders compared with IH subjects (22). To determine whether diminished T cell proliferation in IH subjects was related to elaboration of cytokines, levels of IFN- γ , IL-5, and IL-10 were compared between the two skin test groups. P5 induced both IL-5 and IL-10 production in cultures established from DTH, but not IH responders ($p = 0.003$ (IL-5) and $p = 0.024$ (IL-10)) (Fig. 2). Surprisingly, levels of IFN- γ were not significantly different between skin test groups ($p = 0.322$). The highest levels of IFN- γ (445 pg/ml) induced by P5 corresponded to the strongest proliferative response previously observed to this peptide (standardized index = 4.34, subject 6) (Fig. 2). Mean levels of IFN- γ for cultures stimulated with peptides ranged from 3 pg/ml (P26) to 68 pg/ml (P4) for IH

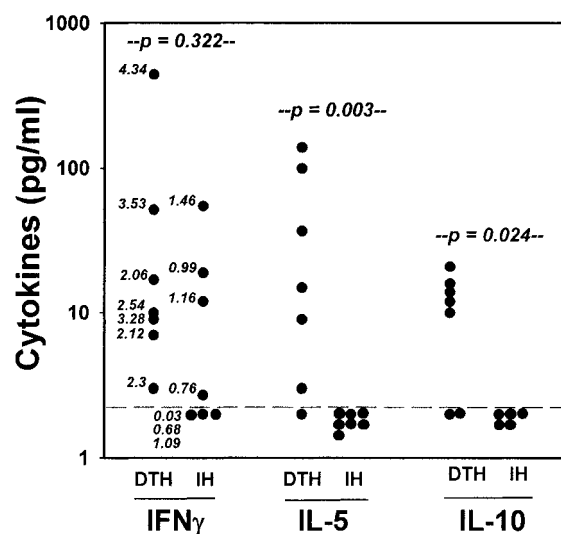


FIGURE 2. Comparison of cytokines induced in P5-stimulated cultures derived from IH and DTH responders. Numbers indicate standardized indexes for T cell proliferation. IL-10 was not measured in two IH responders owing to insufficient sample.

responders, and from 4 pg/ml (P26) to 78 pg/ml (P5) for DTH responders (Fig. 3A). Mean levels of IL-5 ranged from 2 pg/ml (19 peptides) to 35 pg/ml (P16) for IH subjects, and from 2 pg/ml (P28) to 44 pg/ml (P5) for DTH subjects. The most striking difference between the two groups was the absence of measurable IL-5 in cultures established from IH responders that were stimulated with P5, P6, and P7 (Fig. 3B).

Relationship between T cell proliferation and IFN- γ , IL-5, and IL-10 production

The region of Tri r 2 spanned by P11 through P15 was associated with decreased production of IFN- γ and IL-5 in both skin test groups (Fig. 3, A and B); these peptides failed to induce T cell proliferation in cultures established from IH subjects in previous studies (22). Production of IFN- γ , IL-5, and IL-10 was positively correlated with T cell proliferation ($r = 0.384$, $r = 0.444$, $r = 0.327$ ($p < 0.0005$), respectively) (Fig. 4). However, several peptides that stimulated strong T cell proliferative responses (standardized index >2) did not induce IFN- γ or IL-5 production (Figs. 1, 4A, and 4B); conversely, several peptides that induced >20 pg/ml IFN- γ did not stimulate T cell proliferation (standardized index <0.5) (Fig. 4A). Thus, T cell proliferation and production of Th1 and Th2 cytokines were not consistently associated.

Stability of the Tri r 2-specific T cell repertoire

To determine whether the disparity between T cell proliferative responses and cytokine production could be explained by loss of recognition of antigenic determinants over time, T cell epitopes were mapped by standard proliferation assay using methods previously described. Experiments were conducted in one IH (age 76) and one DTH (age 30) responder, and results were compared with those obtained 20 and 22 mo earlier, respectively. The results

FIGURE 1. Profiles of cytokine production in PBMC cultures stimulated with Tri r 2 peptides. Production of IFN- γ , IL-5, and IL-10 was measured in seven DTH (A) and seven IH (B) responders. The final two columns in each plot represent cultures stimulated with rGST (G) and r-Tri r 2 (T). Arrows denote peptides that stimulated strong T cell proliferative responses in previous studies (standardized index >2). P5 (●→) and peptides stimulating Th2-dominated responses (**) are indicated. Cytokines were not measurable in unstimulated wells (no peptide or Ag), with the exception of two individuals in which low levels of IFN- γ were detected. IL-10 was not measured in cultures derived from two subjects (8 and 10) owing to insufficient sample.

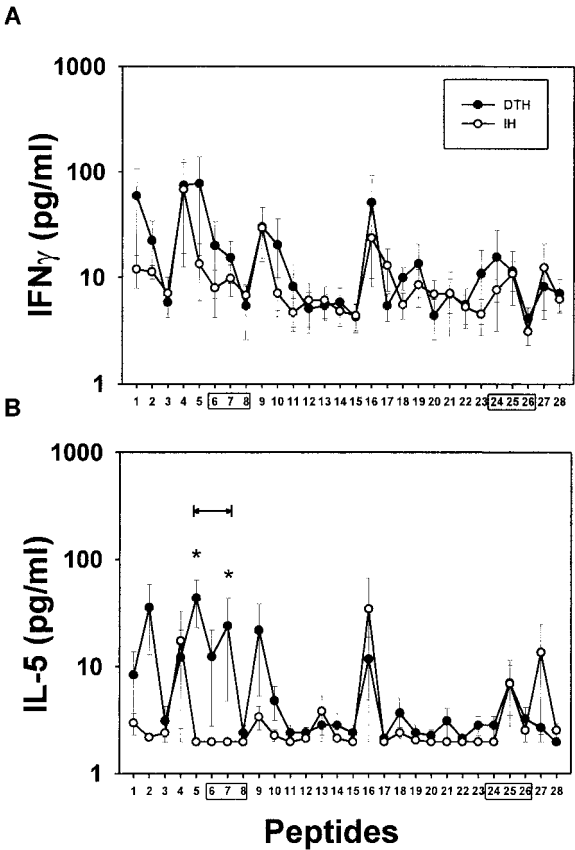


FIGURE 3. Mean levels of IFN- γ and IL-5 in peptide-stimulated cultures. Vertical bars represent SEM. Boxes denote six peptides (P6, P7, P8, P24, P25, P26) spanning two distinct regions of the molecule used previously to predict patient classification (22). The amino-terminal region of the molecule associated with differences in IL-5 production is denoted (<=>); *, $p = 0.003$ (P5) and $p = 0.025$ (P7).

showed that the pattern of T cell epitope recognition was consistent for each subject, with no evidence of selective loss or increased recognition of peptides over time (Fig. 5).

Discussion

The primary goal of these experiments was to examine cytokine production associated with allergen-specific T cell repertoires in humans with distinct skin test reactions to *Trichophyton*. Differences in production of Th1 and Th2 cytokines associated with IH

and DTH were complex. First, culture of PBMC in the presence of the whole protein stimulated both IFN- γ and IL-5 production in DTH and IH responders; however, the majority of these responses were dominated by IFN- γ irrespective of skin test response. Second, r-Tri r 2 stimulated Th2-type responses in two subjects with DTH, but not in any of the IH responders. Finally, production of IL-5 was a major feature of T cell responses to DTH-associated immunodominant epitopes (including P5) in the DTH, but not in the IH skin test group. The unique ability for Tri r 2, unlike other allergens, to elicit distinct skin test reactions in different individuals provides a model for studying the relevance of the Th1/Th2 paradigm to the human system. In keeping with this, we initially hypothesized that differences in T cell proliferation to Tri r 2 peptides between IH and DTH responders would be explained by association of Th1 and Th2 cytokines with DTH and IH responses, respectively. Surprisingly, our findings are contradictory to this theory and suggest that dichotomous immune responses in the human do not adhere to classical Th1 or Th2 profiles. It is our opinion that the complexity of the observed cytokine responses, in large part, reflects the genetic heterogeneity of humans compared with other animal systems that have been used to establish the Th1/Th2 paradigm. Indeed, in murine models of contact sensitivity, regulation of IL-5 production by MHC has been demonstrated (23, 24). Interestingly, in those studies, delayed skin reactions were observed in congenic mouse strains producing high or low levels of IL-5. Similarly, in our studies, levels of IL-5 production were highly variable in subjects with delayed skin tests. Nevertheless, despite the complexity of T cell responses in humans, distinctions between IH and DTH responses, as judged by both T cell proliferation and cytokine production, can be clearly defined.

Obviously, it could be argued that delayed skin responses induced by *Trichophyton* do not reflect classical DTH, but an alternative response. Delayed reactions to *Trichophyton* are typically maximal at 24–48 h after injection of Ag into the skin and, like classical DTH, are characterized by erythema and induration. Variations in the manifestations of DTH responses in patients with concurrent dermatophytosis have been reported, including contact hypersensitivity to *Trichophyton* extract applied to the skin surface and eczematous responses to Ag injected intradermally (12, 25). Furthermore, other types of delayed cell-mediated responses associated with diverse Ags have been described in guinea pigs or humans, including cutaneous basophil hypersensitivity and the Jones-Mote reaction (26–28). Despite characterization of Langerhans cells and T cells at the site of dermatophyte infection, immunohistologic studies of DTH skin tests to *Trichophyton* extract

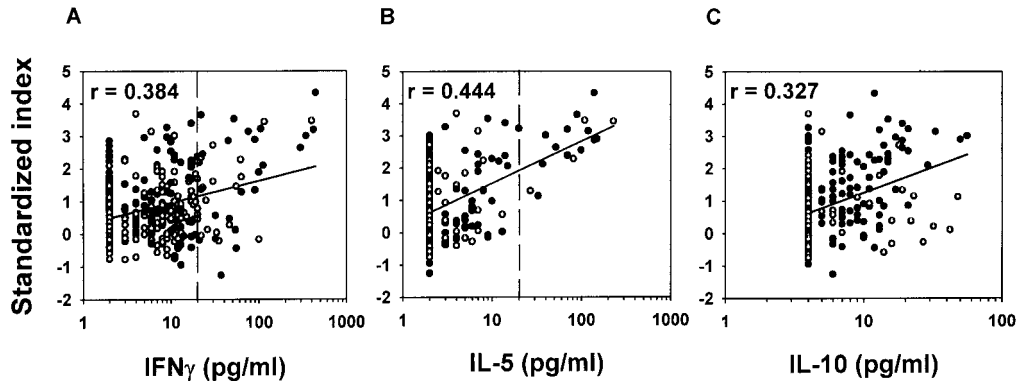


FIGURE 4. Correlation between T cell proliferation and cytokine production in peptide-stimulated PBMC cultures. Points in A and B represent data for each of the 28 peptides in cultures established from seven IH (○) and seven DTH (●) responders. Data in C are for seven DTH and five IH responders. Dashed lines (A and B) are arbitrary reference lines corresponding to IFN- γ and IL-5 levels of 20 pg/ml.

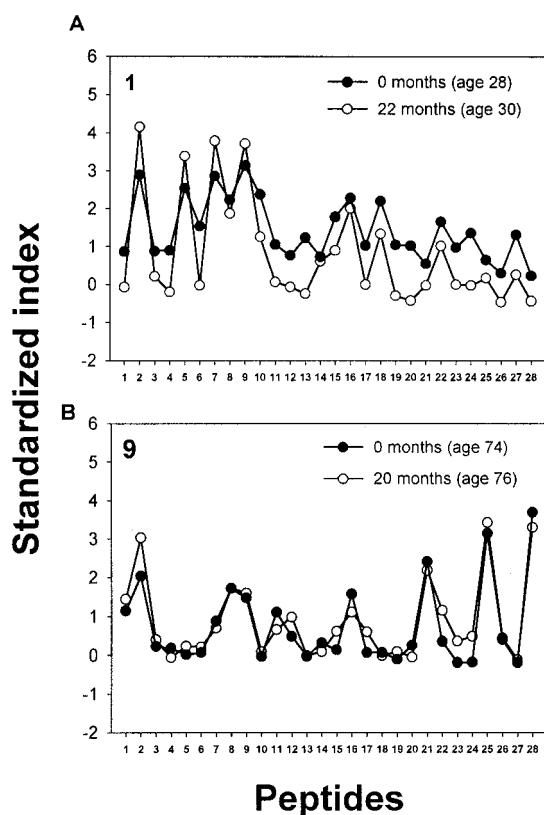


FIGURE 5. Long-term stability of T cell epitope recognition in IH and DTH subjects. T cell proliferative responses were measured in one DTH (A) and one IH (B) responder at 22- and 20-mo intervals, respectively. Standardized indexes for different time points are compared for each Tri r 2 peptide.

have not been conducted (29). Thus, an important next step would be to determine the characteristics of T cells and other cell types in these delayed reactions. Preliminary studies by our group showed that eosinophils were a major component of the cellular infiltrate in delayed skin tests to purified *Trichophyton* Ags (unpublished observations). These findings raise the intriguing possibility of a role for IL-5 in DTH to *Trichophyton*. Indeed, in murine models, there is evidence of a role for both eosinophils and IL-5 in different forms of delayed responses (23, 24, 30). Furthermore, there is mounting evidence to support a role for both type 1 and type 2 cytokines in delayed skin reactions associated with Th1-mediated (nickel sensitivity) and Th2-mediated (atopic dermatitis) allergic conditions (31–35). Our findings are consistent with these observations and underscore the complexity of cellular mechanisms that govern allergic and nonallergic skin test responses. Identification of cell surface markers and intracellular cytokines in *Trichophyton*-induced delayed skin tests could provide insight into the relevance of T cell subsets and eosinophils at reaction sites; however, such studies may be complicated by expression of Th1 markers (e.g., CXCR3) on both eosinophils and T lymphocytes (36, 37).

There is strong evidence that fungal Ags contribute to development of late-onset asthma in a subset of patients with IH to *Trichophyton* (11). Production of IL-5 to major epitopes in DTH responders suggests that the lack of association between DTH and asthma cannot be attributed to a protective classical Th1 response to *Trichophyton*. It seems likely that differences in Ag-specific T cell populations associated with DTH and IH are defined, not solely at the cytokine level, but at the level of TCR specificity (as previously shown), and by other T cell properties. There is evidence to

suggest that expression of the skin-specific homing Ag, cutaneous lymphocyte-associated Ag, is down-regulated in T cells specific for Tri r 2 from patients with chronic dermatophytosis (38, 39). Thus, differences in homing of Ag-specific T cells to anatomical sites could explain the different features of skin infection and manifestations of allergic disease associated with distinct immune responses to *Trichophyton*.

In our system, a subset of peptides induced responses dominated by IL-5 even in the presence of IFN- γ -dominated responses to the whole Ag, suggesting that the frequency of responding cells can modulate cytokine production. It can be assumed that in cultures stimulated with a single peptide, fewer T cells are activated, resulting in lower concentration of cytokines; these conditions may allow cells with a Th2 phenotype to dominate the response. It has previously been reported that the Th1/Th2 balance in Ag-specific systems is dependent on the total number of cells activated (40). Thus, it is reasonable to suggest that the cytokine milieu induced after stimulation with whole Ag reflects complex cellular interactions induced by activation of T cells with multiple specificities and, possibly, different helper phenotypes.

Previous studies by our group showed that T cell lines specific for *Trichophyton* Ags that were established from IH responders had a Th2/Th0 phenotype (20). In that study, a subset of the cell lines derived from IH subjects produced higher amounts of IFN- γ compared with IL-4 or IL-5. The presence of IFN- γ in peptide-stimulated cultures established from IH responders is consistent with previous studies that report induction of IFN- γ by a variety of allergens or peptides (17–19, 41–43). However, the most striking finding associated with IH was a lack of measurable IL-5 in cultures stimulated with peptides spanning the amino-terminal region of Tri r 2, including P5, P6, and P7 (Figs. 2 and 3). This region was previously shown to stimulate markedly reduced T cell proliferation in IH compared with DTH responders (22). Our present results show that decreased T cell proliferation to P5 cannot be explained by diminished IFN- γ production.

We previously proposed that T cell proliferative responses to P5 might promote the development of DTH, thereby preventing progression to chronic dermatophytosis (22). Our cytokine findings suggest that, if this is the case, induction of a Th0 response to P5, as opposed to a Th1 response, may be required to confer protection. More importantly, our results raise the question of how allergen-specific vaccines should be designed for treatment of allergic disease in general. Although IH and DTH responses to Tri r 2 are associated with distinct T cell reactivity to the amino-terminal region of the molecule, it remains to be determined whether administration of a subunit vaccine or a combination of relevant peptides corresponding to this portion would have a protective effect in allergic subjects. IL-10 is a potent antiinflammatory cytokine that inhibits T cell proliferative and cytokine responses, and there is mounting evidence for its role in T cell anergy associated with allergen-specific immunotherapy (44–47). Furthermore, IL-10 has been shown to inhibit DTH responses induced by Th1 clones in mice (48). We hypothesized that production of IL-10 would be increased in cultures established from IH responders who exhibited decreased T cell proliferation to P5. Two IH responders (subjects 12 and 14) showed production of high levels of IL-10 in peptide-stimulated cultures; however, no IL-10 was measurable in P5-stimulated cultures derived from any of the IH responders. Overall, IL-10 production was positively correlated with proliferation. These findings do not provide strong evidence of a role for IL-10 in IH responses to Tri r 2.

In several of the individuals studied in this work, T cell proliferation and cytokine production can be dissociated for a subset of peptides (Figs. 1 and 4). A similar phenomenon has been described

previously using clonal Th cell populations stimulated with altered peptide ligands (49). It is generally accepted that two signals are required for optimal T cell activation (provided by TCR engagement and ligation of a distinct coreceptor). Suboptimal signaling to T cells could occur as a result of low affinity/avidity interactions of specific Tri r 2 peptide-MHC complexes with the TCR, with subsequent uncoupling of distinct signaling pathways required for cell division and cytokine production. Proliferation assays were repeated to investigate whether the disparity between cytokine production and T cell proliferation resulted from loss of epitope recognition over time. The results showed a remarkably consistent pattern over prolonged periods, and also confirmed the reproducibility of the system used. We previously speculated that decreased proliferation to P5 could result from narrowing of the T cell repertoire during aging given the significantly older age of IH responders (22). Our present findings suggest that the allergen-specific T cell repertoire is extremely stable, even in advanced age, and that changes in the repertoire would require many years to develop.

In summary, we have identified distinct features of allergen-specific T cell repertoires associated with immediate and delayed skin reactions based on cytokine production. The amino-terminal major epitope associated with delayed skin tests induces IL-5 production only in T cells from DTH responders. The results demonstrate marked diversity of the allergen-specific T cell repertoire between different subjects. Furthermore, they suggest that, within the same individual, the allergen-specific T cell repertoire comprises T cells with multiple specificities (perhaps with varied affinity or avidity for different epitopes) and with Th1 and/or Th2 characteristics, not closely related to the nature of the skin test response. Stability of the T cell repertoire over time indicates that memory T cells associated with distinct immune response are long-lived, and that decreased T cell proliferative responses to DTH-associated major epitopes in subjects with immediate hypersensitivity are unlikely to be due to selective loss of Ag-specific T cells. Our findings demonstrate the value of detailed studies on T cell responses to allergens, and have important implications for the design of Ag-specific therapies in humans.

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