

The immune response to dermatophytes

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Introduction

Dermatophytosis is the infection of keratinized structures caused by members of the fungal genera *Trichophyton*, *Epidermophyton* and *Microsporum*. These fungi are adapted to infect keratinized tissues by virtue of their ability to utilize keratin as a nutrient source. Dermatophytes therefore differ from most other pathogenic fungi since they are not opportunists and frequently infect healthy individuals. Sites of infection include hair, nails and the stratum corneum of the skin. More than 40 dermatophyte species have been identified which infect humans (anthropophilic), animals (zoophilic) or are present in soil (geophilic). Individual species have distinctive colonial and microscopic morphologies and differ in their predilection for certain anatomical sites. In addition, different *Trichophyton* species may cause a broad spectrum of clinical manifestations which vary in the degree and chronicity of inflammation. The major dermatophyte species are *Trichophyton rubrum*, *T. mentagrophytes*, *E. floccosum*, and *M. canis*. *T. rubrum* and *T. mentagrophytes* are the most common cause worldwide of tinea pedis (athlete's foot) and onychomycosis (infection of the nail beds) (Aly, 1994). It has been reported that 30 to 70% of adults are asymptomatic carriers of these pathogens, an estimate which is in contrast to previous studies stating that dermatophytes were only rarely present on the feet of asymptomatic individuals (Odom, 1993; Cornbleet, 1926; Burgess, 1925). The incidence of symptomatic disease caused by *T. rubrum* and *T. mentagrophytes* increases significantly with age, only rarely affecting children. In contrast, *T. tonsurans* is the most common cause of tinea capitis (ringworm of the scalp) in North America, predominantly affecting children (Aly, 1994). Tinea capitis has increased in prevalence since the 1970s in the United States, creating a significant public health problem (Frieden and Howard, 1994). Although usually regarded as a disease of school-aged children, tinea capitis also occurs in infants and neonates, suggesting that exposure to *Trichophyton* antigens can occur at an early age.

The immune response to *Trichophyton* is unusual in that this fungus can elicit both immediate hypersensitivity (IH) and cell-mediated or delayed-type hypersensitivity (DTH) skin test reactions in different individuals. Furthermore, the nature of the skin test response appears to reflect the efficiency with which infections are cleared. For example, DTH skin tests tend to be associated with the presence of acute, highly inflamed lesions, while chronic infections are more common in subjects with IH responses (Calderon, 1989; Hay and Brostoff, 1977; Jones, 1980; Kerbs *et al.*, 1977; Sorensen and Jones, 1976). Until recently, very little was known regarding the nature of the immune responses to dermatophyte fungi. However, in the last few years, purified antigens have become available which have made detailed studies possible. Elucidation of the mechanisms which govern the development of diverse immune responses in humans is relevant not only to *Trichophyton* but also to a variety of human diseases. In particular, understanding the mechanisms regulating protective cell-mediated immune responses may result in the development of alternative therapeutic strategies. In this chapter, we report on recent studies which shed some light on the dichotomy of the immune response to *Trichophyton* and discuss the potential clinical applications of these findings.

IH to *Trichophyton*

IH is the skin test response related to allergic disease; reactions are characterized by a local wheal and flare occurring 5 to 20 min after injection of antigen into the skin. The effector phase of these responses is mediated by IgE antibody; upon interaction of soluble antigen with mast cell-bound IgE antibodies, the release of histamine and the production of inflammatory mediators is triggered. IH skin tests occurring in the absence of DTH responses appear to be more common among individuals with chronic dermatophytosis, particularly that caused by *T. rubrum* (Hunziker and Brun, 1980; Kaaman,

1978). In addition, it has been known for many years that there is an association between chronic dermatophytosis and atopic disease. Between one third and one half of individuals with chronic dermatophytosis have been reported to be atopic, a proportion larger than that of the general population (Hay, 1982; Jillson and Huppert, 1949; Jones *et al.*, 1973). Asthma presenting in adult life can be very severe and is often said to be "intrinsic" since it may not be associated with a personal or familial history of allergy to inhalant allergens. It is often implied that this "intrinsic" form of asthma is idiopathic. However, in the 1930's, it was suggested that some of these cases might be related to dermatophyte colonization of the feet (Wise and Sulzberger, 1930; Gumowski *et al.*, 1987). In the 1960's, Weary and colleagues first reported an association between chronic tinea pedis and chronic urticaria; the findings of that study were later substantiated by our group (Weary and Guerrant, 1967; Platts-Mills *et al.*, 1987). The possibility that dermatophyte antigens serve as non-specific adjuvants for the production of IgE antibodies resulting in allergic disease seems unlikely. It is more likely that atopic individuals are predisposed to develop chronic dermatophyte infections. This does not, however, necessarily exclude the possibility that dermatophyte antigens play a role in exacerbation of allergic symptoms. For example, our group identified several patients presenting with late onset asthma who also had chronic dermatophytosis, positive bronchial challenge to a Trichophyton extract and high levels of antibodies to *T. tonsurans* of the IgE isotype (Ward *et al.*, 1989). These patients showed a significant improvement in their allergic disease as judged by symptoms, reduced prednisone dosages and decreased bronchial reactivity to Trichophyton extract when treated with long-term systemic anti-fungal therapy (Ward *et al.*, 1994). In addition, there have been many reported cases in which urticaria abated coincident with systemic treatment of *T. rubrum*-induced tinea pedis (Weary and Guerrant, 1967; Charlesworth, 1996).

It should be noted that it is not uncommon to detect IgE antibodies to Trichophyton in the absence of atopy. Leibovici and colleagues reported a group of patients with widespread and chronic dermatophytosis who lacked a clinical history of allergy; these individuals had high levels of total IgE and *T. rubrum*-specific IgG4 antibodies (Leibovici *et al.*, 1995). Furthermore, our group was able to detect IgE antibodies directed towards an 83-kDa *T. tonsurans* glycoprotein (Protein IV) in individuals with a history of athlete's foot who were non-atopic and not currently infected (Woodfolk *et al.*, 1996). Nevertheless, these findings together with the strong association between dermatophytosis and allergic symptoms imply that exposure to dermatophyte antigens can give rise to IgE antibody production and

may also result in sensitization of the airways and symptomatic asthma, or other allergic symptoms in predisposed individuals.

It might be predicted that the superficial location of dermatophyte infections (generally confined to the stratum corneum) would prevent sensitization of the host and result in failure to produce a humoral response. However, the above studies confirm that this is not the case. Antibodies to several Trichophyton species have been measured with varying degrees of success using several different methods including immunodiffusion, counterimmunoelectrophoresis, complement fixation and charcoal agglutination assays (Hay and Shennan, 1982; Grappel *et al.*, 1971; Grappel *et al.*, 1972). We studied the serologic responses to *T. tonsurans* Protein IV in 59 adults who had been skin tested with a Trichophyton extract (Woodfolk *et al.*, 1996). Immediate skin reactions were associated with a positive radioallergosorbent test for IgE antibodies to a Trichophyton extract in 14 of 23 individuals (fig. 1). Antigen-specific IgE and IgG4 antibodies measured by ELISA were also present in most IH subjects. Specific IgG antibodies measured by antigen-binding radioimmunoassay were highest in IH subjects and lowest in those with DTH or negative skin tests. These results are consistent with those reported by Kaaman and colleagues, who reported higher IgG antibodies to dermatophyte antigens measured by ELISA in infected individuals compared with uninfected controls (Kaaman *et al.*, 1981). Furthermore, our findings showed that the prevalence of IgG antibodies increased significantly with age. Considering that IH skin tests tend to be associated with chronic infections, these findings suggest that the humoral response is not protective. Indeed, higher antibody titres among individuals with IH skin tests may simply reflect prolonged antigenic exposure.

Cell-mediated immune responses to Trichophyton

Delayed hypersensitivity is a form of cell-mediated immunity in which the ultimate effector cell is the activated macrophage. Activation of macrophages is dependent upon cytokines produced by the response of sensitized T lymphocytes to antigen. Such T cells recognize and respond to foreign antigen only when it is presented in the form of antigenic peptide complexed with self MHC molecules on the surface of an appropriate antigen-presenting cell. Linear antigenic determinants, often referred to as T-cell epitopes, are thus crucial to cell-mediated responses. In contrast to IH reactions, induration is the hallmark of DTH which is maximal at 24 to 48 h after antigen injection. Induration results from the recruitment of T lymphocytes and blood monocytes into the skin test site, and deposition of fibrin in the extravascular tissue space.

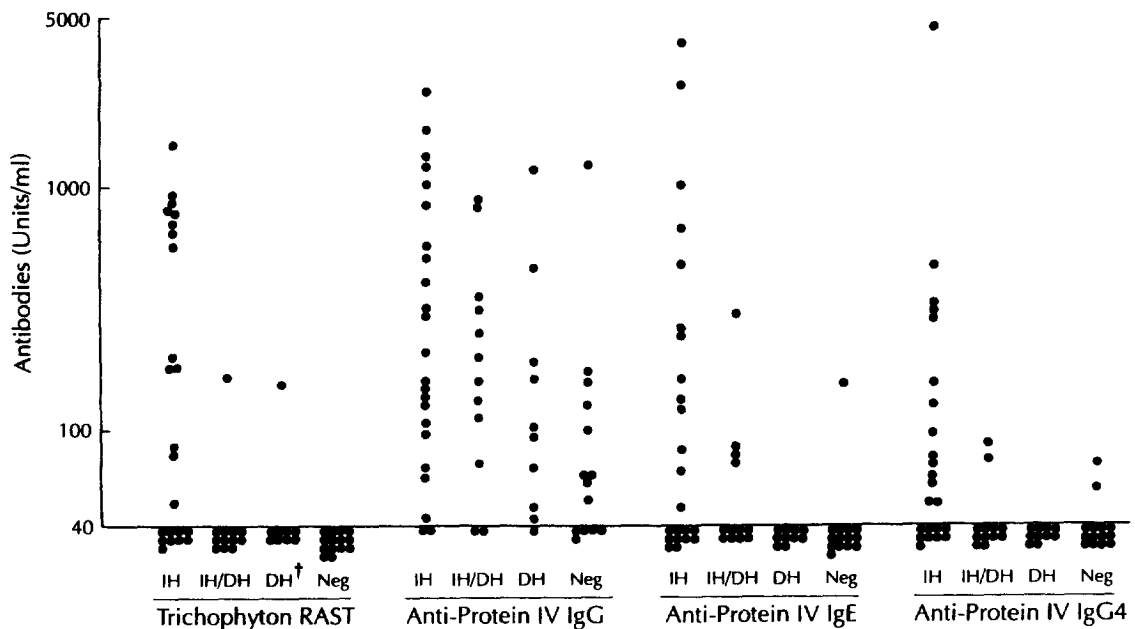


Fig. 1. Quantitative measurements of Trichophyton antibodies in 59 subjects with IH, dual (IH/DH), DH and negative skin test reactions (Neg) to a Trichophyton extract or to purified antigen.

Antibodies measured were IgE antibodies to the 60 to 90% SAS fraction of a *T. tonsurans* extract (Trichophyton RAST), IgG antibodies to Protein IV measured by antigen binding radioimmunoassay and anti-Protein IV IgE and IgG4 antibodies measured by monoclonal antibody-based ELISA. Values were assigned arbitrary units and units for each type of antibody were not equivalent. † One sample was not available for assay by RAST.

Antigen-activated T lymphocytes release a variety of mediators including IFN γ which converts monocytes into activated macrophages. Upon activation, macrophages mediate the effector phase of the response which may include the elimination of foreign antigen and the secretion of enzymes and other mediators injurious to tissues. Antigens such as tetanus toxoid and those derived from *Mycobacterium tuberculosis* which are commonly associated with DTH rarely cause IH reactions. The ability of Trichophyton to elicit these two distinct immune responses is therefore unique.

Delayed hypersensitivity responses following intradermal injections of Trichophyton are considered common in the normal population. Wood and Cruickshank found that 30% of normal uninfected control subjects exhibited DTH responses (Wood and Cruickshank, 1962). It is assumed that most of these responses reflect an immune response to a prior self-limited dermatophytosis. It has been suggested that the inflammatory response induced by a cell-mediated reaction increases the proliferative activity of keratinocytes causing the fungus to be eliminated from the skin. Experimental dermato-

phytosis in animals has confirmed the presence of mononuclear cell infiltrates and perivascular inflammatory cells in the dermis of infected skin (Lepper, 1974). Animal models have also been useful for studying the kinetics of cell-mediated immune responses to Trichophyton; for example, studies in guinea pigs showed that the development of DTH skin tests coincides with the degree of maximal erythema present at the site of primary infection (Kerbs *et al.*, 1977). Green *et al.* demonstrated that cell-mediated immunity is required for maintaining inflammation using guinea pig skin grafted onto nude mice (Green *et al.*, 1982). Two findings which support a protective role for cell-mediated responses to Trichophyton are first, the association of DTH skin tests with acute highly inflamed lesions and second, a resistance to reinfection. Several studies have reported an increased prevalence of delayed skin test responses in subjects with acute compared with chronic infections (Hanifin *et al.*, 1974; Jones *et al.*, 1973; Kaaman, 1981). However, individuals with chronic dermatophytosis do not appear to have deficient cell-mediated responses to other antigens suggest-

ing that the immune "defect" is selective. Lymphocyte transformation *in vitro* has been used to demonstrate cell-mediated immunity to dermatophyte antigens. Some studies have reported reduced lymphoproliferative responses in subjects with chronic dermatophytosis, though most have found normal cellular responses in this group of individuals (Hay and Shennan, 1982; Kaaman *et al.*, 1979; McGregor *et al.*, 1992; Stahl and Svejgaard, 1982). However, as we discuss below, it may be more relevant to distinguish individuals with acute and chronic infections by defining the phenotype of T lymphocytes which proliferate *in vitro*.

The complex interplay between IH and DTH

It has been proposed that active suppression of cell-mediated immunity to Trichophyton antigens occurs in subjects with IH reactions who lack DTH responses. Suppressor T lymphocytes and inhibitory serum factors have been proposed to play a systemic role by modulating cell-mediated responses (Petrini and Kaaman, 1981; Sherwin *et al.*, 1979; Walter *et al.*, 1974). However, no serum factors have been isolated and purified and neither the presence nor function of suppressor T lymphocytes have been confirmed by adoptive transfer experiments. McGregor and colleagues hypothesized that the lack of cutaneous inflammation at the site of chronic *T. rubrum* infection may reflect a local effect of dermatophyte-derived lymphocyte inhibitory factors. The authors were able to demonstrate a direct inhibitory effect of *T. rubrum* on lymphocyte proliferation to recall antigens at high protein concentrations *in vitro* (McGregor *et al.*, 1992). Grando *et al.* demonstrated that the mannan component of *T. rubrum* cell wall binds selectively to monocytes and suggested that this explained the inhibitory influence of mannan on cell-mediated immune function *in vitro* (Blake *et al.*, 1991; Grando *et al.*, 1992). It has also been suggested that antagonism between IH and DTH responses occurs locally, i.e. at the skin test reaction site. It has been reported that skin testing a second time at the same site results in a DTH response in individuals who previously exhibited only IH skin tests (Hunziker and Brun, 1980). Suppression of IH reactions with anti-histamine has also been reported to reveal DTH responses in subjects with chronic dermatophytosis (Jones *et al.*, 1974). In our own experience, repeated skin testing of an individual with DTH to *T. tonsurans* Protein IV resulted in the development of a wheal and flare which preceded the DTH response. The implication of these findings is that antigenic dose is critical in determining the nature of the skin test response and that one must exercise caution when interpreting skin test results in individuals who have been repeatedly tested.

The innate immune response to dermatophytes

Unlike several other fungal pathogens including *Candida albicans* and *Aspergillus fumigatus*, dermatophyte infections never disseminate systemically and do not invade viable epidermis. Non-specific defences have been proposed to play an important role in confining and eliminating infection in the absence of specific immunity. *T. rubrum* cell walls have been shown to activate complement through the alternative pathway and to inhibit fungal growth (Swan *et al.*, 1983; Dahl and Carpenter, 1986). Infiltration of neutrophils occurs before the peak of infection in experimental models, though the role of these cells in dermatophytosis has not been well established. Polymorphonuclear leukocytes adhere to opsonized dermatophyte hyphae *in vitro* and inhibit growth (Dahl, 1994). Furthermore, there is recent evidence for a non-opsonic mechanism of phagocytosis of *T. mentagrophytes* arthroconidia by human neutrophils *in vitro* (Richardson and Bell, 1995). The mechanisms by which neutrophils are recruited to the infection site are unclear. Complement-derived chemotactic factors such as C5a may play a role in addition to soluble factors released by keratinocytes including the chemokine IL8. Another potentially important mechanism for limiting infection is binding of iron by transferrin present in the dermis, thus depriving dermatophytes of an essential nutrient for growth in the deeper layers of the skin. Finally, saturated fatty acids derived from lipids in adult hair have been shown to exhibit fungistatic effects against *Microsporum audouinii*, providing a possible explanation for the lower incidence of scalp infections in older individuals (Rothman *et al.*, 1947).

Mechanisms for dichotomy of the immune response to dermatophyte antigens

Very few studies have addressed the mechanisms of regulation of the immune response to Trichophyton infection. Only recently have these studies become possible, since purified antigens were not previously available. Early studies including experimental infection of animals used a crude antigenic extract of Trichophyton species referred to as "trichophytin". Trichophyton antigens have been described as either glycopeptides, peptides or carbohydrates and each type of antigen has been reported to be associated with different immune responses. For example, Moser and Pollack reported that only a glycopeptide Trichophyton fraction elicited delayed-type hypersensitivity skin tests, while Barker *et al.* suggested that carbohydrates are associated with IH responses (Moser and Pollack, 1978; Barker *et al.*, 1962). In the early 90's, Deuell *et al.* identified a 30-kDa *T. tonsurans*

antigen, designated Tri t 1, which was associated with IH skin tests (Deuell *et al.*, 1991). More recently, our group purified an 83-kDa mannose-rich *T. tonsurans* glycoprotein, designated Protein IV, which was capable of eliciting both IH and DTH skin test reactions (Woodfolk *et al.*, 1996). Subsequent skin testing with Tri t 1 confirmed that this antigen was also capable of producing both types of hypersensitivity reaction, suggesting that diverse immune responses are *not* mediated by distinct antigens. Different Trichophyton species produce different enzyme profiles including lipases, alkaline phosphatases, esterases, α -mannosidases and arylamidases (Brasch and Zaldua, 1994). Keratinases have been detected in lesions of experimental dermatophytosis in guinea pigs using fluorescent antibody probes and also in human tinea pedis by immunoelectron microscopy (Koga *et al.*, 1986; Collin *et al.*, 1973). Despite these studies, very little information is available regarding detailed analysis of individual enzymes. Lambkin and colleagues recently reported the partial purification of a 235,000 Dalton extracellular proteinase derived from *Trichophyton rubrum* which is strongly inhibited by metalloproteinase and chymotrypsin inhibitors (Lambkin *et al.*, 1994). A 34,000 Da cell-associated *T. rubrum* proteinase was also recently purified and characterized by this group (Lambkin *et al.*, 1996). This enzyme exhibited broad substrate specificity and shared 50% amino acid sequence homology at the NH₂-terminus with a serine proteinase derived from *Coccidioides immitis*. Several dermatophyte-derived enzymes have been proposed to play a role in invasion of the host and utilization of tissue as a nutrient source. However, none of these studies have addressed the immunologic potential of such enzymes.

As mentioned above, our group previously reported the isolation and characterization of an 83-kDa *T. tonsurans* glycoprotein which is associated with IH and DTH skin test responses. IH skin tests are associated with chronic dermatophytosis, high antibody titres and failure to clear infection. In contrast, DTH skin tests are associated with acute, highly inflamed lesions which tend to resolve spontaneously, a resistance to re-infection, and low antibody titres. Trichophyton antigens, by virtue of their ability to elicit diverse immune responses, therefore provide us with a unique tool for defining the dichotomy of the immune response in humans. In the mouse, two phenotypically distinct CD4⁺ T helper (Th) cell populations have been described in the regulation of antibody and cell-mediated responses (Mosmann *et al.*, 1986). Th1 cells produce IFN γ and induce cell-mediated immune responses. In contrast, IL4 produced by Th2 cells serves as a growth factor for B cells, induces antibody isotype switching to IgG1 and IgE subclasses, and upregulates cell surface expression of class II

MHC molecules on B cells. Each of the Th cell populations are mutually inhibitory to growth of the other. Furthermore, *in vitro* studies have demonstrated that the presence of IL4 during antigen presentation drives cells towards the Th2 phenotype while the presence of IL12, and to a lesser degree IFN γ , with suboptimal levels of IL4, induces differentiation of cells of the Th1 phenotype.

Although the dichotomy between Th1 and Th2 subsets in humans is not as clearly defined as in mice, the existence of separate populations of T lymphocytes with distinctions similar to those in the mouse is well established (Wierenga *et al.*, 1991; Seder, 1994; Jutel *et al.*, 1995; Kamogawa *et al.*, 1993). Recent evidence strongly suggests that (i) diverse clinical responses to infection are related to different T-cell responses, and (ii) that differences in T helper cell responses to common inhalant allergens underlie the distinction between allergic and non-allergic individuals. For example, in cutaneous leishmaniasis and the tuberculoid form of leprosy, lesions heal spontaneously and are associated with DTH skin test reactions in humans. In contrast, visceral leishmaniasis and lepromatous leprosy are chronic infections which have been linked to increased levels of circulating IL4 (Liew, 1992; Salgame *et al.*, 1992; Kemp *et al.*, 1996). A role for T lymphocytes has also been implicated in both protective and pathogenic host responses to antigens derived from *Mycobacterium tuberculosis*. Only a small fraction (5-10%) of individuals infected with this organism develop clinical illness. Healthy subjects give low antibody responses but high *in vitro* T-cell proliferative responses to the major culture filtrate antigen 85. In contrast, patients with active disease exhibit high IgG antibody levels with reduced lymphoproliferation and IFN γ secretion (Huygen *et al.*, 1988; Munk and Emoto, 1995).

Using Trichophyton Protein IV, we characterized cytokine profiles of circulating antigen-specific T cells from individuals with distinct immune responses. Cell lines established from 5 subjects with IH skin tests produced a cytokine profile characteristic of Th2/Th0 cells upon stimulation with purified Trichophyton antigen or a *T. tonsurans* extract. In contrast, cell lines from DTH subjects generally showed a Th1 profile (Slunt *et al.*, 1996). In these experiments, the production of IL5 (but not IL4) was significantly higher in cell lines derived from IH subjects than cell lines from individuals with DTH (fig. 2). The results confirm that a single defined *Trichophyton* protein can elicit distinct T-cell cytokine profiles. Furthermore, these findings strongly support the view that cells with a Th1 phenotype play a role in cell-mediated responses to dermatophytes, while those with a Th2 phenotype may be involved in perpetuating infection.

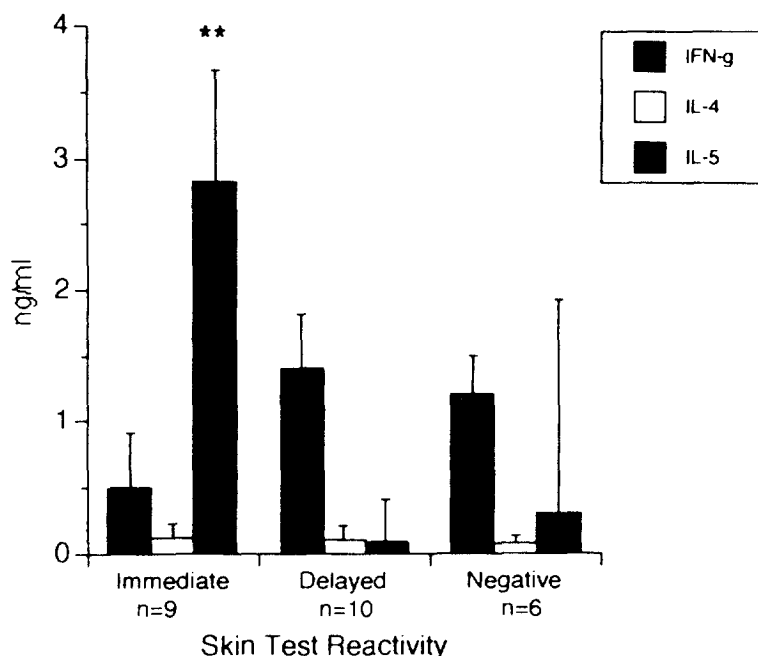


Fig. 2. Geometric mean level of cytokine (IFN γ , IL4, and IL5) production and the standard error of the mean at day seven post-restimulation for antigen specific T-cell lines established from subjects with different skin test reactivity to *Trichophyton*.

Cytokine levels for the IH subject group were 0.51 ± 0.41 ng/ml IFN γ , 0.11 ± 0.09 ng/ml IL4, and 2.8 ± 0.84 ng/ml IL5. The DTH subject group had a mean level of 1.4 ± 0.41 ng/ml IFN γ , 0.10 ± 0.03 ng/ml IL4, and 0.10 ± 0.31 ng/ml IL5. Skin test negative subjects had 1.2 ± 0.28 ng/ml IFN γ , 0.07 ± 0.01 ng/ml IL4, and 0.30 ± 1.60 ng/ml IL5. ** Levels of IL5 were significantly increased among the IH group as compared to the DTH group ($p < 0.001$).

Factors influencing the immune response to dermatophytes

Before attempting to explain how one T helper cell activity preferentially develops during the course of an immune response to an antigen, one should consider factors which may be critical to the outcome; these include properties intrinsic to the antigen, the dose and route of administration of the antigen, and the presence or absence of adjuvants. Factors intrinsic to the host are also critical and include the population of antigen-presenting cells and cytokine milieu present at the site of antigen exposure, and the immune status of the host.

Antigen-presenting cells process antigen and generate antigenic determinants for presentation to T lymphocytes. By virtue of their anatomic location in the skin, dendritic cells may be highly relevant to development of the immune response to dermatophytes. Dendritic cells are professional antigen-pre-

senting cells which present antigen in a highly efficient and broadly specific manner. Dendritic cells capture antigens in peripheral tissues and migrate to secondary lymphoid organs where they acquire the ability to stimulate naive T cells. For this reason, dendritic cells are believed to play a critical role in the induction of T-cell responses. It has recently been demonstrated that inflammatory stimuli aid in maturation of dendritic cells (Cella *et al.*, 1997; Pierre *et al.*, 1997). Indeed, keratinocytes produce a variety of cytokines including IL1, GM-CSF and TNF α which have the potential to influence dendritic cell maturation (Romani *et al.*, 1990). This process is accompanied by markedly altered kinetics of MHC class II molecule synthesis and recycling along with a variety of additional intracellular changes which optimize antigen presentation. The release of distinct inflammatory mediators at sites of acute and chronic dermatophytosis may influence the nature and concentration of antigenic determinants present on the dendritic cell surface, thus

affecting the differentiation of distinct T helper populations. Keratinocytes have been demonstrated to produce a variety of interleukins in addition to those mentioned above including IL3, IL6, IL7, IL8, IL10 and IL12. IL12 induces IFN γ production in resting and activated T lymphocytes, and also in natural killer cells. Furthermore, IL10 mRNA is often expressed in situations of increased antibody responses and suppressed DTH against pathogens, suggesting that IL10 can inhibit DTH (Yamamura *et al.*, 1991; Heinzl *et al.*, 1991; Romani *et al.*, 1993). IL10 inhibits the production of cytokines by Th1 cells and inhibits DTH responses induced by injection of Th1 clones into mouse footpads (Li *et al.*, 1994). Thus, keratinocytes may play a key role in determining the type of T helper immune response during the early stages of antigenic challenge and in modulating the effector stage of DTH responses.

Antigens as molecular tools for studying diverse immune responses

The presence of T lymphocytes with distinct cytokine profiles derived from individuals with diverse immune responses to Trichophyton may be explained by different T-cell repertoires mediating these responses. Molecular characterization of Trichophyton antigens is an obvious first step to studying T-cell antigenic determinants in individuals with different immune responses. Furthermore, definition of immunodominant epitopes associated with protective responses may play an important role in the development of alternative therapeutic strategies for the treatment of chronic dermatophytosis. With these goals in mind, our group has recently cloned and sequenced two Trichophyton antigens; Protein IV, which is present in cultures of both *T. rubrum* and *T. tonsurans*, is a 726-amino acid protein with sequence homology to the prolyl oligopeptidase family of serine proteinases (Woodfolk *et al.*, 1997). This family of enzymes cleaves peptides at proline residues. Members include dipeptidyl peptidase IV, which has been proposed to play a role in influencing the activity of cytokines and growth factors. High yields of recombinant Protein IV have been produced in the yeast *Pichia pastoris*. Cloning and amino acid sequencing have recently revealed a second putative *T. rubrum* serine proteinase belonging to the class D subfamily of subtilases (40 to 58% amino acid sequence identity) (Woodfolk *et al.*, 1998). Production of the recombinant protein has facilitated serologic studies demonstrating IgE and IgG antibody binding capabilities (unpublished). Computer-assisted molecular modelling of the *T. rubrum* subtilase-like putative catalytic domain showed a very similar three-dimensional structure to the structurally conserved regions of *Tritirachium album* proteinase K. Molecular characterization of

antigens derived from Trichophyton provides valuable tools for studying the immune response to dermatophytes and may help us to gain insight into the apparent dichotomy of the immune response to other important pathogens including *Mycobacterium tuberculosis*. Furthermore, gaining an understanding of the immunologic mechanisms and host factors involved in preventing systemic spread of dermatophytes may provide clues regarding mechanisms of spread of invasive fungi.

Therapeutic potential of recombinant Trichophyton proteins

Development of vaccines for the treatment of dermatophytosis has progressed slowly owing, in part, to a lack of encouraging results in early human studies. Recently, the advent of efficacious systemic antifungal drugs such as itraconazole has further reduced interest in this field. However, development of Trichophyton vaccines may be beneficial in certain cases, particularly for those individuals with chronic recurrent dermatophytosis requiring long-term antifungal therapy. TOE is a vaccine still in use today which contains Trichophyton, Oleandomycin and Epidermophyton species. Immunotherapy with "trichophytin" which consists of an antigenic extract of dermatophytes, has been demonstrated to reduce or eliminate delayed skin test responses while having no effect on IH responses (Jillson and Huppert, 1949; Sulzberger and Wise, 1932). In contrast, standard desensitization to common allergens is aimed at reducing clinical symptoms of allergic disease which is often accompanied by a substantial reduction in size of IH skin tests. As mentioned earlier, repeated exposure to Trichophyton antigens in the skin may result in altered skin test reactivity. Therefore, until more is known regarding regulation of the immune response to antigens injected into the skin, it is hard to assess how elimination of DTH skin tests by trichophytin immunotherapy reflects the systemic immune response. Molecular characterization of Trichophyton antigens may facilitate an alternative approach to desensitization. Elucidation of the amino acid sequence of Trichophyton antigens allows T-cell epitope mapping using overlapping peptide methodology for defining antigenic determinants associated with protective immune responses. Incorporation of peptides containing these immunodominant epitopes into vaccines may be a useful alternative approach for modulating the immune response in individuals with chronic dermatophytosis associated with allergic symptoms.

In this article we have reviewed some of the immunologic aspects of dermatophytosis and discussed the relevance of these infections to allergic disease. Trichophyton is unusual in that it elicits

both immediate and delayed hypersensitivity skin test responses. Infection can result in chronic lesions characterized by relatively low grade inflammation or acute highly inflamed lesions. Chronic infections appear to correlate with IH skin tests and a lack of DTH skin reactions. Furthermore, an association between chronic dermatophytosis and atopic symptoms, including urticaria and asthma, is now firmly established. Recent studies have demonstrated higher titres of antigen-specific IgG and IgE antibodies in individuals with IH skin tests compared to those with DTH or negative skin tests. In addition, cell lines with distinct cytokine profiles have been established from individuals with IH and DTH responses, suggesting that different T helper lymphocyte subsets play a role in mediating diverse responses to the same pathogen. Isolation of dermatophyte antigens with enzymatic activity facilitates studies examining the role of such antigens in fungal pathogenesis. In addition, molecular characterization of *Trichophyton* antigens provides us with valuable tools for studying immunologic aspects of this disease. Elucidation of the immunologic mechanisms underlying diverse immune responses to *Trichophyton* has important implications not only for developing alternative therapeutic strategies for the treatment of chronic fungal infections, but also for other chronic diseases caused by a wide range of pathogens.

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Cytokine- and T-helper-dependent immunity in murine aspergillosis

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Introduction

Aspergillus fumigatus is a ubiquitous and opportunistic fungus that elicits respiratory infections, such as allergic asthma, allergic bronchopulmonary aspergillosis, aspergilloma, and invasive pulmonary

aspergillosis (IPA) (Rinaldi, 1983; Bodey and Vartivarian, 1989). The first two disorders are allergic in nature and are caused by exposure to *A. fumigatus* and related species, whereas aspergilloma is a result of saprophytic growth of the fungus in preexisting pulmonary cavities (Rhodes, 1993). In neutropenic

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