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A CLINICAL, MYCOLOGICAL, AND IMMUNOLOGICAL STUDY

OF JUVENILE TINEA CAPITIS

CAUSED BY TRICHOPHYTON TONSURANS

presented by

Dennis E. Babel

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THESIS

# A CLINICAL, MYCOLOGICAL, AND IMMUNOLOGICAL STUDY OF JUVENILE TINEA CAPITIS CAUSED BY *TRICHOPHYTON TONSURANS*

By

Dennis E. Babel

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ABSTRACT

A CLINICAL, MYCOLOGICAL AND IMMUNOLOGICAL STUDY OF JUVENILE TINEA CAPITIS CAUSED BY *TRICHOPHYTON TONSURANS*

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The clinical, mycological, and immunological features of tinea capitis in children were studied. Of the 331 culture proven infections diagnosed during a three year period, 290 (88%) were caused by *T. tonsurans* and 38 (11%) by *M. canis*. When these data were reviewed with respect to sex distribution of patients, it was found that tinea capitis occurred in females almost three times more frequently than in males. Racial occurrence was studied, and it was noted that black children accounted for 95% of all tinea capitis, and 99% of this type of infection was caused specifically by *T. tonsurans*.

Thirty juvenile patients with scalp infections caused by *T. tonsurans* were selected for subsequent studies. They were identified as inflammatory or noninflammatory on the basis of clinical examination of the features of their infection. Cultures of infected material from each patient demonstrated colonies of *T. tonsurans*. These isolates were tested for urease activity. All strains were found to be positive with

those from patients in the inflammatory group requiring 2.1 days of incubation versus 2.2 days in the noninflammatory group. The rapid appearance of enzymatic activity in isolates from all patients might be an indicator of their ability to invade hair.

Twenty-six of 30 isolates of T. tonsurans demonstrated the gross morphology of the sulfureum variety. Twenty-three of these 26 (89%) were able to perforate hair in vitro. This ability indicates that these isolates were of the subvariety perforans. The occurrence of this species subvariety was the same in the inflammatory and noninflammatory groups.

Measurements of T-lymphocyte subpopulations were performed by labeling cells with monoclonal antibodies and analyzing them by flow cytometry. T4/T8 (helper/suppressor) ratios were calculated for all patients and for ten noninfected control subjects. The results showed that the mean T4/T8 ratio of the noninflammatory group was not statistically different from that of the control group. This was interpreted as an indication of T4 inactivation and immune anergy. The mean ratio of the inflammatory group was significantly elevated indicating T4 activation and subsequent immune response. Models for the immune sequences in both of these patient groups are proposed and corrective therapy suggested.

DEDICATION

"If a man does not keep pace with his companions, perhaps it is because he hears a different drummer. Let him step to the music he hears, however measured or far away."

To my wife, Gail, for concurring with Thoreau.

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On a more personal note, thank you to my relatives and numerous friends for their encouragement through the many academic years. My thanks to Dr. Jim Veselanak for taking me under his wing and showing me the ropes of Michigan State University graduate school and to Fritz Simons, my Lansing connection. My affectionate thanks to Dr. Jim Simons, Jr., for teaching me dedication and how to keep my values in proper perspective and to his wife Joanne for all her kindness.

Last, and most importantly, I would like to thank my children, John, Michael, and Kelly, for donating

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TABLE OF CONTENTS

TABL

RESU

TABLE OF CONTENTS Continued

TABLE OF CONTENTS Continued

## LIST OF TABLES

LI

TA

TA

TA

TA

TA

TA

LIST OF TABLES Continued

LIST OF FIGURES

LIS

## LIST OF FIGURES

# INTRODUCTION

Tinea capitis is the most frequent form of dermatophyte infection seen in children today. The presentation of this disease can be an acute, inflammatory process of relatively short duration with a good patient response to antimycotic therapy. Another more common form of this disease is chronic, noninflammatory scalp infection which at times is recalcitrant to appropriate therapy. This clinical variance is related to the dynamics of the host-parasite relationship consisting of a delicate balance between the host's immune capabilities and the pathogenic characteristics of the invading fungus.

The object of this study was to explore this relationship and to try to identify some of the factors which might contribute to the expression of this disease. In the first part of this investigation, the features of the infected juvenile population were reviewed with respect to sex and race as well as the identities of the dermatophytes isolated. In the second part of this study, consideration was given to the most commonly recovered pathogen, T. tonsurans. The ability of these fungal isolates to penetrate hair in vitro and to demonstrate urease activity were compared to the type of disease process they elicited in their respective hosts.

In the final part of this project, the immune response of the patient was studied. The many constituents of the immune system play an important role

in the host's defense against fungal infection. This system was controlled by many checks and balances in the form of soluble cell mediators (Fundenberg et al., 1980). One potential model for the explanation of chronic, noninflammatory dermatophytosis would begin with the fungal antigen being presented by mobile epidermal Langerhan's cells to the vasculature of the upper dermis. Complement would be activated at C3 via the alternate pathway resulting in production of chemotactic factors (CF). The initial influx of cells would include basophils and neutrophils being followed later by lymphocytes and monocytes. T-helper lymphocytes, stimulated by macrophage presented antigen, would elaborate migration inhibition factor (MIF) and lymphokines that stimulate antibody production in B-lymphocytes. The IgE produced by these later cells would complex with the dermatophyte antigens and bind to receptors on basophils and tissue mast cells. The resulting degranulation of these receptor cells would allow the release of vasoactive amines. The histamine produced would be presented directly to the H2 receptors of T-suppressor lymphocytes by a macrophage, or receptor activation might occur indirectly with interleukin I. Thus stimulated, the T-suppressor cells would produce histamine suppressor factor which would further stimulate macrophages to produce prostaglandin E-2 (PGE-2). PGE-2 would then inhibit T-helper lymphocytes from proliferating and prevent their production of MIF, CF, and possibly other lymphokines. These products would cause host

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defense cells to migrate to and remain at the site of infection. In addition, the enzymes associated with dermatophyte keratinolysis of host tissues might also degrade the CF produced by the complement cascade. The resulting immunologic anergy would allow the existence of chronic, noninflammatory tinea.

One critical component of this model that could be tested was the ability of T-helper cells to proliferate during dermatophyte infections. The ratio of T-helper cells to T-suppressor cells could be calculated with the use of monoclonal antibodies and flow cytometry. A significant shift in this ratio would be an indirect means of determining proliferation of one cell type or another. These analyses were performed on patients with inflammatory infections, noninflammatory infections, and noninfected control subjects.

## LITERATURE REVIEW

"Study the past if you would divine the future" - Confucius

DERMATOPHYTOSES

The first recorded description of a dermatophyte infection was authored by Aulus Conelius Celsus in the fifth book of his eight volume work, "De Re Medica" (Rosenthol, 1961). This is the oldest surviving medical document after the Hippocratic writings. Celsus was a "philiatros", a friend of physicians, and one of the first great encylopedists. He lived during the reign of the Roman emperor, Tiberius, and wrote his treatise around 30 A.D. He describes kerion as consisting of "large, painful, furuncular lesions with a number of foramina through which exudes a glutinous and purulent humor." To his honor, the medical term kerion celsi has persisted to this day. Cassius Felix is ascribed to have coined the term tinea about 400 A.D. and the Italian renaissance physician, Mercurialis, designated all diseases of the scalp as "teignes" (tineas) in 1577 (Rosenthal, 1960). Samuel Plumbe in his Practical Treatise of Diseases of the Skin (London, 1824) recognized that scalp infections might produce lesions in other parts of the skin. He stated, "The diseased secretion of the scalp affection is capable of producing by innoculation, the ringworm of the skin on other parts and vice versa" (Rosenthal, 1960). It was not until 1837 that the Polish physician, Robert Remak,

found hyphae in the crusts of the scalp condition known as favus. In 1839, Schoenlein described these filaments as molds and attributed this disease to a plant etiology (Ajello, 1974). The Hungarian physician, David Gruby, first isolated the pathogen of favus on potato slices and was able to reproduce the disease by innoculating the fungus onto normal skin in 1841 (Rippon, 1982). He went on to describe "tinea favosa," "ectothrix and endothrix trichophytosis," and "microsporosis" (McGinnis, et al., 1985). Swedish investigator, Per Hendrik Malmsten, described Trichophyton tonsurans as a causative agent of tinea capitis in 1845 (Subrahmanyam, 1980).

Dermatophyte infections, as a rule, involve only the keratinized tissues of the body, i.e., hair, skin, and nail. These unique pathogens possess the ability to derive their total nutritional needs from these non-viable materials. The digestive process involves elaboration of soluble proteolytic enzymes by the colonizing fungus (Grappel, et al., 1974). The qualitative and quantitative characteristics of the enzymes that are manufactured vary with the individual dermatophyte species (Weary and Canby, 1967). Through this means of superficial habitation, the organism interacts with its host. If the host response is minimal, the pathogen enjoys a state of peaceful equilibrium and a chronic infection is established. When the host response is more significant (acute inflammation), this balance is upset and the fungus is eliminated (Livingood and Pillsbury, 1941).

On rare occasions, dermatophytes demonstrate the ability to traverse the keratinized tissues and invade deeper, viable cells (Head and Smith, 1981). Localized infection can present as subcutaneous nodules (Alteras et al., 1984a), follicular granulomas (Mikhail, 1970), or even draining abcesses (Smith and Head, 1982). Mycetomas caused by various dermatophyte species including T. tonsurans have also been reported (West and Kwon-Chung, 1980). A generalized, deeper chronic infection due to T. rubrum, with bizarre hyphal morphology was reported by Nishiyama et al. (1983), while a generalized acute process involving lymph nodes, testes, vertebrae, and brain was attributed to T. mentagrophytes (Hironaga et al., 1983). In most cases of deep involvement, a predisposition such as defective cell mediated immunity or a concurrent underlying disease is suspected (Abraham et al., 1975; Alteras, et al., 1982; Kamalam, et al., 1977).

TINEA CAPITIS

Ringworm of the scalp is a disease caused by many species in the genera Trichophyton and Microsporum. Fungal structures, such as hyphae and conidia, come in contact with the epidermis of the scalp, germinate, and the resulting hyphae invade the follicles. Jillison (1982a) found that only the actively growing (anagen) hairs are involved.

Tinea capitis can be of the ectothrix type in which the invading hyphae disarticulate into arthroconidia

on the surface of the hair shaft destroying the cuticle in the process (Matsuoka and Gedz, 1982; Takatori et al., 1983). In the course of active tinea capitis, arthroconidiation of some dermatophytes produce a water soluble metabolite called pteridine (pyrimidine-4:5′:2,3-pyrazine) (Rippon, 1982). Hairs infected by these pathogens demonstrate a bright blue-green fluorescence when exposed to a "Wood's lamp" (Krull and Babel, 1976).

The second general type of tinea capitis is of the endothrix form. In this presentation, the hyphae separate into arthroconidia which are retained within the hair shaft and the cuticle remains intact. The hairs involved in this pattern of infection will not fluoresce under Wood's filtered ultraviolet light (Rudolph, 1979). Endothrix ringworm is currently the most frequent presentation of fungal scalp infection in North America with T. tonsurans being the etiologic agent (Babel and Rogers, 1983; Sinski and Flouras, 1984). The clinical presentation of this disease is quite variable resulting in lengthy differential considerations and occasional misdiagnoses (Andrew, 1979). In 1910, Raymond Sabouraud noted "What principally characterized our endothrix infections is a negative feature, it is their lack of distinction" (Gaisin et al., 1977).

Howell et al. (1951), described the course of endothrix tinea capitis in three phases, all of which could occur simultaneously among the distinct areas of

scalp infection. In the epidermal phase, the first phase, the fungus is limited to the epidermis of the scalp with no follicular involvement. In phase two, the follicular invasive phase, the organism moves from the epidermis to the hair shaft penetrating the cortex at Adamson's fringe. The hyphae begin arthroconidiation while the hair continues to grow upward. The much weakened shaft will fracture when it reaches the scalp's surface giving the appearance of a black dot. The third and final phase, the healing phase, results when the hair has left the follicle. This last phase may be retarded in juvenile infection and carried on into adulthood.

The host response is quite variable as witnessed by the clinical symptoms. Barlow and Chattaway (1958), noted that "Trichophyton sulfureum can grow for long periods in the hair follicle without producing any obvious clinical reaction in the host." In this form, alopecia may be nonapparent with a minimal amount of diffuse scaling giving the appearance of seborrhea. In a more active response, well demarkated areas of hair loss with prominent "black dots" might be noted (Gaisin et al., 1977). Pigmented dots on the scalp can be associated with a number of other etiologies however (Chernowsky, 1974). This differential has been assembled in Table 1. In an acute T. tonsurans infection, areas of alopecia demonstrate erythema, scattered pustules and crusting (Graham et al., 1964). If inflammation persists, kerion formation occurs as areas of boggy edema and purulent

TABLE

**TABLE 1. Differential diagnosis of pigmented dots in the scalp.**

I. Black Dots

- Endothrix tinea capitis
- Black piedra
- Cladosporium colonization
- Alopecia areata
- Neurodermatitis
- Trichotelomania
- Comedones
- Discoid lupus erythematosis
- Follicular lichen planus
- Lichen sclerosus et atrophicus
- Follicular keratin casts
- Nevi
- Melanomas
- Lentigines
- Foreign materials (dyes, etc.)

II. Brown or Tan Dots

- Post inflammatory hyperpigmentation after bacterial folliculitis
- Post-electrolysis
- Pediculosis

III. Blue Dots

- Ceruleomycosis due to colonization by Penicillium sp. or Aureobasidium sp.

IV. White or Gray Dots

- Ectothrix tinea capitis
- White piedra
- Tinea amniantaecia
- Guttate psoriasis
- Seborrheic dermatitis

V. Red Dots

- Telangiectasias
- Insect bites

(Adapted from Chernowsky, 1974)

drainage (Vanbreusghem et al., 1978). These edematous areas can be interconnected by canalizing fluctuant tracts that give the appearance of dissecting cellulitis. This extreme form is sometimes referred to as profundus kerion celsi (Subrahmanyam, 1980).

Systemic symptomatology can accompany inflammatory tinea capitis which include cervical lymphadenopathy, malaise, pyrexia, arthralgias, and a hyperimmune state referred to as an "id reaction" (Franks et al., 1952). In a review of dermatophytids, Jillison (1982b) described two distinct forms associated with scalp ringworm. The first was called the "follicular-papular dermatophytid" which consists of follicular papules, some with spines, that appear suddenly on the chest, back, and face. This reaction was sometimes induced after the initiation of griseofulvin therapy. Form two or "erythema nodosum dermatophytid" is closely related to the onset of kerion formation, and occurs as multiple erythematous nodules with the incidence being greater in males.

Tinea capitis is normally caused by a single species of dermatophyte, but occasionally more than one dermatophyte may be involved. Grigoriu and Delacretaz (1982) reported a scalp infection in a 10 year old female which demonstrated both T. tonsurans and M. canis. Histopathologic studies confirmed this duality. Tinea capitis profunda due to T. tonsurans and T. verrucosum was reported in a 9 year old female by Kremple-Lamprecht, et al. (1982).

HISTOPATHOLOGY OF ENDOTHRIX HAIR INVASION

Tosti et al. (1970) performed detailed scanning electron microscopic studies of endothrix hair infection. They found that during the early phases of the invasion, the hyphae advanced along the longitudinal axis of the hair cortex. Keratin fibrils were dissociated in the shaft leaving tunnels containing chains of arthroconidia. The fungus is thought to disassemble the hair framework by enzymatic dissolution of the non-keratinous interfibrilar material. Eventual digestion of the keratin fibril bundles occurs by means of this same enzymatic process.

Hematoxylin-eosin stained biopsy sections from T. tonsurans infected scalps showed areas of perifollicular granulomatous inflammation with many plasma cells, foreign body giant cells, lymphocytes, polymorphonuclear leucocytes, fibrosis, and capillary-endothelial proliferation. Periodic acid-schiff (PAS) staining demonstrated strong PAS reactive intrapilary arthroconidia. These spores were not observed below the middle of the keratinizing zone of the anagen hairs and were confined by an intact cortex cuticle at all levels. Colloidal iron stains demonstrated no hyaluronic acid coating of the arthroconidia for T. tonsurans which is unlike the coating seen with M. audouinii. Alcian blue staining showed the presence of great numbers (20 per high-power field) of mast cells in T. tonsurans specimens. Gross pathological change did not necessarily relate to the histopathology of this disease (Graham, et al., 1964).

The pathogenesis of kerion was studied in great detail by Imamura et al. (1975). With the use of fluorescent microscopy and immunofluorescent staining of kerion biopsy sections, they were able to demonstrate fungal antigens not only within the hair shaft but also within the dermal inflammatory infiltrate. They suggested that if the initial host reaction in tinea capitis induced a strong inflammatory response, the hair follicle would be damaged allowing fungal antigen access to the adjacent dermis. The vascular dermis would be highly sensitive to this antigen resulting in a major inflammatory infiltrate response which would induce the clinical changes associated with the kerion. Zaslow and Derbes (1969) noted that this response is much like that of a dermatitis which produces a Type IV reaction indicating development of delayed hypersensitivity rather than a Type I or a anaphylactic response.

PATHOGENIC MECHANISMS

The means by which dermatophytes invade keratin and induce a host response is probably multi-channeled. As early as 1894, MacFadyen observed proteolytic activity in the culture fluids of T. tonsurans (Subramanyam, 1980). Yu, et al. (1968) were able to isolate three distinct enzymes from T. mentagrophytes. The extracellular enzyme they referred to as "Keratinase I" had the ability to digest guinea pig hair and hydrolize several different peptides. In human skin cell cultures, Hino, et al.

(1982) were able to produce acantholysis by means of this proteolytic enzyme. After 18 hours of incubation, acantholysis was initiated by the separation of the dermo-epidermal junction between the basal cells and the basal lamina. After 72 hours, the basal lamina appeared ruptured and degraded while hemidesmosomes were no longer found on the basal cells. The horny layer of the epidermis and hair follicles remained intact. The changes in desmosomes and dermo-epidermal junction induced by "Keratinase I" were similar to those induced by the enzymes elastase, papain, and trypsin *in vitro*.

Davies and Zaini (1984b) studied the enzymatic activities of *T. rubrum* and their relation to the chemotaxis of polymorphonuclear leucocytes (PMN's). They demonstrated the decomposition of hair by enzymatic removal of membranes and cement substance allowing the keratin fibrils to drop apart. The resulting soluble materials were of nutritive value to the dermatophyte. The specificity of these dermatophyte enzymes were similar to that of trypsin and chymotrypsin. These serine esterases are important in PMN chemotaxis. They were able to activate complement via the alternate pathway at C3 with dermatophyte cell wall. In diseased skin, C3 was demonstrated in the basal and supra basal layers. The subsequent cascade with neutrophil chemotactic factor (NCF) being a product of the cleaving of C3a and C5a would result in an influx of PMN's. *T. rubrum* was also able to induce NCF production in the absence of complement by

producing serine esterase which acts on plasma proteins. The PMN influx resulted in an inflammatory reaction like that seen in acute tinea capitis or kerion. In the absence of an inflammatory reaction, as that seen in seborrheic tinea capitis, they suggested that the continuing high levels of dermatophyte enzymatic activity would repudiate the effect of NCF.

An indirect means of increasing dermatophyte invasion potential was hypothesized by Allen and King (1978). They postulated that the conidia and hyphae of the dermatophyte grow and produce infective units more efficiently under conditions of higher carbon dioxide tensions. They noted that occlusion of the skin raised carbon dioxide concentrations and enhanced the rate of infection. The same phenomena in one of our patients was observed by this author. An 86 year old white male presented with bilateral erythematous scaly lesions on his knees. The patient related that he applied a topical arthritic medication to these joints and then wrapped them in saran wrap on a daily basis. The KOH exam of material from these lesions was floridly positive and the fungal culture was identified as T. rubrum.

PATIENT POPULATION CHARACTERISTICS

It has been noted by a multitude of authors that tinea capitis is primarily a disease of young humans. Consideration must be given, however, to the infecting species of dermatophyte relative to this parameter.

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Pipkin (1952) in his epochal paper delivered before the Seventy-First Meeting of the American Dermatological Association, noted that fungal infection of the scalp, up to that time, was seen primarily in prepubertal children and that the responsible agent was M. audouinii. He also found an alarming increase of a new pathogen, T. tonsurans, in many of the southern states and noted that adults were involved as well as children. Adult disease could be insidious, presenting a diagnostic dilemma. Sabouraud (1910) had pointed out that Trichophyton infections usually heal at puberty because the skin surface is at that time unfit for fungal growth and reproduction. This has not proven to be the case with T. tonsurans. Although a number of species can be involved even in infants with this disease (Alteras et al., 1984; Zaror et al., 1984), rarely are they seen in an adult host (Schiff et al., 1974). Tinea capitis in the elderly patient has almost always been due to T. tonsurans (Pursley and Ranier, 1980). The review by Jillison (1982a) of many patients harboring this organism found a child-to-adult ratio of 10:1 (still far greater than any other species); and among adults, a female-to-male ratio of 70:1. Foged and Sylvest (1981) found that adult infections with T. tonsurans were not restricted to the scalp. In vitro tests by Krause (1976) demonstrated that hair from women and children were less resistent to attack by dermatophytes than hairs from male adults. Prevost (1979) in a detailed review of the patients with tinea

capitis seen in Charleston, South Carolina, during the 1950's, found that 94% were due to Microsporum sp. and only 5% to T. tonsurans. The male-to-female ratio was 3:1 with an almost even distribution between blacks and whites. The data she compiled in the 1970's showed almost a complete reversal. T. tonsurans was implicated in 91% of scalp infections with an even male-to-female ratio among children. All of the infected adults were female, and T. tonsurans was recovered in each case. Review of infection by race, showed 99% of blacks were infected by T. tonsurans compared to 36% of whites. Similar age, race, and sex data with regard to tinea capitis caused by T. tonsurans in the United States were noted by other authors (Babel and Rogers, 1983; Chernosky et al., 1956; Gaisin et al., 1977; Sinski and Flouras, 1984).

In other parts of the world, the prevailing etiologic agent of tinea capitis shows remarkable diversity. Raubitschek (1959) found T. violaceum in 95% of the patients in Israel. In Iraq, Rahim (1966) reported T. schoenleinii to be most prevalent followed by T. violaceum. In Italy, Binazzi et al. (1983) noted a steady decline in T. tonsurans since the 1940's with M. canis being the most frequently recovered pathogen from cases of tinea capitis at the present time. In Spain, Miguens and Espinosa (1980) found a considerable decrease in T. schoenleinii while T. verrucosum and T. mentagrophytes were responsible for the majority of scalp ringworm. From the Region of Butare (Africa), Bugingo (1983) recovered T.

violaceum and M. langeroni (canis) with greatest frequency.

In reviewing the epidemiology of dermatophytoses, Vidotto et al. (1982) noted a higher incidence of tinea capitis in the May-June and September-October time periods. Emmons et al. (1977), in reviewing transmission of T. tonsurans in the United States, noted the anthropophilic nature of this species with humans and their fomites being the natural reservoir. Hair brushes, hair clippers, pillows, furniture, hats, etc., can be the vehicles of the transmission. Tinea capitis due to T. tonsurans is seen most frequently in blacks in the lower socioeconomic groups. MacDonald and Smith (1984) attribute this to the crowded living conditions and poor hygiene associated with poverty. Sehgal et al. (1985) notes a similar socioetiologic correlation in India. The sources of dermatophyte infections can be inapparent and insidious (Beneke, 1978; Faergemann et al., 1983; Lopez-Martinez et al., 1984; Polonelli et al., 1982).

Genetic predisposition to fungal infections has been considered by many authors. Rippon (1982) noted that Asian peoples generally responded to a M. ferrugineum infection in a benign, noninflammatory fashion while native Africans suffered a much more active, acute course. Serjeantson and Lawrence (1977) studied the familial distribution of chronic tinea imbricata in an untreated Melanesian population. They suggested that susceptibility

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to chronic T. concentricum infection was recessively inherited and controlled by genes at a single locus. In work done six years later among this same patient group, Hay et al. (1983) found that 98% of those infected were unable to demonstrate delayed hypersensitivity due to T-cell hyporeactivity. Hay et al. (1983) felt that both genetic predisposition and environmental phenomena contributed to tinea morbidity.

HLA-ABC loci can be found on all lymphocytes while the DR locus is found only on B-lymphocytes. These loci lie on chromosome 6 and are used in tissue typing (Roitt, 1977).

Different investigators have studied HLA antigens as genetic markers of predisposition to fungal infections. Their findings conflict with the previous authors assumptions. Svejgaard et al. (1983) typed for HLA-ABC and DR antigens in patients with chronic T. rubrum infections. They found that the distribution of antigens in this group did not differ from that of the control population; therefore, HLA controlled immune mechanisms in chronic fungal infection seemed unlikely. Kanitakis et al. (1984) completed a similar study in 48 patients with pityriasis versicolor and 134 controls. No statistically significant deviations in HLA patterns were found in the patient group when compared to the control population.

TRICHOPHYTON TONSURANS

Trichophyton tonsurans, Malmsten 1845, was first described by the Swedish researcher Per Hendrick Malmsten in 1845 (Ajello, 1974). It is an anamorph fitting into the form phylum Deuteromycota (Babel and Rogers, 1983; Beneke et al., 1984). Its teleomorph state is unknown and may have been lost through evolution (Rebell and Taplin, 1974; Rippon, 1982). All strains that were tested by the A. simii sex stimulation test were of the (-) mating response (Rippon, 1982). This organism was probably originally endemic in Europe but now has been recovered from 25 different countries (Sinski and Flouras, 1984). Because of variability in colonial morphology and clinical symptoms, there was much taxonomic confusion in the early literature. No less than 17 different species were ascribed to this organism (Beneke et al., 1984; Subrahmanyam, 1980). These binomials are listed in Table 2. Georg (1956a&b) consolidated these synonyms into one species and demonstrated that thiamine is a nutritional requirement for this organism (Swartz and Georg, 1955). McGinnis (1980) reviewed the current nutritional tests used for the identification of the more common species of the genus Trichophyton. His data with respect to T. tonsurans are collected in Table 3.

As early as 1894, MacFadyen observed proteolytic activity in culture fluids of T. tonsurans (Subrahmanyam, 1980) but Nobre and Viegas (1972) found that only 13% of the strains tested had lipolytic activity.

TAB

**TABLE 2.** *Trichophyton tonsurans* **synonyms.**

| | | |
|---|---|---|
| *Trichophyton epilans* | Boucher et Megnin | 1887 |
| *Trichophyton sabouraudi* | Blanchard | 1896 |
| *Trichophyton crateriforme* | Sabouraud | 1902 |
| *Trichophyton flavum* | Bodin | 1902 |
| *Trichophyton acuminatum* | Bodin | 1902 |
| *Trichophyton effractum* | Sabouraud | 1910 |
| *Trichophyton fumatum* | Sabouraud | 1910 |
| *Trichophyton umbilicatum* | Sabouraud | 1910 |
| *Trichophyton regulare* | Sabouraud | 1910 |
| *Trichophyton exsiccatum* | Sabouraud | 1910 |
| *Trichophyton polygonum* | Sabouraud | 1910 |
| *Trichophyton plicatile* | Sabouraud | 1910 |
| *Trichophyton pilosum* | Sabouraud | 1910 |
| *Trichophyton sulfureum* | Sabouraud | 1910 |
| *Trichophyton cerebriforme* | Sabouraud | 1910 |
| *Trichophyton ochropyrraceum* | Muijs apud Papengaaji | 1924 |

**TABLE 3. Reaction patterns of *T. tonsurans***

A. Assimulation of carbon compounds

| Carbon Compound | Positive Isolates |
|---|---|
| L-Arabinose | 0% |
| Dextrin | 18% |
| Erythritol | 100% |
| D-Galactose | 53% |
| D-Glucitol | 100% |
| Glycerol | 18% |
| Maltose | 100% |
| Rabitol | 12% |
| Ribose | 18% |
| Sucrose | 88% |
| Trehalose | 88% |

B. Nutritional requirements[a]

| | Casein | Casein + thiamine |
|---|---|---|
| Growth: | + or - to 1+ | 4+ |

C. Urea[b] Hydrolysis

| Days to become positive | | | | |
|---|---|---|---|---|
| 0-7 | 8-10 | 11-14 | 15-21 | Negative |
| 50%[c] | 30% | 17% | 3% | 0% |

[a]This is a two-tube medium test incubated at room temperature and read after 10 days.

[b]Christensen's urea agar medium.

[c]percentage of positive isolates.

Frey et al. (1979) described the great variation in colonial morphology of this organism. The topography can range from flat or plicate to cerebreform or crateriforme. The texture may appear suede-like, velvety or powdery. The surface pigment can vary from white to pale yellow, red-brown or purple. The colony reverse demonstrates early significant pigment which may be mahogany red, brown, or brown-black, with very rare strains showing diffusion of pigment into the agar.

Microscopic morphology reveals numerous microconidia which may be tearshaped, round, elongate, and occasionally balloon shaped. The attachment to mycelia may be sessile or stalked with the latter appearing like a match stick. Macroconidia are infrequently found and are described as being nonechinulate, thin to moderately thick walled with a variable number of segments. Their shape is less perfectly formed than that seen with the macroconidia of T. mentagrophytes. The hyphal diameter is quite variable, especially with age. Mycelial segments with broader diameters may have sessile microconidia or multiple short branches which give the appearance of a caterpillar (Beneke and Rogers, 1980; Rebell and Taplin, 1974).

Matsumoto et al. (1983) while studying species variants of T. tonsurans described a new subvariety of T. tonsurans var. sulfureum. This subvariety was found to have the ability to consistently perforate hair in vitro. Only 17% of the T. tonsurans var. sulfureum strains tested

had this quality. The new proposed nomenclature based on this characteristic is T. tonsurans var. sulfureum subvar. perforans.

T. tonsurans is considered to be a strictly anthropophilic dermatophyte although infection in a horse and a dog have been reported (Rippon, 1982). Human hair infection by this pathogen is of the endothrix type with large arthroconidia (5-8 microns) being produced only within the pilus of the hair. Glabrous skin as well as nail infections are being seen with increasing frequency (Babel and Rogers, 1983).

IMMUNOLOGY OF DERMATOPHYTOSES

Dermatophytes normally invade only the keratinized or nonviable tissues of the body yet they have the ability to initiate active infection in humans. This highly evolved host-parasite relationship can result in many different forms of disease as mentioned previously. This process is influenced by a number of factors including variation in fungal antigen, host immune system, and external environmental factors. The resulting clinical presentation of this disease ranges from minimal scaling to exaggerated inflammation (Ahmed, 1982).

Measurement of the hosts ability to mount an immune response has been performed in vivo by intradermal skin testing with fungal antigen. Jenner noted in 1798, "It is remarkable that variolous matter when the system is disposed to reject it should excite inflammation...more

speedily than when it produced the small pox... It seems as if a change, which endures through life, had been produced in the action, or disposition to action, in the vessels of the skin" (Bullock, 1976). This first description of delayed hypersensitivity was made before Robert Koch performed the first intradermal skin test with tuberculin almost a century later.

Jones et al. (1973) evaluated patients with dermatophyte infections by skin testing with purified trichophytin. They found that those individuals demonstrating a positive delayed hypersensitivity response within 72 hours were able to eventually eliminate the fungus and demonstrated enhanced resistance to reinfection. The patients presenting with longstanding, chronic infection were unable to produce a delayed hypersensitivity (DH) response but would sometimes give an immediate hypersensitivity (IH) reaction. Experimental dermatophyte infections were initiated in healthy volunteers (Jones et al., 1974a). They found that fungal lesions resolved spontaneously following the emergence of DH responses. Sorensen and Jones (1976) skin tested patients chronically infected with dermatophytes to seven common antigens and found their anergy to be relatively specific for trichophytin. Hay and Brostoff (1977) found a marked reduction in the incidence of DH response in patients chronically infected with T. rubrum as compared to those infected by other dermatophyte species. An increased incidence of chronic infection and occurrence of

IH response was found in individuals with a history of atopy (Jones, 1974b,c). The "atopic chronic dermatophytosis syndrome" usually seen in adults has also been identified in children (Song and Achten, 1984). In a study on the significance of trichophytin reactivity in atopic dermatitis, Rajka and Barlinn (1979) investigated cutaneous reaction to Penicillium, Cladosporium, and Alternaria antigens as well. They found that a positive trichophytin reaction in atopic dermatitis did not necessarily mean sensitization to dermatophytes, but was primarily the sign of a cross sensitivity to other fungi. Hunziker and Brun (1980) tested five atopic patients with chronic tinea and observed strong IH response to trichophytin skin testing but no DH response. When they injected a second dose of trichophytin into the wheal of an immediate reaction, they were able to elicit a strong delayed reaction. They theorized that this phenomena might be caused by the neutralization of injected antigen by serum antibodies during the immediate reaction.

Cozad and Chang (1980) noted an important parallel relationship between host resistance to fungal infection and the prevailing level of delayed hypersensitivity response in an animal model. The clinical significance of the skin testing mechanism was questioned by Kaaman (1978). He compared test results using commercially available trichophytin and an ethylene glycol purified trichophytin. IH reactions occurred with both trichophytin antigens at a similar frequency of cases

while DH reactions occurred significantly more often to the purified trichophytin in patients with dermatophytoses. In a subsequent study, Kaaman (1981) found that dermatophyte species differ in their sensitizing capacity as measured by trichophytin skin testing. Antigen prepared from T. mentagrophytes was more potent than that produced from T. rubrum, while Epidermophyton floccosum gave the least. He also noted that the anatomic location of infection affected the cell-mediated response to trichophytin.

Thus, many investigators have employed intradermal skin testing as a means of evaluating the hosts immune status and fungal infections. Their contraversial results demonstrated the pitfalls associated with this procedure. In a review of this subject, Ahmed and Blose (1983) pointed out the tremendous variation in methods of interpretation of test results, quality of test antigens including degradation of test material due to heat or light exposure, and variation in the patient tested, especially in hypersensitive individuals. They also noted a temporal correlation between clearing of the infection and test application. DH response was noted to decrease with time.

OTHER CELL MEDIATED IMMUNITY MEASUREMENTS

Lymphocyte blastogenic transformation (LT) is thought to be an in vitro correlate of intradermal skin testing. Hanifin et al. (1974) compared skin testing results to

those of LT in patients with dermatophyte infections. They found that positive lymphocyte responses correlated with the presence of delayed, but not immediate, cutaneous responses and that sera from chronically infected patients failed to inhibit LT. The studies of Green and Balish (1979) contradicted these results. They noted suppression of LT when the lymphocytes were cultured with autologous serum. Mobacken and Lindholm (1974) found that suppression of LT in patients with dermatophytosis and candidiasis was specific for these fungi as response to other test antigens was normal. Svejaard et al. (1976) did not observe any differences in LT between patients suffering from chronic or acute tineas. Experimental dermatophyte infections in guinea pigs showed a temporal correlation of LT, skin test responses, and erythema with all three parameters demonstrating maximum activity at 10 days (Kerbs et al., 1977). A similar correlation between severity of lesions and degree of LT stimulation was noted in infected humans (Stahl and Svejgaard, 1982). Hay and Shennan (1982), found that patients infected with T. rubrum had reduced LT activity as compared to control subjects and that the anatomic site of the tinea affected this immune response. Lower levels of LT activity were seen in patients with palmo-plantar infection than those with crural lesions.

Hunjan and Cronholm (1979), using a guinea pig model, experimentally infected these animals with three species of dermatophytes. They showed significantly increased LT

activity and macrophage migration inhibition (MMI) when the lymphocytes of these animals were exposed to homologous antigen. In a subsequent study, Hunjan *et al.* (1981) found that the skin test reactions (DH) were common to the three pathogens used and did not distinguish between the different species. They did find, however, a statistical difference in the *in vitro* leukocyte migration indices of sensitized cells to the homologous and heterologous antigens of these dermatophytes. The leukocyte migration inhibition assay was shown to provide a very specific expression of cell mediated immunity.

It would seem that the *in vitro* assessment of immune response to dermatophyte infection demonstrates the same contradictory results among investigators that were noted with *in vivo* skin testing. A major part of this discrepancy is due to the variation in composition of test antigens used.

The enzyme complex of the dermatophytes has been studied by many researchers. Weary and Canby (1967) studied the *in vitro* keratinolytic ability of three species that include *T. rubrum*, *T. schoenleinii*, and *T. mentagrophytes*. They found significant activity with the first two species but virtually none with the latter. In a subsequent study, Weary and Canby (1969) found that this enzyme complex had the ability to cross membranes and suggested the movement of this soluble hyphal product across the dermal-epidermal barrier allowing contact with

the immune system of the body. In an exhaustive review of the work reported by various investigators to 1974, Grappel et al. delineated the purified antigenic components as glycopeptides, polysaccharides, and keratinases. They noted that glycopeptide antigens were able to elicit both immediate and delayed hypersensitivity in guinea pigs. The carbohydrate moieties were related to the former and the peptide moieties to the latter. The polysaccharide antigens were separated into three groups identified as galactomannas I and II, with glucan as the third group. Their studies showed that the galactomannan I was the most antigenically active of these polysaccharides. The keratinases (peptide moieties) were divided into extracellular keratinase I and cell-bound keratinase II and keratinase III. Extracellular keratinase I was found to be the most active in eliciting DH. In an attempt to improve the quality of antigen extracted from dermatophytes, Ottaviano et al. (1974) tested organism growth in both a defined and a complex medium. The antigens produced from their extractions reportedly gave no false positive reactions while commercially prepared trichophytin gave 25% false positive or negative reactions.

Hellgren and Vincent (1976) studied the antigenic properties of the fatty acids occurring in the lipid fraction of the dermatophytes. They found that the middle-chain fatty acids (C10-C12) showed a high allergenicity and gave rise to DH reactions when

introduced into guinea pigs. They suggested that these fatty acids acted as contact allergens and might be partially responsible for the inflammatory skin reactions seen in tineas.

Christiansen and Svejgaard (1976) studied the antigenic structure of four species of dermatophytes using crossed immunoelectrophoresis. They noted at least 25 distinct antigens with each organism and two antigens common to T. rubrum and each of the other species. Their findings indicated an amazingly complex immunochemical structure for these fungi. Similar results were noted by DeMontelos and Guinet (1982) with quantitative crossed immunoelectrophoresis.

Moser and Pollack (1978) isolated glycopeptides with skin test activity from T. mentagrophytes, T. rubrum, and M. canis. These antigens were extracted by an ethylene glycol method using submerged cultures. The isolated glycopeptides were divided into two chemical groups: glucopeptides and mannopeptides. When these antigens were introduced into immunized guinea pigs, it was found that only the mannopeptides were capable of eliciting DH responses.

Kaaman and Wasserman (1981) studied cross-reactive, cell mediated immune response both in vivo and in vitro to purified dermatophyte antigen preparations. The antigens were recovered from three different fungal species and were tested in sensitized guinea pigs. Their results showed a cross-reactivity between the different antigens

although homologous antigen gave the strongest response. This was noted in both the *in vivo* skin testing and *in vitro* LT studies.

Takiuchi *et al*. (1982) isolated an extracellular proteinase (keratinase) from *M. canis*. Disc electrophoresis of this enzyme showed a single protein band and the activity of this enzyme was completely neutralized by antibody. This keratinase had the ability to actively degrade hair, and it was suggested that it might play a pathogenic role in tinea capitis by both mechanical destruction and by initiating a hypersensitive reaction in the host. In a subsequent study, Takiuchi *et al*. (1984) partially characterized this keratinase and indicated that it was probably a serine proteinase. Hattori *et al*. (1984) isolated a similar enzyme from *Candida albicans*. Hintner *et al*. (1985) found that keratin intermediate filaments from human tissues were degraded by serine proteinases and that the enzymatic degradation products could function as effective immunogens causing the formation of high titer antibodies.

Ashai *et al*. (1982) purified an antigen from *T. mentagrophytes* mycelia by picric acid precipitation. Chemical analysis determined this product to be of a peptide nature suggesting that the protein fraction carried the antigenic activity. Disc electrophoresis demonstrated a considerable heterogeneity in its molecular size but the activity was the same throughout. When tested with peritoneal exudate cells from sensitized

guinea pigs, an inhibitory effect was noted on the cell migration of macrophages.

Holden et al. (1981) developed a method for identification of dermatophyte antigens "in situ" by immunoperoxidase staining. The hyphal cell wall was shown to be the major antigenic determinant. Ultrastructurally, the location of this antigenicity appeared as a continuous granular deposition on the inner and outer aspects of the mycelial wall. San-Blas (1982) demonstrated that the hyphal walls of dermatophytes are composed of three layers; an amorphous galactomannan sandwiched between two layers of fibrils, the outer one made of beta-glucan and the inner one of chitin. The author concluded that galactomannans were primarily responsible for the immunogenic response to hyphal invasion.

HUMORAL IMMUNITY

Many authors have reported the appearance of serum antibodies in response to dermatophyte invasion. Grappel et al. (1971) were able to recover antibody from the sera of patients infected with T. schoeleinii by means of charcoal agglutination and immunodiffusion. In later work, Grappel et al. (1972) found that these antibodies persisted in the sera of patients only during early infection. Svejgaard and Christiansen (1979) using crossed immunoelectrophoresis demonstrated antibody in only 9.5% of patient sera. Interestingly, these subjects demonstrated both extremes of tinea infection ranging from

kerion celsi to noninflammatory chronic dermatophytosis. They also noted the disappearance, with time, of serum antibody in acute infections and the persistence of antibody in chronic infections. Svejgaard et al. (1984) in subsequent studies recovered IgE and IgG antibodies in some patients infected with T. rubrum. Attapattu and Clayton (1982) noted that anatomic site, extent of body involvement, nature, and duration of the infection, all played a part in determining the presence of detectable circulating antibodies whereas age, race, and sex seemed to have no influence. Precipitating antibodies appeared early in the course of infection and complement fixing antibodies later on. Sohnle et al. (1983) also noted that age did not seem to play a role in the production of dermatophyte antibody. Kaaman et al. (1981) by means of enzyme linked immunosorbent assay (ELISA) analysis showed a significantly higher IgG response to trichophytin in tinea patients than in a noninfected control group of humans. IgM antibody levels were the same in both groups. Honbo et al. (1984), using both polysaccharide (SAC) and peptide (PEP) dermatophyte antigens, evaluated the sera from chronically infected patients, noninfected adults, and noninfected children. They found all sera reactive to both antigens with Ig levels only slightly higher in the infected group. IgG and IgA were detected by both SAC and PEP while IgE was detected by SAC alone.

Hopfer et al. (1975) found low titers of antidermatophyte antibodies with affinity for host

epithelial tissue in the sera of 80% of patients with chronic dermatophytosis. These antibodies were identified as IgM immunoglobulins and were thought to be produced in response to particulate hyphal antigens. This unusual affinity of tinea antibodies for host epithelial tissue might partially explain the short duration of antibodies in the circulation during fungal infection. Nielson (1984), while studying subjects with chronic infections, noted that those who possess tissue antigens which cross-react with dermatophytes seem to have an immunologic tolerance which may facilitate ongoing fungal infection. Montes and Wilborn (1985), in reviewing the fungus-host relationship in candidiasis, found the formation of multilayers of antigen-antibody complexes on the yeast cell wall where it was speculated they might protect the fungus against the aggressive action of the host. Gotz et al. (1978) assayed infected subjects for IgA, IgG, IgM, and IgE. Although unable to detect elevations in IgG, IgA, and IgM, a significant increase in IgE was identified in some patients with dermatophytosis.

The role that antibody response plays in dermatophyte infections leaves much room for debate. Immunoglobulin assisted destruction of hyphae is questionable and elevated levels of IgE has been associated with persistence of this disease.

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CELL MEDIATED IMMUNITY

The other branch of the immune response is cell mediated immunity (CMI) and is suggested by many investigators to be the main avenue by which fungal disease is eliminated. Graybill and Mitchell (1979) performed *in vivo* studies using a mouse model and *Cryptoccus neoformans* and suggested that CMI played a major role in host defense against the yeast. Rasmussen and Ahmed (1978) skin tested children with tinea capitis caused by *T. tonsurans* to trichophytin and recorded their cutaneous responses. They found that those children with the noninflammatory scalp infections were unable to demonstrate DH reactions. The subjects presenting with an inflammatory (acute) process gave a DH reaction of greater than 10mm. DH response is an *in vivo* means of measuring CMI. Those patients able to elicit this response were also able to eliminate their infection, thus indicating the importance of CMI in host defense. Cox *et al.* (1982) evaluated CMI in patients with coccidioidomycosis. They noted hyperproduction of IgE associated with depression of CMI in subjects with active disseminated disease and suggested a defect in T-lymphocyte population which regulates antibody synthesis.

Buckley (1976) studied the function and measurement of human B and T-lymphocytes in the immune response. She noted that B-lymphocyte differentiation into antibody secreting plasma cells was affected by the activities of T-lymphocytes. B-cells were associated with humoral

response and T-cells with CMI. Evans and Lazarus (1978) defined two functionally distinct subpopulations of human T-cells that collaborated in the generation of cytotoxic cells responsible for cell mediated lympholysis.

SERUM FACTORS

Walters et al. (1974, 1976) investigating diminished CMI response in patients with chronic dermatophyte infections found diminished leukocyte activity when patients' cells were cultured in vitro with autologous sera. They proposed the existence of a serum blocking factor. Yu and Grappel (1972) were able to inhibit the keratinase activity of T. mentagrophytes with sera from both infected and noninfected subjects. Immunoelectrophoretic analyses of sera indicated that this inhibitor resided in the alpha-globulin fraction and was finally identified as alpha-2-macroglobulin. King et al. (1975) identified this serum dermatophyte inhibitory component as unsaturated transferrin. It was suggested that this factor inhibited fungal growth by binding iron which the mycelia needed for growth. Shiraishi and Arai (1979) demonstrated that transferrin's inhibitory effect was nonspecific and that its activity directly related to the unsaturated iron binding capacity of this serum component. Artis and Jones (1980) studied the effect of human lymphokine on the in vitro growth of T. mentagrophytes. They suggested that although lymphokine active against mammalian cells was not directly

antagonistic to the growth of this dermatophyte, the possibility still existed that activated lymphocytes could release an iron chelator such as transferrin that could inhibit fungal growth. Artis et al. (1983), in a subsequent study, found that the fungistatic activity of transferrin was dependent on both the quantity of fungus and the particular species.

Allen et al. (1977) described a case of generalized infection caused by M. audouinii. The patient also had depressed CMI response associated with a missing plasma factor required for lymphocyte blastogenesis. They were not able to characterize this factor and noted that the patients' transferrin levels were within normal limits. The disease process was finally eliminated by the use of an antifungal agent (Amphotericin B) and plasma infusions from normal donors. In another case of widespread dermatophytosis, Sherwin et al. (1979) noted the presence of an undefined immunosuppressive serum factor. They found that this factor specifically suppressed patient T-cell function and that this function was restored after antifungal therapy. Their hypothesis was that this widespread fungal infection was not the result of primary immunosuppression but rather that the dermatophytosis, once established, was the source of an immunosuppressive effect on the host. Cohen (1985) noted that fungi are capable of manufacturing immunosuppressive products. Cyclosporine was given as an example. Nelson et al. (1984) suggested that the loss of CMI in chronic

mucocutaneous candidiasis might be related in part to a plasma inhibitory factor released from the cell wall of the pathogen. This cell wall component was found to be mannan polysaccharide.

Swan et al. (1983) evaluated the role of serum complement in dermatophytoses. Their studies indicated that T. rubrum could activate the complement cascade by the alternate pathway. The generation of anaphylatoxins, chemotaxins, and opsonins would be important in the resulting inflammatory reaction and ultimate host defense against the dermatophyte. They suggested that dermatophyte antigen might also trigger the complement cascade via the classical pathway if high enough titers of antifungal complement-fixing antibodies were present in patient sera.

T-LYMPHOCYTE SUBPOPULATIONS

Reinherz and Schlossman (1980) noted that human T-cell subpopulations had been defined on the basis of differential expression of receptors for the Fc portion of immunoglobulins and other specific cell-surface antigens. In their study, they utilized antibodies to characterize the subpopulations of T-gamma (IgG) and T-mu (IgM) Fc receptor-bearing cells, as well as OKT4 and OKT5 monoclonal antibodies to identify lymphocytes bearing those receptors.. Their results showed that the T-mu cells contained both inducer (OKT4) and cytotoxic/suppressor (OKT5) populations whereas the

T-gamma contained few T-lymphocytes. They concluded that there was little correlation between T-cell subsets defined by monoclonal antibodies and those defined by Fc receptors.

Monoclonal antibodies were used by other investigators in immunomorphological studies with skin lesions and quantitative analysis of peripheral blood lymphocytes (Schmitt and Thivolet, 1982; Faure and Thivolet, 1982). It was found that subjects with atopic dermatitis had a decrease in total peripheral blood T-cells (OKT3) and an abnormal ratio of helper/suppressor (OKT4/OKT8) cells. This was attributed to a decrease in suppressor cell (OKT8) level. (Many patients with chronic dermatophyte infections are atopic). Baran (1984) noted that proper immune function results from a balance between T-helper and T-suppressor subsets. An increase in helper T-cells may result in an autoimmune state while an increase in suppressor cells may signal an immunodeficient state.

Stobo et al. (1976) reviewed the effect of suppressor thymus-derived lymphocytes in fungal infection. They noted that patients with localized or disseminated fungal infections manifest deficiencies in T-cell reactivity although they did not determine whether the defect preceded or was secondary to the infection. Watson and Collins (1979) demonstrated a similar phenomena in mice infected with Mycobacterium. DH response was seen 14 days after innoculation but was later followed by a persisting

anergy. Their study indicated that this anergy was mediated by a population of suppressor T-cells. Gupta et al. (1979) studied the mobility of T-cells in patients with chronic mucocutaneous candidiasis. They found that patients with impaired lymphocyte migration had abnormally low helper/suppressor cell ratios.

Petrini and Kaaman (1981) studied T-lymphocyte subpopulations in patients with chronic dermatophytosis. They noted a relationship between severity of disease and decreased proportion of T-mu cells with concomitant increase of T-gamma cells. They then labeled patient cells with monoclonal antibodies for T-helper and T-suppressor receptors and found only one case where this ratio was abnormal as compared to controls.

Brahmi et al. (1980) reviewed both T and B- cell numbers in patients with chronic dermatophytosis. Their results showed normal B-cell levels but reduced T-cell numbers in peripheral blood. IgE levels were significantly elevated in most of their patients.

Fedotov (1982) developed clinico-immunological groupings of patients with T. rubrum infections by enumerating their T and B-cells, immunoglobulins, and skin test reactions to specific antigen. In a subsequent paper, Fedotov et al. (1982) noted a statistically significant decrease of T and B- lymphocytes which continued during the course of the patient infection. In a third paper, Fedotov (1984) associated the intensity of papular and vesicular reactions to trichophytin skin

testing and the quantitative and qualitative characteristics of patient lymphocytes as well as duration of infection.

Modlin et al. (1985) examined T-lymphocyte subpopulations in patients with leprosy. They found that those subjects with nonreactional lepromatous leprosy (anergic) had decreased numbers of total T-cells but normal helper-suppressor T-cell ratios, while individuals with reactive erythema nodosum leprosum had more cells of the helper-inducer T-cell phenotype and fewer of the suppressor-cytotoxic T-cell phenotype.

Balogh et al. (1981) studied serum IgE levels and T-cell counts in cases of chronic dermatophytosis. Their results showed an inverse correlation between serum IgE levels and the total number of peripheral T-cells. Patients with chronic infections showed significantly lower levels of T-cells and marked elevation of IgE. Hay et al. (1983) noted the same inverse correlation in patients with chronic tinea imbricata.

Jones (1980) reviewed the clinical and laboratory aspects of the relationship between atopy and chronic dermatophytosis. He proposed that IgE, the mast cell, and histamine acted in a localized fashion within the connective tissue of the skin to inhibit T-helper cell function and the inflammatory response to the pathogen. In a subsequent article, Jones (1983) associated widespread chronic tinea infections to mild IgE elevations. This class of antibody was noted to be

specific for the invading dermatophyte as patients showed normal immune mechanisms to other mycotic, bacterial and viral antigens. Most patients demonstrated IH responses to trichophytin but their DH responses were absent.

Henney et al. (1972) used the C57 BL mouse to study the role of cyclic 3′,5′ adenosine monophosphate (CAMP) in the specific cytolytic activity of lymphocytes. They noted a direct correlation between increased intracellular levels of CAMP and the decrease of cytolytic activity of a lymphocyte population. Rocklin (1976) also found that increased levels of CAMP decreased lymphocyte production of migration inhibitory factor (MIF). In subsequent work, Rocklin (1977) found that histamine added to in vitro cultures of sensitized lymphocytes suppressed their antigen-induced production of MIF. He suggested the production of a histamine-induced suppressor factor (HSF) by a population of lymphocytes bearing H2 receptors. In further work, addressing the mechanism of action of histamine on lymphocytes, Rocklin et al. (1978) found that this agent did not interfere with antigen binding by macrophages or macrophage presentation of antigen to the lymphocytes. They proposed the existence of two types of T-suppressor cells responsive to histamine, one whose function is inhibited and another whose function is augmented. By this means, histamine could enhance antibody production as well as suppress CMI. Rocklin et al. (1979) proposed a model to explain the regulatory role of histamine as part of a negative feedback mechanism for

CMI. In this model, histamine released from tissue mast cells or basophils by an IgE antibody trigger, would dampen immune response by decreasing the production or release of MIF from lymphocytes.

Reinherz and Schlossman (1980) in studying the regulation of the immune response noted the importance of balance between the T-helper cells and T-suppressor cells. T-helper cells provide the inducer function in T-cell to T-cell, T-cell to B-cell, and T-cell to macrophage interactions. T-suppressor cells negate this activation and may play a role in the anergy seen in some fungal infections. Sohnle and Collins-Lech (1978) found significantly less MIF produced by lymphocytes from patients with pityriasis versicolor when stimulated by specific fungal antigen.

Beer and Rocklin (1984) proposed a model in which histamine, in the presence of monocytes or interleukin-I, activated T-suppressor cells bearing H2 receptors. These activated cells produce HSF which is capable of stimulating mononuclear phagocytes to synthesize prostaglandins that ultimately suppress T-inducer cell proliferation and lymphokine production.

The sources of immunosuppressive histamine are mast cells and basophils as mentioned previously. Graham et al. (1964) demonstrated that mast cells were present in great numbers in histopathology sections of biopsys taken from patients with tinea capitis due to T. tonsurans. Nabel et al. (1981) provided evidence that T-inducer

cells stimulate production of mast cells and suggested that mast cell-T cell interactions comprise yet another part of the immunoregulatory circuit. Clamann (1985) described an increased number of mast cells in graft-versus-host disease and suggested a similar regulatory relationship between T-inducer, mast cell, histamine, and T-suppressor cells.

Poulain et al. (1980), while studying the resistance to infection by T. mentagrophytes, noted the early influx of basophils to the upper dermis close to the basal membrane of the epidermis. Green et al. (1980) found that basophils comprised about one- third of the leukocytes infiltrating tinea lesions at 24-48 hours. They noted the rapid degranulation of these cells which resulted in histamine release. Espersen et al. (1984) found that peptidoglycans of Staphylococcus aureus were able to induce histamine release from human basophils by means of a nonimmunological mechanism. As previously mentioned, hyphae of the dermatophytes also have a peptidoglycan component.

Another major cell type involved in this complex immune response is the macrophage. The function of this cell type varies with the anatomic site from which it was derived (Black et al., 1985; Schaffner et al., 1983). Braathen and Thorsby (1983) found the human epidermal Langerhans cell (LC) to be more potent than blood monocytes in inducing antigen-specific T-cell responses. They speculated that the LC might participate as an

antigen-presenting cell or release interleukin I or both. In a separate study, Braathen and Kaaman (1983) demonstrated that LC's induce CMI to trichophytin in dermatophytosis. This is significant because the dermatophyte hyphae which normally grow only in the upper epidermis have no recognized means of exposure to the immune system to stimulate immune response. They proposed that the mobile LC's found in the epidermis transport and present dermatophyte antigens to the T-lymphocyte constituting the afferent phase in the cutaneous cellular immune response in dermatophytosis.

Transport of dermatophyte antigens by the LC's to the epidermal basal membrane could also result in activation of complement as some C3 is found in this area. The resulting influx of polymorphonuclear leukocytes (PMN's) could be associated with the acute response seen in some dermatophyte infections. PMN's play an important role in host defense against fungi (Cohen et al., 1981). Wright and Nelson (1985) demonstrated the candicidal activity of myeloperoxidase, the primary enzyme produced by PMN's. The secondary effect of endogenous pyrogen and release of lactoferrin by neutrophils resulted in hyperthermia which is found to be deleterious in many fungal diseases (Mackowiak, 1981).

Davies and Zaini (1984a) in studies on PMN chemotaxis induced by T. rubrum, noted that PMN infiltration of chronic dermatophyte infections is rare. They suggested that high enzymatic activity associated with keratin

dissolution, especially in tinea capitis, might result in degradation of NCF. Inhibition of PMN chemotaxis has also been associated with griseofulvin (Shulman et al., 1982). This drug is the therapeutic mainstay in juvenile tinea capitis.

Increased epidermal activity in acute dermatophytosis was demonstrated by Berk et al. (1976). These authors found that invasion of the stratum corneum by dermatophytes led to an eczematous reaction in the epidermis. The resulting abrogation of the dermal-epidermal barrier could allow the influx of inflammatory cells and serum factors which would affect the invading hyphae. The increased rate of epidermal turnover would allow a more rapid shedding of the pathogen. Sohnle et al. (1976) noted the same increase in keratinization in C. albicans infections. They noted the rapid accumulation of PMN's and lymphocytes in the upper dermis and suggested that the release of lymphokines from the later cells caused an increase in mitosis of epidermal cells.

Chronic, noninflammatory dermatophyte infections can be resistant to antifungal therapy. Jones (1982) suggested that this resistance might be associated with immune unresponsiveness in the patient. One means to counter this anergy, would be with the administration of an H-2 antagonist such as cimetidine (Jorizzo et al., 1980). The cimetidine would bind to the H-2 receptor of the T-suppressor lymphocyte blocking its stimulation by

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histamine. Ultimately T-inducer function would not be diminished and CMI response reinstituted. Presser and Blank (1981) were able to effect a cure in a girl who had a two year history of tinea capitis due to T. tonsurans. The patient was treated with 300 mg. of cimetidine four times a day and 1.5 gm of griseofulvin daily. The process cleared entirely after two months of therapy. In vivo, resistance to griseofulvin alone has been studied by other authors (Hay, 1979; Hay and Brostoff, 1977); Jones, 1982; Robertson et al., 1982).

Topical antidermatophyte agents more recently evaluated included undecylenic acid (Landau, 1983), undecanoic acid (Das and Banerjee, 1983), and selenium sulfide (Allen et al., 1982). None of these agents demonstrated a major effect. Oral ketoconazle was found to be effective in some cutaneous tineas (Cox et al., 1982a; Stratigos et al., 1983), and was found effective in a comprised patient with invasive T. rubrum infection (Baker and Para, 1984). One patient with subcutaneous mycetomas caused by M. audouinii was treated with griseofulvin, amphotericin B, and ketoconazole before resolution of his infection (West, 1982). Zaslow and Derbes (1969) suggested the use of immunosuppressants in the treatment of highly inflammatory tinea such as kerion celsi. Vaccination against dermatophytosis for the most part has met with little success (Lewis and Hopper, 1937; Hussin and Smith, 1983).

## I. CLINICAL STUDIES

An epidemiological review of all patients with juvenile tinea capitis presenting at Henry Ford Hospital, Detroit, Michigan, was performed from 1983 to 1985. Thirty patients from this group were selected for additional studies. Ten noninfected juvenile controls of normal health were evaluated along with the patients investigated.

### A. MATERIALS AND EQUIPMENT

1. Woods light
2. Microscope slides, 25 mm x 75 mm (Am. Sci. Prod. #M6162)
3. Cover glass, 22 x 22 mm (Am. Sci. Prod. #M 6045-2)
4. #15 scalpel blades (Bard-Parker #H8294-001115)
5. 100 mm Mycosel Fungal Plates (BBL #21178)
6. Chlorazol fungal stain (Derm Lab Supply)
7. Light microscope with 10x, 45x, 97x objectives (AO Spencer, Model 10)

### B. PROCEDURE

Juvenile patients with suspected tinea capitis were evaluated in a sequential manner as they appeared in clinic. These subjects ranged in age from 1-14 years old.

1. Wood's Light Examination

   A wood's light examination is performed to determine the presence or absence of

fluorescent hairs. Utilizing a darkened room, the ultraviolet light from a Wood's lamp (3650 angstroms) is directed towards the areas of scalp alopecia. A positive fluorescence is determined by the appearance of bright, blue-green, broken hairs. Certain dermatophyte species are able to induce this pathology.

2. Microscopic Examination

   a. Patient specimens are obtained by scraping infected areas with a sterile scalpel blade (Onsberg, 1979).

   b. This material is placed on a clean glass microscope slide.

   c. A few drops of chlorazol solution is added to the slide.

   d. A cover glass is then placed on top.

   e. This slide preparation is then gently heated for two seconds.

   f. The slide is then examined with a light microscope using the 10x objective.

   g. The presence of hyphae and/or arthroconidia in or on the hair fragments or epithelial cells is considered positive.

3. Pathogen Isolation

   a. Patient specimens are obtained as noted under 2a.

   b. This material is innoculated onto Mycosel agar plates medium in plates.

   c. These plates are incubated at 25 degrees C (DeVries, 1971).

   d. Each culture is examined twice each week for the appearance of fungal growth.

e. All plates are held for four weeks before being discarded and recorded as negative.

4. Patient Data

a. Patient information including age, sex, and race is obtained on all subjects.

b. These data are compared to each respective dermatophyte isolated.

c. Analysis of this information is incorporated into tables.

d. A subgroup of the above population are selected for additional studies. All members of this later group demonstrate endothrix tinea capitis by means of microscopic examination. In each case, the pathogen recovered is Trichophyton tonsurans.

e. A more detailed history form (Appendix 1) is completed on each of these subjects and a patient consent form (Appendix 2) obtained. Each patient is identified by a sequential study number to protect their privacy.

II. MYCOLOGICAL STUDIES

Identification of the fungal pathogens isolated are performed on each culture by means of macroscopic and microscopic morphologic evaluation. Urease production and hair performation testing are performed on cultures from patients in the additional study group.

A. MATERIALS AND EQUIPMENT

1. Light microscope with 10x, 45x, and 97x objectives (AO Spencer, Model 10)

2. Microscope slides, 25 mm x 75 mm (Am. Sci. Prod. #M6162)

3. Cover glass, 22 x 22 mm (Am. Sci. Prod. #M6045-2)

4. Highland transparent tape (3M Co. #5910)

5. Autoclaved juvenile hair clippings

6. Christensen's urea agar medium in tubes (Difco #0283-34-8)

7. 100 x 15 mm sterile disposable petri dishes (Falcon #1029)

B. REAGENTS

1. Sterile water for irrigation USP (McGraw #R5000)

2. Yeast extract (BBL #11929)

3. Lactophenol cotton blue soln (LPCB). (Derm Lab, Inc.)

C. WORKING REAGENTS

Yeast extract solution.

1. Dissolve 10.0 gm of yeast extract in 90.0 ml distilled water.

2. Sterilize by filtration.

3. Date and store at 4-8 degrees C.

D. PROCEDURES

1. Pathogen Identification

a. Macroscopic morphology
Gross colony features are observed including surface topography, texture and pigment as well as colony reverse pigmentation.

b. Microscopic morphology

1. Place a drop of LPCB on a clean glass slide.

2. Touch the adhesive side of a 1" piece of tape to the colony surface.

3. Stretch the tape specimen over the LPCB drop.

4. Place a cover glass on top of this preparation.

5. The slide is then examined with a light microscope using the 45x objective.

6. The morphology of the hyphae and conidia are noted.

2. Urea Hydrolysis Test
(Beneke and Rogers, 1980)

a. Christensen's urea agar medium in tubes is innoculated with material from primary isolation plates by means of a sterile innoculating needle.

b. These tubes are incubated at 25 degrees C and examined daily for urease activity (color change).

c. Tubes are held for four weeks before being discarded and recorded as negative.

d. The positive test results are recorded relative to incubation time. (days required before a reaction is observed)

e. T. rubrum is innoculated as a negative control and T. mentagrophytes as a positive control.

3. Hair Perforation Test
(McGinnis, 1980)

a. 15-20 sterile hairs are placed in a sterile 100 mm petri dish.

b. 25 ml of sterile distilled water and 0.1 ml of sterile yeast extract solution are then added to the dish.

c. A small amount of the colony growth from the primary isolation plate is then transferred to the dish by means of a sterile innoculating needle.

d. The test dish is incubated at 25 degrees C and examined weekly for four weeks before being discarded and recorded as negative.

e. Hairs are removed from the culture plate and placed in a drop of LPCB on a clean glass slide.

f. A cover glass is added to this preparation.

g. The slide is examined by light microscopy using the 10x objective.

h. A test is considered positive if wedge-shaped erosions are noted in the hair fragments.

i. T. rubrum is tested as a negative and T. mentagrophytes as a positive control.

III. IMMUNOLOGICAL STUDIES

CELL SEPARATION PROCEDURES FOR LYMPHOCYTE STUDIES

PRINCIPLE

A method for isolating mononuclear cells from peripheral blood was described by Boyum (1968). Mixtures of polysaccharide and radiopaque contrast medium were used in these procedures. Blood specimens were layered on this medium and centrifuged. Ficoll sedimented erythrocytes and granulocytes leaving the lymphocytes at the plasma-hypaque interface (Fig. 1). This resulted in a 70-90% yield of mononuclear cells with a high degree of purity for lymphocytes. Small subpopulations of lymphocytes could, however, still be lost. This is a modification of Boyum's original procedure.

**FIGURE 1. Ficoll-hypaque separation of lymphocytes before and after centrifugation.**

I. BEFORE CENTRIFUGATION

a. Heparinized whole blood diluted 1:1 with saline

b. Ficoll-hypaque solution

II. AFTER CENTRIFUGATION

c. Plasma-saline dilution

d. Lymphocyte interface

e. Hypaque solution

f. Red blood cells and granulocytes aggregated by Ficoll

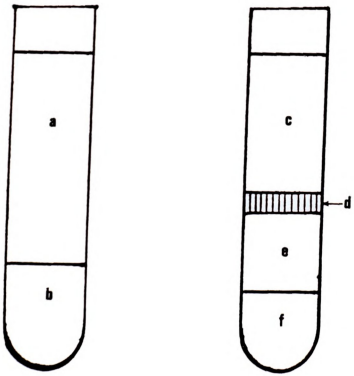


FIGURE 1.

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## BUFFY COAT SEPARATION

### A. SPECIMEN COLLECTION

Peripheral blood is collected from antecubital veins by means of venipuncture in a 10 cc sodium heparin tube (venoject green stopper #T-200SKA) from subjects in the additional test group. Samples should be processed as soon as possible but, when necessary, may be held overnight but must be kept at room temperature.

### B. MATERIALS AND EQUIPMENT

1. Discard can.
2. Pasteur pipettes, 5 3/4" (Am. Sci. Prod. #5211-1).
3. 15 ml sterile screw cap centrifuge tubes (Corning #25310).
4. Sterile disposable pipettes (1 ml, 5 ml, 10 ml). (Corning #7077-10N).
5. Disposable 1 ml microtubes (Fisher #04-978-145).
6. Rubber bulbs for pasteur pipettes.
7. International Clinical centrifuge (ICC) (Model 8452C).
8. Fisher Micro-centrifuge (FMC) (Model 59A).

### C. REAGENTS

1. Hanks' balanced salt soln (Hanks BSS) 10X w/Ca++. Mg++, phenol red, w/o sodium bicarbonate (Gibco #310-4060, 100 ml/bottle). Stock soln used to prepare 1x Hanks' BSS.
2. 7.5% sodium bicarbonate soln (Gibco #670-5080, 100 ml/bottle).

3. 1 M Hepes buffer (pH 7.3). (Gibco #380-5630, 100 ml/bottle).

4. Penicillin-streptomycin soln (penicillin-10,000 units/ml-streptomycin-10,000 mcg/ml). (Gibco #600-5145, 20 ml/bottle).

5. Ficoll Paque, sterile, sp. grav. 1.077 + or - 0.002 (Pharmacia Co.) NOTE: Ficoll Paque is light sensitive. DO NOT LEAVE EXPOSED TO LIGHT FOR MORE THAN 10 MINUTES. USE AT ROOM TEMPERATURE.

6. Sterile water for irrigation USP (American McGaw #NDC 0264-2101-00).

7. 0.9% Sodium chloride irrigation USP (American McGaw #NDC 0264-2201-00).

8. Dulbecco phosphate buffered saline (PBS) 10X without Ca++ and Mg++ (Gibco 310-4200).

D. <u>WORKING REAGENTS</u>

1. Hanks' BSS, 1X (PREPARE ASEPTICALLY)

   a. Dilute 100 ml stock 10X Hanks' BSS w/886.4 ml sterile water.

   b. Add 4.6 ml sterile sodium bicarbonate.

   c. Add 10 ml 1M Hepes buffer (pH 7.3).

   d. Add 20 ml pencillin-streptomycin solution.

   e. Aliquot into sterile 250 ml bottles. Store at 4-8 degrees C.

2. Dulbecco PBS 1X without Ca++ and Mg++ (PREPARE ASEPTICALLY).

   a. Dilute 100 ml Dulbecco PBS 10X with 900 ml sterile water.

   b. Adjust solution to pH 7.2 to 7.4 with 1N NaOH.

   c. Label and date working reagent.

d. Store at 4-8 degrees C.

E. CONTROLS

Blood from normal, healthy individuals are evaluated in parallel with the patient samples.

F. PROCEDURE

1. Dilute heparinized blood 1:1 with 1X Dulbecco PBS.
2. Place 4 ml of Ficoll (RT) into a 15 ml centrifuge tube.
3. Carefully layer 8 ml of diluted blood on top of Ficoll. (wet side of tube w/Ficoll first for easier layering).
4. Centrifuge layered tube at 400 g for 35 min. (ICC).
5. To five 1 ml microcentrifuge tubes add 3/4 ml Hanks' BSS.
6. To these add interface layer from Ficoll tube to vol. of 1 ml
7. Mix gently with pasteur pipette.
8. Centrifuge microcentrifuge tubes at 1500 RPM x 1 min. (FMC).
9. Discard supernatant and concentrate pellets from 5 tubes to 2.
10. Add Hanks' BSS to the 2 tubes to make 1 ml.
11. Mix gently with pasteur pipette.
12. Centrifuge tubes at 1500 RPM x 1 min. and discard supernatant.
13. Resuspend pellets with Hanks' BSS to 1 ml.
14. Mix gently with pasteur pipette.
15. Centrifuge tubes at 1500 RPM x 1 min. and discard supernatant.

16. Pool cell suspensions from 2 tubes into 1 and dilute to 1 ml with Hanks' BSS.

## LABELING OF LYMPHOCYTE SUBPOPULATIONS BY THE INDIRECT METHOD (Baran, 1984)

### PRINCIPLE

Human lymphocytes can be separated into subsets on the basis of maturation and/or biological function. A number of subpopulations have been defined by the presence of specific antigenic determinants on the cell surface. This labeling procedure is based on the ability of a monoclonal antibody to bind to the surface of viable cells which express the unique antigenic determinant. In this indirect method, purified lymphocytes are first coated with monoclonal antibody (MCA) and then tagged with a fluorescein-isothiocyanate (FITC) labeled goat anti-mouse immunoglobulin. The lymphocyte subpopulations identified by their respective MCA's in this study included: T-helper (T4), T-suppressor (T8), total T-cell (T11), total B-cell (B1), total B-cell and activated T-cell (IA) populations.

A. PATIENT SAMPLE

A purified suspension of 6 million lymphocytes/ml in Hanks' BSS is used for each immune profile panel of monoclonal antibodies.

B. MATERIALS AND EQUIPMENT

1. AO Spencer, double line hemocytometer and cover glass (Am. Sci. Prod. #B3180).

2. Adjustable micropipettes, 5-20 µl, 20-200 µl (Fisher #21-185).

3. Disposable 5-200 µl micropipette tips (Fisher #21-244-1).

4. Microcentrifuge tubes, 1 ml (Fisher #04-978-145).

5. Pasteur pipettes, 5 3/4" (Am. Sci. Prod. #5211-1).

6. Microscope slides, 25 mm x 75 mm (Am. Sci. Prod. #M6162).

7. Rubber bulbs for pasteur pipettes.

8. Discard can.

9. Ice bath.

10. Fisher microcentrifuge Model #59A.

C. REAGENTS

1. Hanks' BSS 10x (Gibco #310-4060).

2. Dulbecco PBS 10x without Ca++ and Mg++ (Gibco #310-4200).

3. Bovine albumin soln, 30% w/v, sterile. (Am. Sci. Prod. #4840-6).

4. Sterile water for irrigation, osmolality 0.00. (McGraw #R5000).

5. Monoclonal antibodies (MCA)

   a. T4 (Coulter #660239)
   b. T8 (Coulter #6602310)
   c. T11 (Coulter #6602308)
   d. B1 (Coulter #6602311)
   e. MsIgG (Coulter #6602394)
   f. Ia (New England Nuclear #NEI-011)

6. GAM-FITC, conjugated goat anti-mouse IgG antibody, heavy and light chain specific. (Coulter #6602159).

7. Tago-FITC, conjugated goat anti-mouse IgG and IgM antibodies gamma mu light chain specific. (Tago Inc. #6253)

8. Trypan blue (0.5% in saline). (Allied chem. #508) STOCK SOLN USED TO PREPARE DILUTING FLUID FOR WHITE CELL COUNT.

9. Paraformaldehyde (Fisher #T-353).

10. 0.9% Sodium chloride (McGraw #R5200).

11. 7.5% Sodium bicarbonate soln (Gibco #670-5080).

12. 1M Hepes buffer (pH 7.3). (Gibco #380-5630).

13. Penicillin-streptomycin soln (Gibco #600-5145).

D. WORKING REAGENTS

1. White cell count diluting fluid

   a. Place 900 µl 0.9% sodium chloride in a microtube.

   b. Add 100 µl 0.5% trypan blue reagent to this tube and mix.

   c. Prepare a fresh dilution each test day.

2. Hanks' BSS 1x
   (prepare as described under cell separation procedures).

3. Dulbecco PBS 1x
   (prepare as described under cell separation procedures).

4. PBS with 2% bovine albumin

   a. Add 6.7 ml, 30% bovine albumin soln to 93.3 ml Dulbecco PBS 1x.

   b. Date and store at 4-8 degrees C.

5. 1% Paraformaldehyde soln

   a. Add 1 gm paraformaldehyde to 100 ml of PBS 1x.

   b. Date and store at 4-8 degrees C.

6. Coulter (MCA)

   a. Add 500 µl of sterile, distilled water T4, T8, T11, B1, and MsIgG stock vials.

   b. Date and store at 4-8 degrees C.

7. Coulter GAM-FITC
   (Prepare as described in step 6)

8. Ia MCA

   a. Add Ia stock vial to 25 ml PBS 1x.

   b. Date and store at 4-8 degrees C.

E. CONTROLS

Coulter MsIgG, normal mouse immunoglobulin specific for non-human tissue is added to patient cells as a negative control. Cells from normal healthy individuals are evaluated in parallel with patient samples as a positive control.

F. PROCEDURE

1. Cell Dilution.

   a. Add 40 µl of Hanks' BSS 1x, 40 µl of Trypan blue working dilution and 20 µl of 1 ml separated cell suspension in a microcentrifuge tube and mix.

   b. Pipette 10 µl of this suspension into each hemocytometer chamber.

   c. Count one large square (16 small squares) in each chamber and add total number of cells.

   d. Multiply this number x 50,000 and divide by 2.

   e. This result is the number of viable cells/ml.

f. Adjust this suspension to 6 million cells/ml.

g. If greater than 5% of cells are stained by trypan blue (nonviable) discard specimen.

2. Primary Labeling

a. Mark 6 microcentrifuge tubes with each MCA and negative control to be used.

b. Add 190 µl of suspension containing one million cells to T4, T8, T11, B1, and MsIgG tubes.

c. Add 100 µl of suspension containing one million cells to Ia tube.

d. Add 10 µl of T4, T8, T11, B1 and MsIgG MCA's to each corresponding tube.

e. Add 100 µl of Ia MCA to Ia tube.

f. Mix and incubate tubes in ice bath for 30 min.

g. Dilute each tube to 1 ml with cold Hanks' BSS 1x and mix gently.

h. Centrifuge tubes at 1500 RPM x 1 min. in microcentrifuge.

i. Discard supernatant.

j. Repeat steps g, h, and i two more times.

3. Secondary Labeling

a. Resuspend pellets in each tube with 195 µl of PBS 1x.

b. Add 5 µl of GAM-FITC to T4, T8, T11, Ia, and MsIgG tubes.

c. Add 5 µl of Tago-FITC to B1 tube.

ANALYSI

PRINCIP

d. Mix and incubate tubes in ice bath for 30 minutes.

e. Dilute each tube to 1 ml with cold Dulbecco PBS with 2% bovine albumin and mix gently.

f. Centrifuge tubes at 1500 RPM x 1 min. in microcentrifuge.

g. Discard supernatant.

h. Repeat steps e, f, and g two more times.

i. Resuspend pellet in 0.5 ml of 1% parformaldehyde.

j. Cap tubes and store at 4-8 degrees C.

ANALYSIS OF LYMPHOCYTE SUBPOPULATIONS
(Goldstein et al., 1982)

PRINCIPLE

Flourescently labeled cells suspended in an electrically conductive medium produce an electrical resistance pulse, the amplitude of which is proportional to the cell volume when passed through a detection area of a flow cytometer. Optical signals are produced as the cells intercept focused light from a laser and emit a fluorescent signal. Multiple detectors allow the simultaneous measurement of forward angle light scatter, a parameter of size, and 90 degrees scatter, a parameter of cellular granularity or cell surface structure density. These signals are converted into electronic data which is then analyzed by the computer and a numerical value generated.

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A. MATERIALS AND EQUIPMENT

1. Epics V flow cytometry system (Coulter Corp.).
2. EASY analysis computer (Coulter Corp.)
3. Dysan 8" floppy disketts (Dysan #800528)
4. 0.5 ml of labeled cells (1 million cells/ml) in 1% paraformaldehyde.

B. PROCEDURE

1. Filtered cell suspensions are processed by the flow cytometer after standardization of equipment.
2. Cell populations to be studied are gated.
3. Ten thousand cells are counted.
4. Data generated are analyzed by the computer and a numerical value calculated.

C. CONTROLS

Positive and negative controls are run with the patient panel.

STATISTICAL METHODS

The results generated from the flow cytometry analyses gave T4/T8 ratios for each person in the control group, inflammatory group, and noninflammatory group. The mean responses of the three groups were compared using the "analysis of variance (ANOVA)." For the ANOVA procedure to be appropriate, the variances of each group are assumed to be equal (Neter and Wasserman, 1974).

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EPIDEMIOLOGY OF TINEA CAPITIS

Juvenile patients with suspected tinea capitis were evaluated in a sequential manner as they appeared in clinic. These subjects ranged in age from 1-14 years old. Scalp lesions were examined by means of Wood's light, microscopic examination of infected tissue, and culture methods. Patient data with respect to race and sex were also obtained. This three year study was performed between January 2, 1982, and December 31, 1984.

During this time period, 331 children demonstrated culture proven tinea capitis. T. tonsurans was the pathogen recovered in 290 (88%) of these cases and M. canis in 38 (11%). A three-fold increase in M. canis was noted between 1983 and 1984 (Table 4).

When these data were reviewed with respect to sex distribution among juvenile patients, it was found that females were infected more frequently than males by a ratio 2.6:1 (Table 5). This ratio remained fairly constant during the three year review period when all species of dermatophytes were considered.

The sex distribution of tinea capitis among juvenile patients caused by T. tonsurans gave a consistent female-to-male ratio of 3.3:1 (Table 6). When the second most frequently recovered pathogen, M. canis, was reviewed, this ratio showed an inverse change with time (Table 7). In 1982, the female-to-male ratio

TABLE 4. Dermatophytes recovered from juvenile patients with tinea capitis.

TABLE 4. Dermatophytes recovered from juvenile patients with tinea capitis.

| YEAR | *T. tonsurans* | | *M. canis* | | Other | | Total | |
|---|---|---|---|---|---|---|---|---|
| | #[a] | %[b] | # | % | # | % | # | % |
| 1982 | 94 | (90) | 8 | (8) | 2 | (2) | 104 | (100) |
| 1983 | 88 | (91) | 8 | (8) | 1 | (1) | 97 | (100) |
| 1984 | 108 | (83) | 22 | (17) | 0 | (0) | 130 | (100) |
| Total | 290 | (88) | 38 | (11) | 3 | (1) | 331 | (100) |

[a]number of positive cultures

[b]percent of total cultures

*TABLE 5.* *Sex distribution among all juvenile patients with tinea capitis.*

TABLE 5. Sex distribution among all juvenile patients with tinea capitis.

| YEAR | Female | | Male | | Total | | F/M Ratio[c] |
|---|---|---|---|---|---|---|---|
| | #[a] | %[b] | # | % | # | % | |
| 1982 | 77 | (74) | 27 | (26) | 104 | (100) | 2.9:1 |
| 1983 | 72 | (74) | 25 | (26) | 97 | (100) | 2.9:1 |
| 1984 | 90 | (69) | 40 | (31) | 130 | (100) | 2.3:1 |
| Total | 239 | (72) | 92 | (28) | 331 | (100) | 2.6:1 |

[a]number of patients with tinea capitis

[b]percent of patients with tinea capitis

[c]female to male ratio

TABLE 6. Sex distribution of juvenile patients with tinea capitis due to T. tonsurans.

TABLE 6. Sex distribution of juvenile patients with tinea capitis due to T. tonsurans.

| YEAR | Female | | Male | | Total | | F/M Ratio[c] |
|---|---|---|---|---|---|---|---|
| | #[a] | %[b] | # | % | # | % | |
| 1982 | 72 | (77) | 22 | (23) | 94 | (100) | 3.3:1 |
| 1983 | 68 | (77) | 20 | (23) | 88 | (100) | 3.4:1 |
| 1984 | 83 | (77) | 25 | (23) | 108 | (100) | 3.3:1 |
| Total | 223 | (77) | 67 | (23) | 290 | (100) | 3.3:1 |

[a]number of patients with tinea capitis

[b]percent of patients with tinea capitis

[c]female to male ratio

Table 7. Sex distribution of juvenile patients with tinea capitis due to *M. canis*.

Table 7. Sex distribution of juvenile patients with tinea capitis due to M. canis.

| YEAR | Female | | Male | | Total | | F/M Ratio[c] |
|---|---|---|---|---|---|---|---|
| | #[a] | %[b] | # | % | # | % | |
| 1982 | 5 | (62) | 3 | (38) | 8 | (100) | 1.6:1 |
| 1983 | 4 | (50) | 4 | (50) | 8 | (100) | 1:1 |
| 1984 | 7 | (32) | 15 | (68) | 22 | (100) | 1:2 |
| Total | 16 | (42) | 22 | (58) | 38 | (100) | 1:1.4 |

[a]number of patients with tinea capitis

[b]percent of patients with tinea capitis

[c]female to male ratio

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was 1.6:1, but by 1984 this ratio had shifted to 1:2. This was due to a five-fold increase of scalp infections caused by M. canis in juvenile males. A summary of all data with regard to pathogen and sex distribution is listed in Table 8. These data show that 93% of all female tinea capitis was caused by T. tonsurans as compared to 75% in males.

The racial distribution of juvenile patients with tinea capitis occurring during this period was also reviewed. Because of the large number of black children diagnosed as having tinea capitis and the relatively small number of infected juveniles in other racial populations, the latter were simply grouped together and identified as "non-blacks". Ninety-five percent of all tinea capitis identified in this study occurred in black children (Table 9). These data may be biased and may simply reflect geographic location; however, they are in agreement with the findings of Prevost (1979) and Sinski and Flouras (1984).

Comparison of the incidence of sex and race with respect to scalp infections caused by T. tonsurans and M. canis can be found in Table 10. Tinea capitis in black females was caused by T. tonsurans in 95% of cases, while scalp infections due to this dermatophyte were not observed in non-black males.

*TABLE 8. Distribution of T. tonsurans and M. canis by patient sex.*

TABLE 8. Distribution of T. tonsurans and M. canis by patient sex.

| | Female | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|
| YEAR | T. tonsurans | | M. canis | | T. tonsurans | | M. canis | |
| | #[a] | %[b] | # | % | # | % | # | % |
| 1982 | 72 | (94) | 5 | (6) | 22 | (88) | 3 | (12) |
| 1983 | 68 | (94) | 4 | (6) | 20 | (83) | 4 | (17) |
| 1984 | 83 | (92) | 7 | (8) | 25 | (63) | 15 | (37) |
| Total | 223 | (93) | 16 | (7) | 67 | (75) | 22 | (25) |

[a]number of patients of this sex infected by this dermatophyte

[b]percentage of patients of this sex infected by this dermatophyte

TABLE 9. Distribution of juvenile patients with tinea capitis by race.

TABLE 9. Distribution of juvenile patients with tinea capitis by race.

| YEAR | Blacks | | Non-Blacks | | Total | |
|---|---|---|---|---|---|---|
| | #[a] | %[b] | # | % | # | % |
| 1982 | 99 | (95) | 5 | (5) | 104 | (100) |
| 1983 | 95 | (98) | 2 | (2) | 97 | (100) |
| 1984 | 120 | (92) | 10 | (8) | 130 | (100) |
| Total | 314 | (95) | 17 | (5) | 331 | (100) |

[a]number of patients with tinea capitis

[b]percent of patients with tinea capitis

TABLE 10. Distribution of juvenile patients by sex and race with tinea capitis caused by T. tonsurans and M. canis.

| YEAR | Black Females | | | | Black Males | | | | Non-black Females | | | | Non-black Males | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T. tons.[c] | | M. canis | | T. tons. | | M. canis | | T. tons. | | M. canis | | T. tons | | M. canis | |
| | #[a] | %[b] | # | % | # | % | # | % | # | % | # | % | # | % | # | % |
| 1982 | 69 | (93) | 5 | (7) | 22 | (92) | 2 | (8) | 3 | (100) | 0 | (0) | 0 | (0) | 1 | (100) |
| 1983 | 68 | (96) | 3 | (4) | 20 | (87) | 3 | (13) | 0 | (0) | 1 | (100) | 0 | (0) | 1 | (100) |
| 1984 | 82 | (95) | 4 | (5) | 25 | (74) | 9 | (26) | 1 | (25) | 3 | (75) | 0 | (0) | 6 | (100) |
| Total | 219 | (95) | 12 | (5) | 67 | (83) | 14 | (17) | 4 | (50) | 4 | (50) | 0 | (0) | 8 | (100) |

[a]number of patients with tinea capitis due to this dermatophyte

[b]percent of patients with tinea capitis due to this dermatophyte

[c]T. tonsurans

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EVALUATION OF CLINICAL FORMS OF TINEA CAPITIS

In a subsequent study, 30 juvenile patients with ringworm of the scalp caused by T. tonsurans were evaluated. These children were of normal health in all other respects as determined by history and physical examination. Patients with a history of atopy or any other concurrent disease were excluded. Patients receiving systemic antimycotic therapy and those on corticosteriod or other immunomodulating therapies at the time of initial visit were also excluded.

Patients were evaluated with respect to their clinical presentation of disease and placed into one of two groups based on the following criteria. Group one was identified as "inflammatory" with the members demonstrating alopecia, scaling, erythema and pustules. In some extreme cases, a boggy kerion with a purulent exudate was also observed (Figures 2,3).

Group two was identified as "noninflammatory" and was composed of those patients demonstrating diffuse or well demarkated alopecia and scaling without the presence of erythema, edema, or pustules (Figure 4). All patients showed endothrix hair invasion on microscopic examination of infected tissue (Figure 5) and their cultured specimens grew species and varieties of T. tonsurans.

FIGURE 2. Inflammatory tinea capitis with erythema and multiple pustules.

FIGURE 3. Inflammatory tinea capitis with edema and erythema and areas of boggy kerion formation.

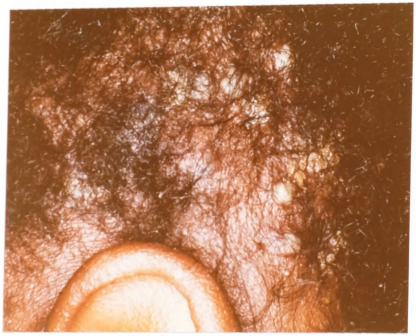

FIGURE 2

FIGURE 3

FIGURE 4. Noninflammatory tinea capitis with widespread alopecia.

FIGURE 5. Microscope slide preparation demonstrating endothrix hair invasion (450X).

a. Arthroconidia within the hair shaft

b. Infected hair fragment

c. Epithelial cells

FIGURE 4

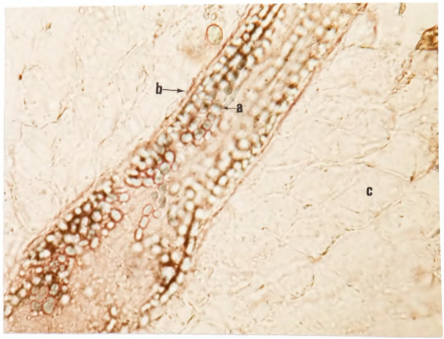


FIGURE 5

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## UREASE TESTING OF CLINICAL ISOLATES OF *T. TONSURANS*

The fungal pathogen isolated from each patient was tested for appearance of urease activity relative to time. These results are listed in Table 11 for the inflammatory group and Table 12 for the noninflammatory group. All cultures of *T. tonsurans* demonstrated a positive urease test which is in agreement with the findings of McGinnis (1980). Fungal isolates recovered from patients with inflammatory infections required an average of 2.1 days incubation before showing a positive reaction versus 2.2 days in the noninflammatory group. These response times are suprisingly rapid when compared with the results of McGinnis. Only 50% of his isolates were positive in one week, with 21 days of incubation being required before all of his test strains showed urease activity. The positive controls were positive in three days while the negative controls were still negative at seven days.

## IN VITRO HAIR PERFORATION TESTING OF CLINICAL ISOLATES OF *T. TONSURANS*

The same fungal isolates were tested for their ability to perforate hair *in vitro* by means of the hair perforation test (McGinnis, 1980). These results are listed in Tables 13 and 14. Eight of the ten strains (80%) of *T. tonsurans* recovered from patients in the inflammatory group were hair perforation positive versus 15 of 20 (75%) in the noninflammatory group. The mean

TABLE 1

| Pati |
|---|
| P-1 |
| P-5 |
| P- |
| P- |
| P- |
| P- |
| P- |
| P- |
| P- |
| P |

Posi
at

aspec

binc

TABLE 11. Urease activity of isolates of T. tonsurans recovered from patients with inflammatory tinea capitis.

| Patient | T. tonsurans var.[a] | Days to (+)[b] |
|---|---|---|
| P-1 | sulfureum | 3 |
| P-5 | sulfureum | 3 |
| P-14 | sulfureum | 1 |
| P-15 | sulfureum | 2 |
| P-18 | sulfureum | 2 |
| P-19 | sulfureum | 2 |
| P-21 | sulfureum | 3 |
| P-23 | sulfureum | 2 |
| P-26 | sulfureum | 1 |
| P-29 | sulfureum | 2 |
| | Average days | 2.1 |

Positive control (+) in 3 days, negative control still (-) at 7 days.

[a]species variety of T. tonsurans

[b]incubation time in days to produce a positive reaction

TABLE 12

| Patien |
|---|
| P-2 |
| P-3 |
| P-4 |
| P-6 |
| P-7 |
| P-8 |
| P-9 |
| P-10 |
| P-11 |
| P-12 |
| P-13 |
| P-16 |
| P-17 |
| P-20 |
| P-21 |
| P-24 |
| P-2 |
| P-2 |
| P-2 |
| P-3 |

Positi
at 7 d

[a]speci

[b]incub

TABLE 12. Urease activity of isolates of T. tonsurans recovered from patients with noninflammatory tinea capitis.

| Patient | T. tonsurans var.[a] | Days to (+)[b] |
|---|---|---|
| P-2 | sulfureum | 3 |
| P-3 | sulfureum | 2 |
| P-4 | sulfureum | 3 |
| P-6 | sulfureum | 3 |
| P-7 | sulfureum | 2 |
| P-8 | sulfureum | 3 |
| P-9 | sulfureum | 3 |
| P-10 | sulfureum | 1 |
| P-11 | sulfureum | 2 |
| P-12 | red | 1 |
| P-13 | red | 4 |
| P-16 | sulfureum | 1 |
| P-17 | sulfureum | 1 |
| P-20 | sulfureum | 1 |
| P-22 | sulfureum | 3 |
| P-24 | sulfureum | 2 |
| P-25 | sulfureum | 2 |
| P-27 | red | 2 |
| P-28 | sulfureum | 2 |
| P-30 | red | 3 |
| | Average days | 2.2 |

Positive control (+) in 3 days, negative control still (-) at 7 days.

[a]species variety of T. tonsurans

[b]incubation time in days to produce a positive reaction

TABLE 13.

| Patient |
|---|
| P-1 |
| P-5 |
| P-14 |
| P-15 |
| P-18 |
| P-19 |
| P-21 |
| P-23 |
| P-26 |
| P-29 |

The pos
4 weeks
[a]species
[b]time of

**TABLE 13.** *In vitro* hair perforation ability of isolates of *T. tonsurans* recovered from patients with inflammatory tinea capitis.

| Patient | *T. tonsurans* var. [a] | Hair perf. | Days[b] |
|---|---|---|---|
| P-1 | sulfureum | + | 9 |
| P-5 | sulfureum | + | 16 |
| P-14 | sulfureum | - | |
| P-15 | sulfureum | + | 13 |
| P-18 | sulfureum | + | 16 |
| P-19 | sulfureum | - | |
| P-21 | sulfureum | + | 16 |
| P-23 | sulfureum | + | 16 |
| P-26 | sulfureum | + | 16 |
| P-29 | sulfureum | + | 16 |
| | Total (+) strains | 8 (80%) | 15 day average |

The positive control perforated hair within 4 weeks, negative control did not.

[a]species variety of *T. tonsurans*

[b]time of incubation required for a positive result

TABLE 14.

| Patient |
|---|
| P-2 |
| P-3 |
| P-4 |
| P-6 |
| P-7 |
| P-8 |
| P-9 |
| P-10 |
| P-11 |
| P-12 |
| P-13 |
| P-16 |
| P-17 |
| P-20 |
| P-22 |
| P-24 |
| P-25 |
| P-27 |
| P-28 |
| P-30 |

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[a]speci

[b]time

TABLE 14. *In vitro* hair perforation ability of isolates of *T. tonsurans* recovered from patients with noninflammatory tinea capitis.

| Patient | *T. tonsurans* var.[a] | Hair perf. | Days[b] |
|---|---|---|---|
| P-2 | sulfureum | + | 16 |
| P-3 | sulfureum | + | 16 |
| P-4 | sulfureum | + | 16 |
| P-6 | sulfureum | + | 13 |
| P-7 | sulfureum | + | 16 |
| P-8 | sulfureum | + | 16 |
| P-9 | sulfureum | + | 16 |
| P-10 | sulfureum | + | 16 |
| P-11 | sulfureum | + | 9 |
| P-12 | red | - | |
| P-13 | red | - | |
| P-16 | sulfureum | + | 16 |
| P-17 | sulfureum | + | 16 |
| P-20 | sulfureum | + | 16 |
| P-22 | sulfureum | - | |
| P-24 | sulfureum | + | 16 |
| P-25 | sulfureum | + | 16 |
| P-27 | red | - | |
| P-28 | sulfureum | + | 16 |
| P-30 | red | - | |
| | Total (+) strains | 15 (75%) | 15 day average |

The positive control perforated hair within 4 weeks, the negative control did not.

[a]species variety of *T. tonsurans*

[b]time of incubation required for a positive result

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incubation time required in both groups was 15 days. Positive controls perforated hair while negative controls did not.

All of the isolates from the inflammatory group and 16 of 20 isolates from the noninflammatory group demonstrated the colony morphology of T. tonsurans var. sulfureum. This gave a total of 26 isolates of this species variety from both groups. Of these 26 isolates, 23 isolates (89%) were hair perforation positive indicating that they were of the subvariety perforans.

LYMPHOCYTE SUBPOPULATION MEASUREMENT BY FLOW CYTOMETRY

Lymphocytes were isolated from the patients in both groups by means of ficoll-hypaque centrifugation of heparinized peripheral blood. The lymphocytes from ten healthy, non-infected children were used as controls. These cells were labeled with monoclonal antibodies and analyzed by flow cytometry. A histogram of the contour plot of two parameter analysis was used to indicate the distinct cell populations in each sample (Figure 6). The X-axis measures the forward angle light scatter (FALS2) which is an indication of cell size. The Y-axis measures 90 degree light scatter (LI90) which is an indication of cell granularity and surface structure density. Figure 7 demonstrates cross sections of the contour plot in the previous figure. The cell population to be counted is selected from this cross section by the placement of four intersecting lines called gates (Fig. 8). Only the cells

FIGURE 6. Contour plot of two parameter analysis. This plot demonstrates the separation of the granulocyte population (g) and lymphocyte population (l).

## TWO PARAMETER ANALYSIS

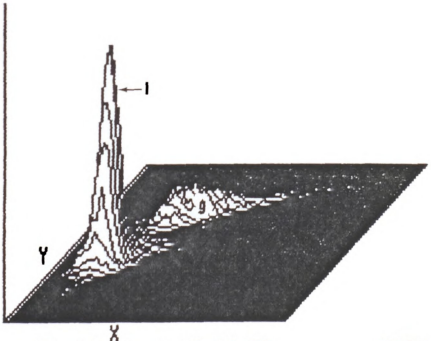



FIGURE 6.

FIGURE 7. Cross section of the contour plot of two parameter analysis. This plot demonstrates distinct cell populations consisting of granulocytes (g), monocytes (m), and lymphocytes (l).

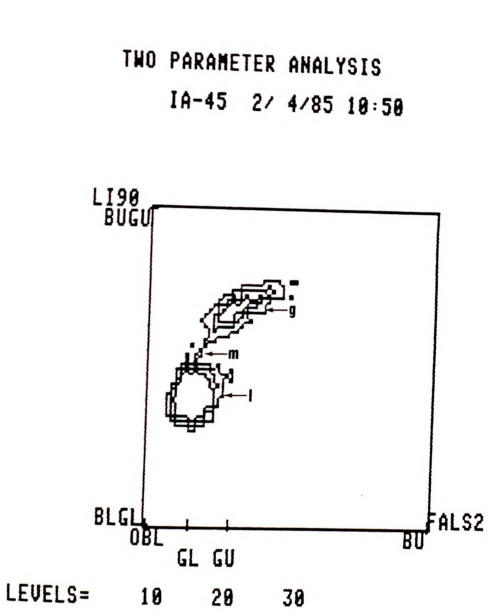

TWO PARAMETER ANALYSIS
IA-45 2/ 4/85 10:50
LI90
BUGU
g
m
l
BLGL
OBL
GL GU
BU
FALS2
LEVELS= 10 20 30

FIGURE 7.

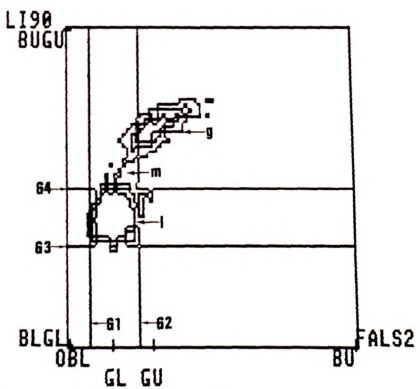
FIGURE 8. Cross section of the contour plot of two parameter analysis with gates. The forward angle light scatter (FALS 2) gates are set at channels 18 (G1) and 62 (G2). The ninety degree light scatter gates (L190) are set at channels 80 (G3) and 126 (G4). The population confined within these gates are lymphocytes (l) with monocytes (m) and granulocytes (g) being excluded.

LEVE

FALS
L190

TWO PARAMETER ANALYSIS

IA-45 2/ 4/85 10:50




LEVELS= 10 20 30

FALS2 18 62
LI90 80 126

FIGURE 8.

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within the gated square (lymphocytes) are measured for fluorescence. Each lymphocyte subpopulation within this square is individually measured for fluorescence. The single parameter histograms of T4 (helper), T8 (suppressor), T11 (pan-T), B1 (pan-B), IA (pan-B, macrophages and activated T-cells) and control cells are depicted in Figures 9, 10, 11, 12, 13, and 14 respectively. Two gates are placed on the single parameter histogram to select the population of fluorescent cells to be counted thus eliminating nonspecific fluorescence (Figure 15). Ten thousand cells in this gated population were measured for fluorescence and the positive cells computed. This number was then converted into percentage of total cells. The fluorescence of the same lymphocyte subpopulation from two different patients was compared in Figure 16 and overlayed in Figure 17.

COMPARISON OF T4/T8 RATIOS BETWEEN INFLAMMATORY, NONINFLAMMATORY, AND CONTROL GROUPS

The results of the flow cytometry analysis of lymphocytes from the inflammatory, noninflammatory, and control groups are listed in Tables 15, 16, and 17 respectively. The mean of the T4/T8 ratio in the inflammatory group was 2.76 compared to 1.715 in the noninflammatory group and 1.60 in the control group. These results indicate that the mean responses for the three groups are not equal with $p<0.001$. Pairwise comparisons indicate that the control group ($X=1.60$) is

FIGURE 9. Single parameter analysis of fluorescence of T4 subpopulation (a) and nonspecific fluorescence (n).

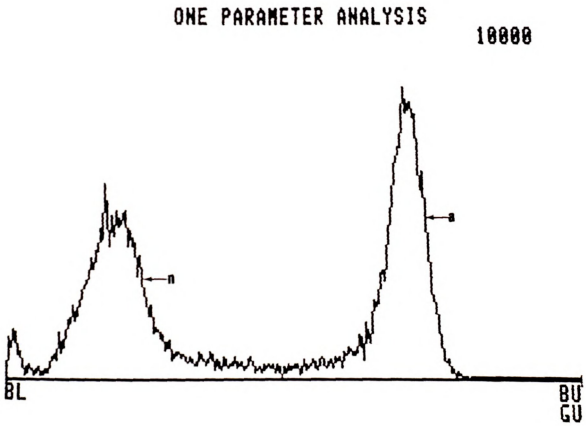
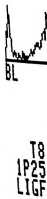

ONE PARAMETER ANALYSIS
10000
n
a
BL
BU
GU
T4
1P256
LIGFL
16/ 4/85 9:45
/FALS2,LI90

FIGURE 9.

FIGURE 10. Single parameter analysis of fluorescence of T8 subpopulation (a) and nonspecific fluorescence (n).



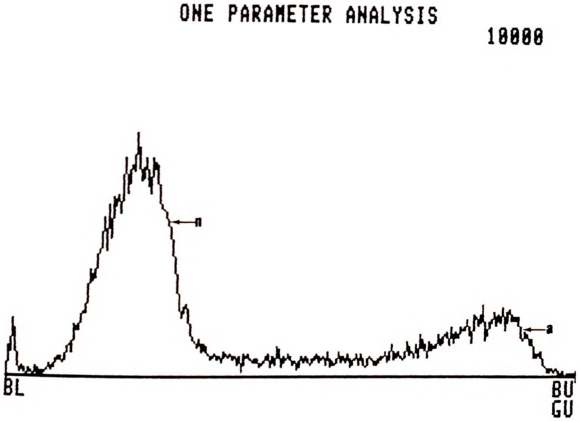

ONE PARAMETER ANALYSIS
10000
a
a
BL
BU
GU
T8
1P256
LIGFL
27/ 3/85 14:22
/FALS2,LI90

FIGURE 10.

FIGURE 11. Single parameter analysis of fluorescence of T11 subpopulation (a) and nonspecific fluorescence (n).

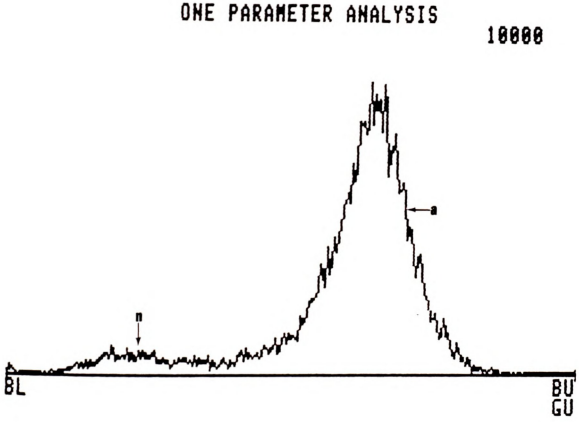

ONE PARAMETER ANALYSIS
10000
BL
BU
GU
T11
1P256
LIGFL
27/ 3/85 14:24
/FALS2,LI90

FIGURE 11.

FIGURE 12. Single parameter analysis of fluorescence of B1 subpopulation (a) and nonspecific fluorescence (n).

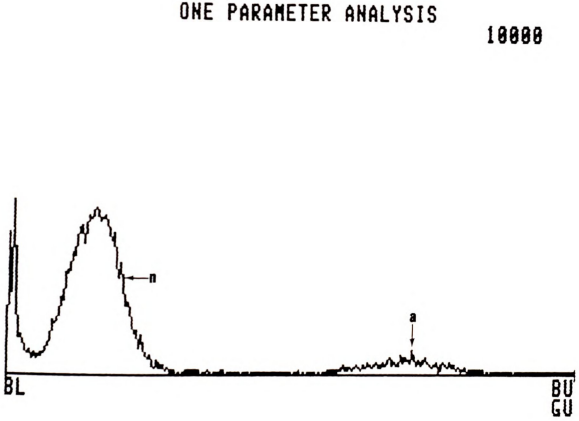

ONE PARAMETER ANALYSIS
10000
n
a
BL
BU
GU
B1
1P256
LIGFL
19/ 4/85 14:20
/FALS2,LI90

FIGURE 12.

FIGURE 13. Single parameter analysis of fluorescence of IA subpopulation (a) and nonspecific fluorescence (n).

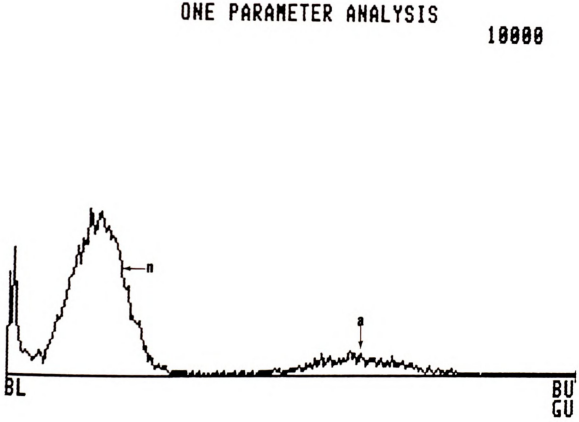

ONE PARAMETER ANALYSIS
10000
n
a
BL
BU
GU
IA
1P256
LIGFL
19/ 4/85 14:44
/FALS2,LI90

FIGURE 13.

FIGURE 14. Single parameter analysis of fluorescence of negative control (a) and nonspecific fluorescence (n).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LIG

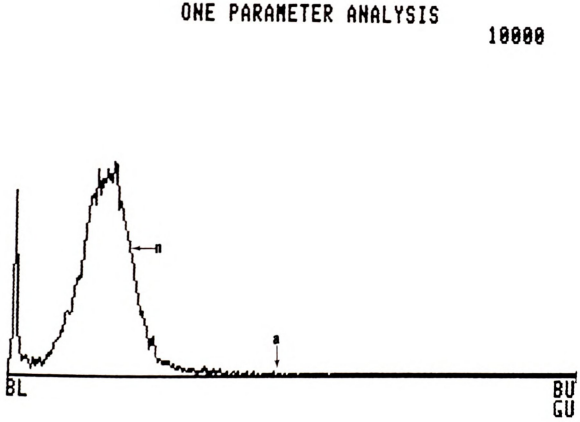
ONE PARAMETER ANALYSIS
10000
BL
BU
GU
CONTROL
1P256
LIGFL
27/ 3/85 14:36
/FALS2,LI90

FIGURE 14.

FIGURE 15. Single parameter analysis of selected cell population. The gates set at channels 96 (G1) and 255 (G2) demarcate the cell population (c) to be counted.


LIG

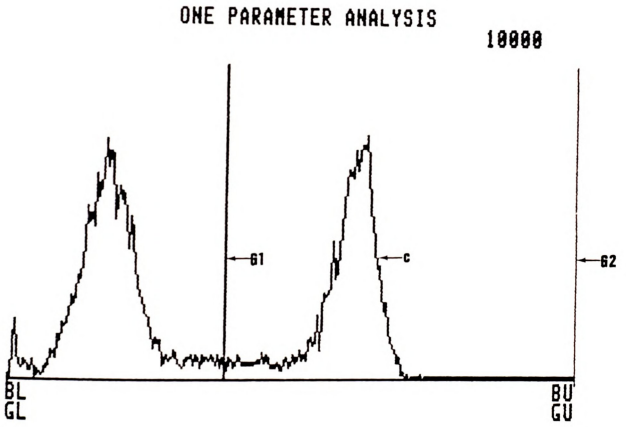
ONE PARAMETER ANALYSIS
10000
G1
C
G2
BL
GL
BU
GU
LIGFL /FALS2,LI90
CHANNEL 96 TO 255
INTGRL 4650

FIGURE 15.

FIGURE 16. Two-histogram comparison of the T8 populations of two different patients.

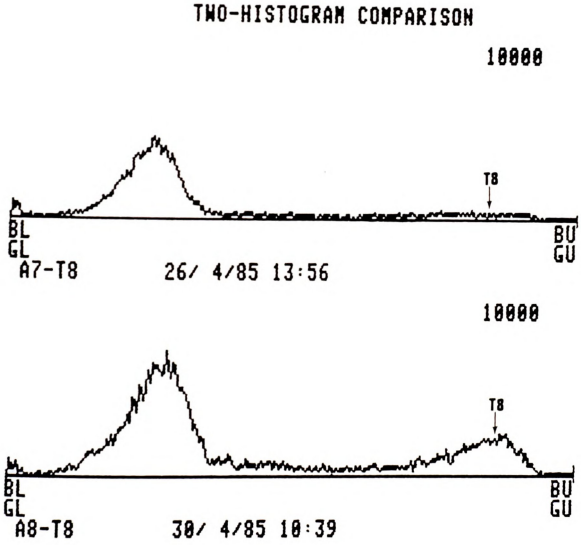
TWO-HISTOGRAM COMPARISON
10000
T8
BL
GL
BU
GU
A7-T8
26/ 4/85 13:56
10000
T8
BL
GL
BU
GU
A8-T8
30/ 4/85 10:39

FIGURE 16.

FIGURE 17. Overlay of histograms of two different patients comparing their T4 populations (a).

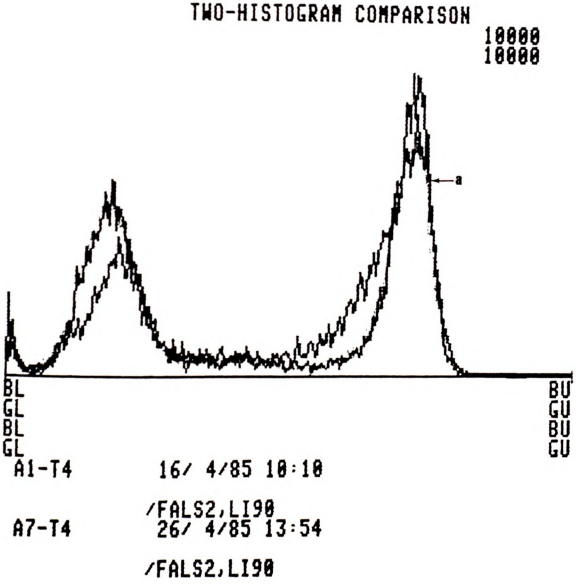
TWO-HISTOGRAM COMPARISON
10000
10000
a
BL
GL
BL
GL
BU
GU
BU
GU
A1-T4
16/ 4/85 10:10
/FALS2,LI90
A7-T4
26/ 4/85 13:54
/FALS2,LI90

FIGURE 17.

TABLE 15

| Patie |
|---|
| P-1 |
| P-5 |
| P-1 |
| P-1 |
| P-1 |
| P- |
| P- |
| P- |
| P- |
| P- |

[a]Mono
subp

[b]Rati

[c]T-h

[d]T-s

[e]Tot

[f]Tot

[g]To

[h]Ne

TABLE 15. Flow cytometry analysis of lymphocyte subpopulations from patients in the inflammatory group.

| | Monoclonal Antibody Panel[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| Patients | T4[c] | T8[d] | T11[e] | B1[f] | IA[g] | -C[h] | Ratio[b] |
| P-1 | 62 | 18 | 90 | | | 1 | 3.4 |
| P-5 | 58 | 24 | 86 | 6 | 9 | 2 | 2.4 |
| P-14 | 62 | 20 | 84 | 5 | 8 | 2 | 3.1 |
| P-15 | 62 | 19 | 82 | 4 | 8 | 2 | 3.3 |
| P-18 | 49 | 17 | 82 | 15 | 22 | 4 | 2.9 |
| P-19 | 49 | 22 | 89 | 10 | 13 | 3 | 2.2 |
| P-21 | 48 | 18 | 81 | 10 | 15 | 3 | 2.7 |
| P-23 | 48 | 21 | 81 | 5 | 10 | 1 | 2.3 |
| P-26 | 64 | 22 | 89 | 8 | 12 | 3 | 2.9 |
| P-29 | 55 | 21 | 86 | 8 | 13 | 3 | 2.6 |

X= 2.76

[a]Monoclonal antibodies used to label specific lymphocyte subpopulations; results in percent (%)

[b]Ratio of T4/T8 (helper/suppressor) lymphocyte subpopulations

[c]T-helper cell population

[d]T-suppressor cell population

[e]Total T-cell population

[f]Total B-cell population

[g]Total B-cell and activated T-cell populations

[h]Negative control

TABLE 16

| Patie |
| --- |
| P-2 |
| P-1 |
| P- |
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TABLE 16. Flow cytometry of lymphocyte subpopulations from patients in the noninflammatory group.

| | Monoclonal Antibody Panel[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| Patients | T4 | T8 | T11 | B1 | IA | -C | Ratio[b] |
| P-2 | 52 | 28 | 92 | 2 | 12 | 1 | 1.9 |
| P-3 | 57 | 33 | 89 | 4 | 9 | 2 | 1.7 |
| P-4 | 53 | 26 | 85 | 5 | 16 | 3 | 2.0 |
| P-6 | 37 | 29 | 90 | 4 | 8 | 2 | 1.3 |
| P-7 | 35 | 28 | 89 | 3 | 6 | 2 | 1.25 |
| P-8 | 51 | 24 | 84 | 8 | 13 | 3 | 2.1 |
| P-9 | 48 | 27 | 84 | 6 | 11 | 3 | 1.8 |
| P-10 | 52 | 29 | 88 | 9 | 12 | 3 | 1.8 |
| P-11 | 51 | 28 | 90 | 10 | | 4 | 1.8 |
| P-12 | 48 | 28 | 84 | 12 | 17 | 4 | 1.7 |
| P-13 | 50 | 27 | 86 | 11 | 17 | 4 | 1.85 |
| P-16 | 46 | 29 | 86 | 10 | 16 | 4 | 1.6 |
| P-17 | 51 | 32 | 80 | 11 | 2 | 4 | 1.6 |
| P-20 | 40 | 20 | 83 | 11 | 16 | 2 | 2.0 |
| P-22 | 44 | 20 | 82 | 9 | 18 | 1 | 2.2 |
| P-24 | 39 | 20 | 91 | 5 | 8 | 1 | 2.0 |
| P-25 | 47 | 29 | 84 | 11 | 15 | 2 | 1.6 |
| P-27 | 48 | 31 | 83 | 12 | 18 | 5 | 1.5 |
| P-28 | 45 | 28 | 85 | 9 | 15 | 4 | 1.7 |
| P-30 | 47 | 30 | 84 | 10 | 15 | 3 | 1.6 |

X = 1.715

[a]Monoclonal antibodies used to label specific lymphocyte subpopulations; results in percent (%)

[b]Ratio of T4/T8 (helper/suppressor) lymphocyte subpopulations

TABLE 17

| Cont |
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TABLE 17. Flow cytometry analysis of lymphocyte subpopulations from subjects in the control group.

| | Monoclonal Antibody Panel[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| Control | T4 | T8 | T11 | B1 | IA | -C | Ratio[b] |
| C-1 | 46 | 31 | 71 | 13 | 17 | 2 | 1.5 |
| C-2 | 38 | 34 | 81 | 5 | 9 | 1 | 1.1 |
| C-3 | 37 | 21 | 67 | 9 | 19 | 1 | 1.8 |
| C-4 | 41 | 35 | 81 | 9 | 19 | 7 | 1.2 |
| C-5 | 43 | 23 | 71 | 17 | 22 | 3 | 1.9 |
| C-6 | 51 | 27 | 87 | 8 | 12 | 1 | 1.9 |
| C-7 | 47 | 28 | 82 | 4 | 12 | 1 | 1.7 |
| C-8 | 45 | 32 | 80 | 7 | 11 | 2 | 1.4 |
| C-9 | 48 | 27 | 86 | 5 | 9 | 2 | 1.8 |
| C-10 | 46 | 29 | 83 | 10 | 14 | 2 | 1.6 |

X = 1.60

[a]Monoclonal antibodies used to label specific lymphocyte subpopulations; results in percent (%)

[b]Ratio of T4/T8 (helper/suppressor) lymphocyte subpopulations

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not significantly different from the noninflammatory group ($\bar{X}$=1.715) with $p>0.05$. All other pairwise comparisons are significant. In particular, the noninflammatory group ($\bar{X}$=1.715) has a significantly lower mean response than does the inflammatory group ($\bar{X}$=2.76) with $p<0.05$.

For this statistical analysis to be appropriate, the variances of each group are assumed to be equal. The observed variances in this study were inflammatory group 0.196, noninflammatory group 0.06, and control group 0.07, which are not significantly different with $p>0.07$.

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## DISCUSSION

Tinea capitis is a dermatophyte infection of the scalp and is seen most frequently in children. It can be caused by a number of fungal species in the genera Trichophyton and Microsporum. The form that the disease takes is associated with the specific invading organism and the ability of the host to deal with this pathogen. A complex interaction between host and parasite occurs and can result in the rapid elimination of the infective process or its continuance as a low grade chronic ringworm.

In the first part of this study, clinical data was collected from all juvenile cases of tinea capitis diagnosed in the Dermatology Clinic at Henry Ford Hospital, Detroit, Michigan, between January, 1982, and December, 1984. The incidence of patient, sex, and race, and dermatophyte species isolated was compiled. It was found that T. tonsurans was the most frequently recovered pathogen (88%) followed by M. canis (11%). These data are certainly different from those reported in the 1950's (Pipkin, 1952). At that time, M. audouinii was the primary pathogen isolated from ringworm of the scalp and was responsible for epidemic disease among school aged children. Only one case of tinea capitis caused by this fungus was diagnosed during the 1982-84 period. The reason for the virtual disappearance of one species and the dramatic increase of another is not well

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understood. The temporal change in primary pathogens coincides with the initial use of griseofulvin, a systemic antimycotic agent. Possibly differential drug sensitivity played a role in this evolution.

In this study, scalp infections caused by T. tonsurans were noted most frequently among black children (99%). These findings are in agreement with those of Prevost (1979) although she observed and even female-to-male distribution versus a 3:1 ratio in this study. MacDonald and Smith (1984) attributed this racial disparity to the crowded living conditions and poor hygiene associated with poverty in lower socioeconomic groups. Sehgal et al. (1985) noted a similar correlation in India. Another factor which may play a role in this racial unbalance is cultural. The scalps of black children can become dry and scaly. It was also found that mothers of black children routinely dress the scalps of their offspring with heavy oils such as vasoline petroleum jelly to eliminate the unsightly appearance. This application was especially common in young black females. The heavy oils might act as an adherent which would retain hyphal fragments and arthroconidia acquired from an anthropophilic or other environmental source. The prolonged contact with the scalp would allow more time for germination of the fungus and eventual invasion of the hair follicles.

Thirty juvenile patients with tinea capitis caused by T. tonsurans were selected for subsequent

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studies. The appearance of their infection was assessed for alopecia, scaling, pustule formation, erythema, lymphadenopathy and kerion formation. They were then assigned to either the inflammatory or noninflammatory groups based on these findings. Rasmussen and Ahmed (1978) reviewed pediatric cases of tinea capitis caused by T. tonsurans and found that 85% were of the noninflammatory type. They observed that some noninflammatory infections could evolve into the inflammatory type resulting in more rapid resolution. They did not note the reverse pattern, with inflammatory infection changing into the noninflammatory type. These developments were not observed among the thirty study patients possibly because antimycotic therapy was initiated at the time of their first visit, and they were then re-examined at four week intervals until resolution of their disease. It was noted, however, that those patients with inflammatory tinea capitis had a much shorter disease duration than children with noninflammatory disease.

T. tonsurans var. sulfureum was isolated from all patients in the inflammatory group (10) and in 16 out of 20 in the noninflammatory group. Twenty-three of these 26 isolates (89%) were hair perforation positive indicating that they were of the subvariety perforans. Matsumoto et al. (1983) found only 17% of the T. tonsurans var. sulfureum isolates that they tested were of the subvariety perforans. These studies suggest the

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possibility that this subvariety is more often associated with tinea capitis. Seventy-seven percent of the patients in this study grew isolates of this subvariety. Alternately, the incidence of this strain may simply be due to differential geographic distribution. No significant difference was found in fungal strains between the two study groups.

The fungal isolates were tested for urease activity. All strains were positive, requiring a mean incubation time of 2.1 days in the inflammatory group and 2.2 days in the noninflammatory group. McGinnis (1980) found that only 50% of the isolates he tested were positive by seven days. The rapid urease reaction time of patient isolates indicates a strong activity by this enzyme. Possibly greater enzymatic activity by these isolates contribute to their hair infection potential. However, other dermatophyte enzymes from these isolates would have to be evaluated. Rippon (1982) noted, for example, that only the strains of M. gypseum producing elastinase had the ability to invade hair. Takiuchi et al. (1984) found that M. canis produced a keratinase that they characterized as a serine proteinase that could separate the keratin fibrils of hair.

The role that the pathogen, T. tonsurans played in the host-parasite relationship with infected patients was considered in this study. Two factors were evaluated. The specific strain was determined by the hair performation test, and the urease test was used as one

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measure of enzymatic activity. Though limited, these results indicate that the strains isolated in this study from juvenile patients with tinea capitis are somewhat unique when compared to other isolates tested in previous studies. Their increased invasive potential is a considered possibility. No relation to severity of disease was noted with these studies.

The host's ability to deal with the fungal pathogen was investigated as a function of immune response. Peripheral blood was collected from patients in both clinical groups as well as from ten subjects in a control group. Lymphocytes were isolated from each blood specimen, labeled with five different monoclonal antibodies (MCA), and analyzed by flow cytometry for lymphocyte subpopulation composition (Reinherz et al., 1979). T-lymphocyte helper/suppressor ratios were calculated and their mean established for the inflammatory, noninflammatory, and control groups. Analysis of variance (ANOVA) was used to statistically compare the mean responses of the three groups. The mean helper/suppressor ratio of the control group ($\bar{X}=1.60$) was not significantly different from that of the noninflammtory group ($\bar{X}=1.715$) with $p>0.05$. The mean of the inflammatory group ($\bar{X}=2.76$) was significantly different from both the control and noninflammatory groups with $p<0.05$. These data indicate an increase of T-helper cells in the inflammatory group and a lack of proliferation of T-helper cells in the noninflammatory

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group. Comparison of patient helper/suppressor ratios to their clinical presentation of disease are noted in Tables 18 and 19. These results are in agreement with those of Petrini and Kaaman (1981) who performed lymphocyte subpopulation studies in patients with chronic noninflammatory T. rubrum infections. In their study, patient cells labeled with MCA's gave similar helper/suppressor ratios to those seen in control subjects. In a comparable study, Modlin et al. (1985) observed normal helper/suppressor ratios in patients with noninflammatory leprosy and greatly increased ratios in patients with reactive or inflammatory leprosy.

One possible sequence of events that might occur in the immune response of patients with inflammatory tinea capitis begins with the fungal invasion of the hair shaft. As the hyphae separate into arthroconidia within the hair, enzymes such as the serine esterases (Davies and Zaini, 1984b) or serine proteinases (Hinter et al., 1985) are released and begin to disassemble the keratin fibrils.

The soluble antigenic enzymes are then transported by Langerhan's cells (Braathen and Kaaman, 1983) through the surrounding epidermis to the dermis. The antigens then activate the C3 component of complement found in this tissue, triggering the complement cascade via the alternate pathway. C3a and C5a, products produced by this cascade, are neutrophil chemotactic factors which cause an influx of PMN's to the site of infection (Davies and Zaini, 1984a). The PMN's release their potent enzymes

TABLE 18

| Patient |
| --- |
| P-1 |
| P-5 |
| P-1 |
| P-1 |
| P-1 |
| P- |
| P- |
| P- |
| P- |

TABLE 18. Clinical symptoms and T4:T8 ratios of patients in the inflammatory group.

| Patient | Scaling | Alopecia | Pustules | Drainage | Kerion | Lymphadenopathy | ID Reaction | Infect. Sibs | Ratio |
|---|---|---|---|---|---|---|---|---|---|
| P-1 | + | + | + | - | - | + | + | - | 3.4 |
| P-5 | + | + | + | - | - | + | - | - | 2.4 |
| P-14 | + | + | + | + | - | + | + | - | 3.1 |
| P-15 | + | + | + | + | + | + | + | - | 3.3 |
| P-18 | + | + | + | + | + | + | - | + | 2.9 |
| P-19 | + | + | + | - | - | - | - | + | 2.2 |
| P-21 | + | + | + | + | + | + | + | - | 2.7 |
| P-23 | + | + | + | + | - | + | - | - | 2.3 |
| P-26 | + | + | + | + | + | + | + | - | 2.9 |
| P-29 | + | + | + | + | - | + | - | - | 2.6 |

TABLE

| Patient |
| --- |
| P- |
| P- |
| P- |
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| P |
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TABLE 19. Clinical symptoms and T4:T8 ratios of patients in the noninflammatory group.

| Patient | Scaling | Alopecia | Pustules | Drainage | Kerion | Lymphadenop. | ID Reaction | Infect. Sibs | Ratio |
|---|---|---|---|---|---|---|---|---|---|
| P-2 | + | + | - | - | - | - | - | - | 1.9 |
| P-3 | + | + | - | - | - | +/- | - | - | 1.7 |
| P-4 | + | + | - | - | - | - | - | - | 2.0 |
| P-6 | + | + | - | - | - | - | - | + | 1.3 |
| P-7 | + | + | - | - | - | - | - | + | 1.25 |
| P-8 | + | + | - | - | - | + | - | - | 2.1 |
| P-9 | + | + | - | - | - | - | - | - | 1.8 |
| P-10 | + | + | - | - | - | - | - | - | 1.8 |
| P-11 | + | + | - | - | - | - | - | - | 1.8 |
| P-12 | + | + | - | - | - | + | - | - | 1.7 |
| P-13 | + | + | - | - | - | + | - | - | 1.85 |
| P-16 | + | + | - | - | - | - | - | - | 1.6 |
| P-17 | + | + | - | - | - | + | - | - | 1.6 |
| P-20 | + | + | - | - | - | - | - | + | 2.0 |
| P-22 | + | + | - | - | - | - | - | + | 2.2 |
| P-24 | + | + | - | - | - | + | - | - | 2.0 |
| P-25 | + | + | - | - | - | - | - | - | 1.6 |
| P-27 | + | + | - | - | - | - | - | - | 1.5 |
| P-28 | + | + | - | - | - | - | - | - | 1.7 |
| P-30 | + | + | - | - | - | - | - | - | 1.6 |

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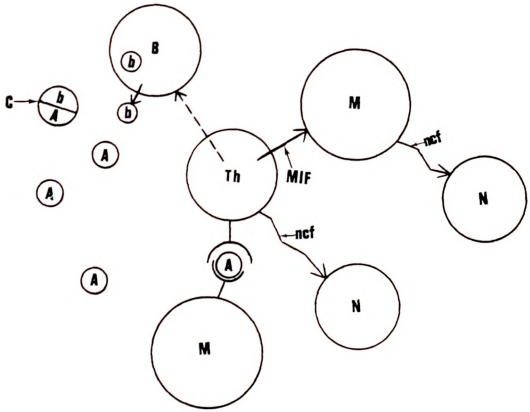
causing damage to the dermal-epidermal barrier. At the same time, fungal antigen is presented to T-helper cells by macrophages causing their activation and proliferation. Activated T-helper cells release lymphokines which stimulate B-cells to produce antibody (Gotz et al., 1978). T-helper cells also produce MIF causing macrophages to remain at the site of infection. Antibodies (probably IgG and IgM) complex with fungal antigen and are phagocytized and destroyed by macrophages and neutrophils (Cohen et al., 1981). The cell products released by this destruction result in additional damage to the dermal barrier (Mackowiak, 1981). The privileged nature of the stratum corneum is now compromised (Jones et al., 1974a) and unsaturated serum transferrin can move into the area of hyphal invasion and compete with the dermatophyte for iron (Artis et al, 1983). The rate of epidermal proliferation is increased and the infected hair and fungus are shed (Berk et al., 1976; Hino et al., 1982).

Many other factors and interactions may also be involved in this simplified hypothetical model. The key cell type in the regulation of this model is the T-lymphocyte (Figure 18). The feedback mechanisms between T-helper and T-suppressor cells have a critical balance (Goldstein et al., 1982). The immune response anergy seen in patients with noninflammatory tinea capitis may be a result of this association. The T4/T8 ratios (helper/suppressor) in patients with noninflammatory

FIGURE 18. Normal immune response model. In normal immune response, fungal antigen (A) is presented to T-helper cells (Th) by macrophages (m). Production of macrophage inhibition factor (MIF) keeps macrophages (m) at the site of infection while neutrophil chemotactic factor (ncf) attracts neutrophils (N) to this area. Another lymphokine produced by Th stimulates B-cells (B) to produce antibody (b) which will complex (C) with fungal antigen.

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ringworm were no different from those observed in the noninfected control group.

Jones (1980) noted a similar anergy in atopic patients demonstrating chronic T. rubrum infections. He proposed the inhibition of T-helper cell activation by the T-suppressor cells. Rocklin (1977) noted that the suppressor factor elaborated by these cells diminished the production of MIF by the T-helper (T4) population. Jorizzo et al. (1980) proposed a model for the immune anergy associated with chronic mucocutaneous candidiasis. In this model, IgE would bind to basophils and mast cells causing degranulation and histamine release. Histamine would then bind to the H2 receptors of T-suppressor cells causing them to produce a lymphokine which would inactivate T-helper cells. Elevated levels of IgE have been noted by many authors (Balough et al., 1981; Brahmi et al., 1980; Hay et al., 1983) in patients with chronic, noninflammatory dermatophyte infections.

This mechanism might apply to patients with noninflammatory tinea capitis. Serum IgE would complex with the antigen produced by T. tonsurans. This complex would bind to basophils and mast cells at the site of infection (Tosti et al., 1970). Subsequent cell degranulation would release histamine which would bind to the H2 receptors of T-suppressor cells. Suppressor cells would then produce a product which would directly or indirectly prevent T-helper cell proliferation and

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production of products such as MIF which are necessary for the inflammatory response.

Presser and Blank (1981) found that cimetidine administration to patients with chronic, noninflammatory tinea capitis, caused their infection to become inflammatory and subsequently resolve. Cimetidine is an H2 antagonist and would compete with IgE-antigen complexes for the H2 binding sites. Rocklin et al. (1978) found that H2 antagonists such as cimetidine would negate the suppressive effects of histamine. They were able to demonstrate this by restoring the MIF production of T-helper cells in vitro. In theory, this drug would have a similar effect in the patients with noninflammatory tinea capitis who were unable to demonstrate an elevation in T4/T8 ratios. This model is demonstrated in Figure 19.

FIGURE 19. Proposed model for immune anergy. In this model, fungal antigen and host IgE complexes (A) and binds to mast cells (MC). This results in degranulation of the MC and release of histamine (h). Histamine binds to the H2 receptor of the T-suppressor cells (Ts) stimulating production of suppressor factors (sf). This factor shuts down production of macrophage inhibition factor (mif), neutrophil chemotactic factor (ncf) and B-cell stimulating factor (bsf) resulting in immune anergy.

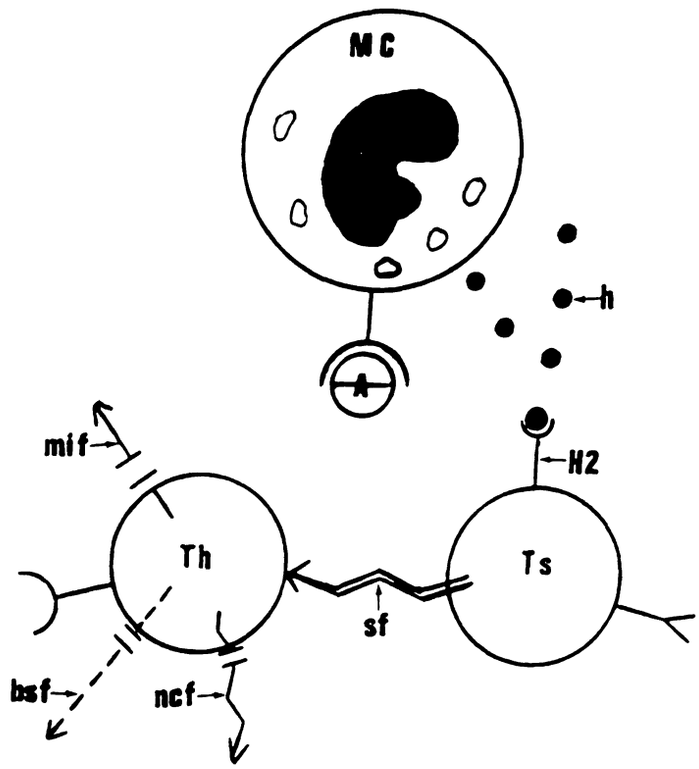


FIGURE 19.

## SUMMARY

The epidemiology of juvenile tinea capitis was studied with respect to infective agent and host, sex, and race. Of the 331 culture proven infections diagnosed, 290 (88%) were caused by T. tonsurans and 38 (11%) by M. canis. A three-fold increase in the incidence of M. canis infections between 1983 and 1984 was noted. Females demonstrated tinea capitis almost three times more frequently than males which is the reverse of the ratio observed in the 1950's. Ninety-five percent of all tinea capitis reviewed in this study occurred in black children. This population may be biased, however, because of geographic location. If only T. tonsurans infections were considered, this number increased to 99%. Some factors which might contribute to this racial disparity include crowded living conditions among those in lower socioeconomic groups, poor hygiene, and grooming practices.

Thirty juvenile patients with tinea capitis were selected for subsequent studies. They were placed into either the inflammatory group or noninflammatory group based on the characteristics of their scalp infection. T. tonsurans was the dermatophyte isolated from all patients. Each isolate was tested for urease activity and hair perforation ability. All isolates were urease positive requiring a mean incubation time of 2.1 days in the inflammatory group and 2.2 days in the noninflammatory

group. The brief incubation time required before demonstration of enzyme activity is surprising and suggests the possibility of an enzymatic contribution to the pathogenic mechanisms of these strains in tinea capitis. T. tonsurans var. sulfureum was recovered in 26 of the thirty patients. Twenty-three of these 26 (89%) gave positive results in the hair perforation test indicating they were of the subvariety perforans. The high occurrence of this subvariety was equal in both patient groups.

T-cell helper/suppressor ratios were performed on the lymphocytes of all patients and ten noninfected controls. These studies were performed to indirectly measure the immune response in patients infected by T. tonsurans. These results showed that the helper/suppressor ratio of the noninflammatory group was not statistically different from the control group. This indicates the lack of host ability to initiate an immune response to the invading dermatophyte. The inflammatory group showed a significant increase in this ratio as compared to the other two groups, indicating stimulation of their T-helper cell population by the fungal antigen. The anergy observed in the noninflammatory group is attributed to T-helper cell inactivation by T-suppressor cells.

# APPENDIX 1

# PATIENT DATA SHEET

Henry Ford Hospital
DERMATOLOGY

Date

TINEA CAPITIS STUDY

Patient ID Number _____

☐ Acute ☐ Chronic

Age ___ Race ___ Sex ___

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A. CLINICAL: ☐ Alopecia ☐ Scaling ☐ Pustules ☐ Drainage

☐ Kerion ☐ Id Rxn ☐ Lymphad. ☐ Inf. Sibs

_____

_____

_____

B. LAB DIAGNOSIS: KOH Exam _____ Culture _____

Hair Perforation ( )

C. LYMPHOCYTE STUDIES: Urease Test ( ) Days ( )

1. Blood volumn obtained Date: Vol.: _____
2. Ficoll grad. separation Date:
   a. Total # WBC'S recovered _____ b. % Lymphs _____
3. Procedure comments: _____

_____

4. Flow Cytometry
   a. Monoclonal panel

| Parameter | | % WBC'S |
|---|---|---|
| T4 (helper) | | |
| T8 (supp.) | | |
| T11 (pan T) | | |
| B-1 (pan B) | | |
| IA (B&act.T) | | |
| C (control) | | |

   b. Comments: _____

_____

APPENDIX 2

PATIENT CONSENT FORM

Henry Ford Hospital
DERMATOLOGY
Consent To Participate
In A Research Study

DATE
MRN
NAME

PRHRC APPROVAL DATE: ___/___/___

Protocol Title: "T-Cell Response in Dermatophytoses"

1) I have been asked to participate in a research study which will involve the obtaining of two additional tubes of blood.

2) I am aware that reasonable forseable discomforts & risks include no more than having my blood drawn for routine tests.

3) The benefits which I may reasonably expect include: potentially a better understanding of my infection by my physician so that he might be better able to deal with my problem.

4) I understand that study infromation identifying me will remain confidential and will not be disclosed outside the hospital except with my written permission or as required by law.

5) I have discussed this study with _____ and he/she has offered to answer any questions I may have. I am aware that I should contact Dennis Babel at 876-2159 and/or the Research Office at 876-2024 if I have any questions regarding the research, my rights, or my participation in the study and its outcome.

6) In giving my consent, I acknowledge that my participation in this research study is voluntary, is without additional cost and that I may withdraw from it at any time without prejudice to me.

| | SIGNATURE | DATE |
|---|---|---|
| Patient: | _____ | ____ |
| Parent/Guardian: | _____ | ____ |
| Witness: | _____ | ____ |
| Investigator: | _____ | ____ |

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