EXPERIMENTAL HUMAN TRICHOPHYTON MENTAGROPHYTES INFECTIONS*

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ABSTRACT

Inflammatory dermatophytosis was induced on the ankles, thighs, or forearms of 40 men using purified spore suspensions of a granular strain of Trichophyton mentagrophytes. Standard reproducible infections resulted from the application of a quantitated dose of spores to a measured skin area, followed by continuous occlusion with a plastic patch for 4 days. Alternate methods of inducing infections included the use of infected skin scales as a source of spores and the substitution of damp clothing for the occlusive patch. The infections healed spontaneously within 90 days of onset. None spread to other body areas or to other persons.

Details are provided on the methods of preparing purified spore preparations, quantifying inocula, and applying occlusive patches.

In 1909, Bloch and Massini [1] showed that dermatophytosis could be experimentally induced in human subjects and that infection was accompanied by characteristic immunologic changes. Many investigators have since attempted to study the pathogenesis and immunology of dermatophytosis using model infections in humans; however, their results have been difficult to interpret because of varying techniques, small numbers of infections, poor reproducibility, and lack of standardization of the inoculum [2]. No method has been successful enough to become widely accepted.

The high incidence of dermatophytosis among U.S. combat forces in Vietnam [3] stimulated our interest in inducing similar infections in volunteers under controlled laboratory conditions. Based on pilot studies by Taplin and Katz (unpublished data), we devised several new techniques for initiating experimental infections on the glabrous skin using a granular strain of Trichophyton mentagrophytes [2]. Our test organism was originally isolated in Vietnam from an American soldier with a moderately inflammatory case of dermatophytosis (unpublished data).

This report details the methods used to induce standard, reproducible T. mentagrophytes infections on human skin.

MATERIALS AND METHODS

Source of inoculum. Two-to-three-week-old cultures of Trichophyton mentagrophytes (Arthroderma benhamiae mating type “a”) (American Type Culture Collection No. 18,748) on Dermatophyte Test Medium (DTM) [4] were used for preparing the infecting spore inoculum. Adequate virulence was assured by using subcultures of primary isolates from active lesions in guinea pigs or humans. Spores for initiating infections were harvested from the first subculture on DTM.

Preparation of inoculum. An outline of the method employed to isolate individual spores for the infecting inoculum is presented in the Figure. The details are as follows: 5.0 ml of sterile physiologic saline containing 0.01% Tween-40 was pipetted onto the surface of a 2- to 3-week-old culture of T. mentagrophytes. The culture was removed from the agar surface using a heavy-gauge, flamed nichrome loop. The culture suspension was poured into a sterile 250-ml Erlenmeyer flask containing sufficient No. 3000 (6 mm) glass beads to form a layer 3 beads in depth. The culture bottle was rinsed twice with 5.0 ml of sterile physiologic saline-0.01% Tween-40 solution. The rinses were added to the Erlenmeyer flask. The flask was shaken on a New Brunswick Scientific Co. Model G-2 laboratory rotator at 275 rpm for 30 min to homogenize the culture and free the spores from the hyphae.

After shaking, the homogenized suspension was poured into a sterile syringe to which was attached a sterile, stainless-steel Micro-Syringe filter holder, (XX 30-025 00), with a stainless-steel extension barrel, (XX 025 16) (Millipore Corp.), containing 2.0 gm of sterile Pyrex No. 3850 glass wool. The flask was rinsed twice with 5.0 ml of sterile physiologic saline-Tween-40 solution; this was also added to the syringe. The homogenized spore suspension and the flask washings were slowly percolated through the glass-wool filter into a sterile 30-ml polycarbonate centrifuge tube. When the syringe barrel emptied, the plunger was inserted and allowed to move slowly by gravity to a point 3 cm from the bottom of the syringe to force the remaining liquid out of the filter. The spore suspension in the polycarbonate centrifuge tube was centrifuged at 15,000 × g for 20 min at 20°C in a Sorvall RC2-B centrifuge (Ivan Sorvall Corp.). After centrifugation, supernatant fractions were decanted and the spores resuspended in an equal volume of membrane-filtered (Millipore HA 0.45), sterilized antibiotic wash solution containing 300 mg cyclohexamide, 100 mg chloramphenicol, and 100 mg of tetracycline HCI per liter of distilled water. The resuspended spore pellet was triturated 10 times and the spores recentrifuged and washed again with the antibiotic solution. The washing procedure was repeated a third time, after which 10 ml of the washed spore suspension was transferred to a sterile, screw-cap test tube and mixed vigorously for 2 min on a Vortex mixer. After mixing, the suspension was allowed to stand for 30 min and then the top 5 ml of the spore suspension were removed and transferred to another sterile, screw-cap test tube. This suspension contained at

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A medical history was taken from each volunteer and a physical examination was performed. Scrapings of each toe web space, the groin, and any areas of dermatitis were taken for microscopic examination in 10% potassium hydroxide (KOH) solution and culture on DTM.

Skin tests were performed by the intradermal injection of 10 µg of Crucikshank's purified trichophytin [5] in 0.1 cc of sterile saline. Delayed reactions were observed at 48, 72, and 96 hr.

**RESULTS**

In a pilot experiment, *T. mentagrophytes* infections were induced on the ankles of five men by creating a microenvironment similar to that which was responsible for high rates of infection among American combat forces in Vietnam [3]. Patches measuring 5 x 5 cm were cut from military cotton-wool socks, sewn into the uppers of canvas jungle boots, impregnated with suspensions containing 10^6 spores (i.e., 4 x 10^6 spores/cm²), and worn against the ankle continuously for 5 days. Sterile water was added periodically to keep the patches damp and flush against the skin. Inflammatory infections, similar to those described from Vietnam by Blank and others [6], developed at the occluded sites within 3 days following removal of the patches.

After demonstrating that Vietnam-type infections could be induced in the laboratory under conditions simulating those in Southeast Asia, a second group of five men was infected on the ankle using the occlusive patch described in Materials and Methods. All five had a history of dermatophytosis; however, physical examinations, cultures, and trichophytin skin tests were negative. Infecting inocula contained 250,000 spores (i.e., 10^4 spores/cm²). Occlusion was maintained for 4 days. The infections were monitored daily. Their courses were like those described by us in a previous paper [7], and can be summarized as follows: (1) during the first 2 days following removal of the occlusive patch, no signs of infection were present; (2) from the 3rd to the 10th day, erythema, edema, and small vesicles appeared, and the degree of inflammation steadily increased; (3) from the 11th to the 22nd day, the lesions enlarged to fill nearly the entire area originally overlain by the occlusive patch; (4) from the 23rd to the 45th day, the erythema and edema were replaced by scaling, and discrete follicular infections became apparent in three of the six subjects; (5) from the 46th to the 90th day, the infections healed spontaneously. Scrapings taken from the lesions at twice-weekly intervals were consistently culture positive until the end of the third week. Drops of pus expressed from the follicular infections yielded pure cultures of *T. mentagrophytes*.

Seven months after induction of the primary ankle infections in the second group of subjects, the previously infected sites were covered with sterile occlusive patches for 4 days. These sites had shown no signs of infection and had been culture negative for at least 4 months. There was no
recurrence of infection in any of the five subjects following reocclusion.

Infections were also induced on the volar surface of the forearm in each of 24 men using 10^9 spores/cm^2 and on the lateral lumbar region of each of three men using 2 × 10^2 spores/cm^2. The spores were applied to 5 x 5 cm patches and occlusion was maintained for 4 days, as in the previous experiment. The forearm infections were similar to the ankle infections in appearance and course; however, the point of maximum inflammation was reached during the 9th to the 13th rather than the 11th to the 22nd days, and the lesisons healed within 60 rather than 90 days. Like the ankle infections, they were culture positive until the 22nd day. The infections on the back showed minimal erythema and no scaling; they were culture positive from the 6th through the 13th day; and they healed spontaneously between the 16th and 21st day.

Scales harvested from healing infections were used as an alternative source of infecting inocula. After air-drying at room temperature and humidity (23°C, 45% RH) for 1 month prior to use, pieces of scale measuring 2–3 mm in diameter were held against normal skin with Blenderm tape or an occlusive gauze patch for 4 days. Inflammatory infections developed on the ankles, thighs, and forearms of each of three subjects tested.

**DISCUSSION**

The model infection described in this paper provides a useful new tool for researchers interested in the biology of human dermatophyte infections. Its simplicity and reliability permit definitive experimental studies to be conducted with little effort and a minimum number of subjects. Besides being useful for studying pathogenesis and immune responses, it can also be employed to evaluate antifungal agents with a degree of standardization not possible by other means [7].

Induction of standard infections depends on control of three factors—spore dose, duration of occlusion, and immune status of the experimental subject. The severity of infection is influenced by the spore dose and the duration of occlusion (JH Reinhardt, AM Allen, unpublished data); therefore, it is important for standardization to ensure that spore doses and durations of occlusion be identical for all subjects. Immune factors should also be considered in the experimental design, since there is evidence that patients with atopic dermatitis differ from normal subjects in response to infection [8], and that subjects with a positive skin test response to purified trichophytn antigen react differently to infection than those with negative skin tests [5].

A monospore isolate (i.e., a colony derived from 1 spore) of a single strain of *T. mentagrophytes* was used as the standard infecting agent in all of our experiments. Other sporulating strains would probably have been just as effective, since the organism we used was the least virulent of six different granular *T. mentagrophytes* isolates tested in guinea pigs. It remains to be determined whether dermatophyte strains differ significantly in virulence when tested under standard conditions in human subjects, and whether the differences, if any, correlate with elaboration of fungal enzymes, toxins, or allergens.

All but one of our subjects were able to carry on normal work and home life during the experiments, and they experienced little or no discomfort at the infected sites. The exception was a man who developed an unusual amount of inflammation at the site of occlusion. Administration of griseofulvin brought his infection rapidly and completely under control, and he suffered no recurrence.

In no instance did infection spread beyond the areas originally covered by occlusive patches, nor did infection spread to family members or laboratory personnel.

Other investigators have shown that inflammatory *T. mentagrophytes* infections can be induced in humans by inoculating and occluding unscarified skin; however, their methods and results differed from ours in several important respects, including the size and type of inoculum, the method of producing occlusion, and the severity of the inflammatory reaction. Slorper [9], working in Malaya, used unquantified amounts of culture material and scrapings from infected lesions as inocula, and suggested that the hot, humid climate favored his methods. Blank and Taplin (unpublished data) induced infection in a volunteer by seeding his boots and socks with spores and keeping them wet with swamp water for 5 days. In this instance, overt infection occurred only in areas subjected to friction and pressure (i.e., boot tops, Achilles tendon, dorsum of foot), and the subject was disabled because of painful, swollen feet and femoral lymph nodes. We have extended these studies by quantifying the spore dose, showing that occlusion by wet boots and socks is exactly equivalent to other methods of occlusion, and demonstrating that scales desquamated from lesions remain infective for as long as a month. The clinical and epidemiologic significance of such information has been commented upon previously [3,6,10].

**REFERENCES**


