The Production of Experimental Dermatophyte Lesions in Guinea Pigs

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Experimental dermatophytoses were induced in virgin and previously infected guinea pigs by the quantitated application of spores to plucked and shaved areas of skin. Lesions could be consistently induced without occlusion with 7 dermatophytes—Trichophyton mentagrophytes, T. rubrum, T. tonsurans, Microsporum canis, M. gypseum, M. persicolor and Epidermophyton floccosum. The progress of lesions was monitored visually and their infectivity determined using a hair brush sampling technique. Airborne spread of fungal elements from infected animals to other animals housed in the same area and to the atmosphere was also examined.

With 2 dermatophytes, T. mentagrophytes and M. canis, it was found that the infectivity of lesions correlated well with the clinical progress of the disease after primary infection and on reinfection. This was not the case with the other fungi investigated where factors such as absence of hair invasion, hair invasion wholly or mainly endothrix in nature, or the development of a thick scab which trapped hairs and fungal elements, resulted in the development of lesions of low infectivity.

Dissemination of spores into the air and onto neighboring noncontact animals occurred readily with M. canis, and to a lesser degree with T. mentagrophytes. Airborne spread of fungal elements from lesions due to the other dermatophytes was negligible.

On reinfection, some spores germinated and hyphal growth occurred but lesions appeared earlier, healed more rapidly and were markedly less infective.

This experimental model with T. mentagrophytes, would seem an ideal system for investigating the effects of various procedures, e.g., antimonycotic therapy, vaccination, on the progress and infectivity of dermatophyte lesions.

Experimental dermatophyte infections have been produced with varying success on the skin of man and lower animals for many years [1]. A variety of techniques has been used, but only in the last few years have standardized procedures been devised for experimental guinea pig [2,3] and human infections [4]. Oclusion of the inoculated area for 3–4 days after application of the inoculum is an essential feature of these. They permit direct observation of the way lesions develop in virgin and previously infected individuals, and give some idea of the number of spores required to initiate infection. The guinea pig model has also been used to correlate the development of cellular hypersensitivity, as measured by lymphocyte blastogenic and skin test assays, with the intensification of lesion erythema [5].

We have devised an experimental guinea pig model which allows a number of additional features of the pathogenesis of dermatophyte infections to be studied. A preliminary note on this technique has appeared elsewhere [6].

MATERIALS

Animals

Guinea pigs around 6 mo of age were used in all experiments. They were kept in one room in separate cages and fed pellets and water as required. The colony, from which animals were obtained, was housed in a separate building. Regular sampling of this colony and individual animals prior to inoculation, revealed all animals to be free of dermatophyte spores. Sampling was carried out using brushes and the techniques described below.

Fungi

Eight dermatophytes were included in the investigation—Trichophyton mentagrophytes var. erinacei, recovered from a hedgehog; Trichophyton mentagrophytes var. mentagrophytes, recovered from a rat; Trichophyton rubrum, isolated from a human leg lesion; Trichophyton tonsurans, recovered from man; Microsporum canis, isolated from scalp lesions in a child; Microsporum gypseum, recovered from an apparently normal rat; Microsporum persicolor, isolated from a vole in England, and Epidermophyton floccosum, recovered from human groin lesions.

Preparation of Spore Inocula

The aerial growth from 2–3 week Trichophyton cultures grown at 30°C on Sabouraud’s dextrose agar (Difco) was removed by flooding the surface with sterile saline containing 0.01% Tween-80 and scraping off the fungal growth with a sterile blade. The suspension was then pipetted into a sterile universal and shaken in a Vortex mixer for about 5 min in an attempt to separate spores from the mycelium. After shaking, the suspension was poured into a sterile 10 ml syringe in which approximately 1.5 cm of sterile nonabsorbent cotton wool was loosely packed. After expression through the syringe, the suspension (containing mainly aleuropores) was centrifuged at 2500 rpm for 5 min. The deposited spores were then washed once in phosphate buffered saline (PBS) pH 7.2 containing chloramphenicol (50 μg/ml) and twice in plain PBS before being resuspended in the required amount of PBS to give approximately 10^6 microaleuriospores/ml as determined by standard viable count techniques.

Since M. canis failed to produce macrospores in adequate numbers when grown on Sabouraud’s dextrose agar, spores were harvested from the growth obtained on a rice medium which had been incubated at 30°C for 20 days. The preparation procedure was similar to that described above except that the filtration step was omitted. Suspensions of approximately 10^6 viable units/ml were normally obtained by this method and were used without further dilution.

With M. gypseum, M. persicolor and E. floccosum the procedure was as described for the Trichophyton species except that the cotton wool filtration step was omitted.

Initiation of Infection on Guinea Pigs

Primary infection: Infections were initiated on naked skin areas on both flanks of each guinea pig. After hand plucking, the area to be inoculated was clipped with scissors and shaved. A square (2 x 2 cm) was then marked on each flank; care being taken to avoid obviously damaged skin. One hundred μl of the appropriate spore suspension was then slowly applied dropwise
onto the specified area, care being taken to prevent the inoculum from spreading beyond the marked region. The inoculated area was left uncovered throughout the period of the experiment.

Reinfection: After the clearance of primary lesions, animals were reinjected (secondary infection) at the same site using the same technique.

Observation and evaluation of infections: Animals were examined and sampled at 2-day intervals.

Using sterile forceps, skin/hair samples were removed and examined for fungal elements in a KOH/ink wet preparation (20% KOH in 1:1 ratio with blue-black Parker Quink ink).

A modification of the hairbrush sampling technique of Baxter [7] was used as a quantitative measurement of in vivo fungal growth. Briefly, circular plastic scalp massagers (brushes), 8 cm in diameter and containing 125 spikes (bristles), were gently brushed over one inoculated area from neck to tail, 10 times. Each brush was then placed in a clean envelope and transported to the laboratory. Here the bristles were pressed firmly into plates of Sabouraud’s dextrose agar (2% agar) containing chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml), giving a multiple inoculation on the surface of the medium. The plates were then incubated at 30°C for at least 3 weeks before being discarded. The number of dermatophyte colonies appearing on each plate was recorded. Normally these were visible within 7 days of inoculation. After use, the brushes were immersed in 1% Savlon solution overnight, scrubbed, washed in clean hot water, and allowed to dry. Periodic “sterility” tests on the brushes demonstrated the effectiveness of this washing process.

During the investigations, animals were caged separately in the same room and care was taken to minimize possible cross-infection during the sampling procedure. One species of dermatophyte was investigated at a single time. This eliminated any problems which might have arisen due to competition for colonial growth and differentiation of individual colonies.

Dissemination of spores from infected animals to neighboring animals and to the environment was also investigated at each period. Noninfected animals were “brushed” as for inoculated animals, while the air of the animal room was sampled by leaving antibiotic supplemented Sabouraud’s dextrose agar plates opened at the sampling bench for 90 min after each sampling.

The reproducibility of results obtained using the hair brush sampling technique were assessed by simultaneously inoculating 5 guinea pigs with T. mentagrophytes var. erinacei. These were then sampled as described above and the results analysed statistically using the Student’s t-test.

RESULTS

Clinical Characteristics of Lesions after Primary Infection

The sequence of events following inoculation with each of the dermatophytes was essentially similar although the time sequence varied between species. In general, lesions followed the following course: (1) the appearance of discrete red inflamed areas which coalesced to form a single large erythematosus area covering the inoculation site; (2) scaling which gradually expanded over the entire infected area; (3) the progressive development of a thick crust which on desquamation left smooth alopecic areas; (4) regrowth of hair. In some animals, additional foci of infection de veloped and healed within this time period. No crust formation or alopecia were seen with the fungi incapable of invading hair in vivo—M. persicolor and E. floccosum.

With var. erinacei (the only fungus in which the development of lesions was consistently examined in depth), lesions became visible around day 3, appeared to be most severe from days 12-27 when extensive scaling and laterly crust formation were evident, and resolved around day 37. Fungal elements were visible microscopically in cutaneous scrapings from days 2 to 28. With all fungi tested, hyphae and/or arthrospores were visible microscopically in cutaneous scrapings for at least 16 days postinoculation. In the case of M. canis, arthrospores were still visible on day 58.

If these clinical observations for var. erinacei are compared with the results shown in Fig 3 and 4, it becomes obvious that the recovery of fungal elements (7 spores) from experimental lesions and from the hair of normal neighboring animals correlates well with the clinical progress of lesions. Lesions seemed most infective when extensive scaling and crust formation was evident.

After reinfection, the duration of all infections was shorter than that of the first infection. Lesions appeared earlier and ran
their course at a much faster rate. In all cases, spores appeared to germinate although fungal elements were difficult to detect in direct smears. All secondary infections were microscopically negative by day 16.

Recovery of Fungi from Active Lesions

The numbers of colonies isolated from animals infected with each fungus are shown in Fig 1 and 2. Reproducibility of the technique was found to be satisfactory as revealed in Fig 3. The graphs shown in Fig 1 and 2 are the results from single animals. In each case, repeat experiments gave similar results. On reinfection, lesions were much less infective; all fungi following a similar pattern to that shown in Fig 4 for T. mentagrophytes var. erinacei. There was also some indication that immunity to reinfection decreased with time.

Dissemination of Fungi from Active Lesions

Dissemination of spores into the air and onto noncontact animals was particularly obvious following M. canis infection, but also occurred to some degree with T. mentagrophytes (Fig 4, 5). Apart from one example, lesions were not encountered in carrier noncontact animals. This one exception was in an animal which had been shaved and which developed a lesion at the site of skin injury (with T. mentagrophytes var. mentagrophytes).

DISCUSSION

As with other recent experimental animal systems [1-5], our guinea pig model allowed observations on the clinical and microscopic progress of lesions to be followed after primary and subsequent inoculations. Consistent and reproducible results were obtained with a variety of dermatophytes using inocula of 10^5-10^6 spores, although in additional experiments, var. erinacei and Microsporum racemosum lesions could be regularly produced with as few as 10 spores (J.M.B. Smith, unpublished data). While previous authors have stressed the importance of occlusion for successful experimental inoculation, we had no difficulty in initiating infection without this procedure.

Microscopic observations of cutaneous scrapings revealed fungi to be growing initially in conoid-like structures; a feature especially evident with T. rubrum. It is possible that the smudging procedure used in the preparation of the inoculum site, provided moist chambers (empty/damaged hair follicles) suitable for the initial germination of spores.

There did not appear to be any clear evidence of why some species induce apparently more severe and pronounced lesions than others. A number of suggestions have been made that the mating type [8] and the production of hydrolytic and keratinolytic enzymes [8,9] are correlated with the virulence of the fungus as judged by associated host response. However, little information is available concerning the factors responsible for variances in the appearance of lesions caused by zoophilic, geophilic and anthropophilic species. Recent observations indirectly suggest that the ability of fungi to proliferate extensively in hairs and hair follicles is associated with highly inflamed lesions [10]. Our findings that the 2 dermatophytes incapable of invading hairs produced minimal scaling and erythema, supports this contention. Thus it seems not unreasonable to suggest that apart from host factors such as cellular hypersensitivity [5], the features and severity of ringworm infections depend mainly on the ability of the fungi to invade hair follicles. Where invasion occurs in association with a minimal inflammatory response, lesions and the fungus are likely to persist for longer periods than in cases where the host response is intense.

As well as permitting visual observations, our model demonstrated that at least with T. mentagrophytes and M. canis, additional information concerning the infectivity of lesions and progress of in vivo growth could be obtained using a hair brushing technique. With these 2 dermatophytes, the appearance of lesions correlated well with their infectivity as determined by colony counts. The infectivity of lesions produced by the other 5 dermatophyte species examined appeared to be extremely low. Possible explanations for this include: a lack of hair invasion (M. persicolor, E. floccosum); hair invasion solely or predominantly endothrix in nature (T. tonsurans and T. rubrum respectively); and the formation of a thick scab in which hair stubs and fungal elements became embedded (M. gypseum, T. tonsurans).
to an increase in epidermal turnover and desquamation rate [11]. Some spores apparently germinate, but subsequent growth is restricted; possibly by the rapid induction of the host inflammatory response. Soluble factors: antibodies [13], lymphokines [14], and transferrin [15], released into the area of fungal proliferation apparently restrict the growth of the fungus.

Our experiments also revealed that dissemination of infective fungal elements (arthrospores) occurred readily from *M. canis*, and to a lesser extent *T. mentagrophytes*, lesions. These results support the suggestions that airborne transmission of infection must be considered a possibility with many zoophilic tinea [16]. Although our findings would suggest that airborne spread of fungi such as *E. floccosum* and *T. rubrum* is of minor epidemiological significance, these fungi have been recovered from the air of clinics in which patients with groin lesions were being examined [17].

At present, experiments are in progress using guinea pigs and *T. mentagrophytes* var. *erinacei*, to determine the effects of systemic and topical corticosteroids, topically applied antimycotics, and vaccination procedures on the establishment, progress and infectivity of dermatophyte lesions. This system would seem an ideal model for investigating these rather controversial aspects of the pathogenesis of dermatophytoses.

REFERENCES