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DERMATOPHYTE PATHOGENICITY AS A FUNCTION OF MATING TYPE AND ASSOCIATED ENZYMES*

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ABSTRACT

Severity of ringworm infections appears to be related to the production of proteolytic enzymes which in turn is related to mating type in two species tested *T. mentagrophytes* (*A. benhamii* (a) and (A)) and *Microsporum fulvum* (*Nannizzia fulva* (+) and (−)). No difference in experimental guinea pig infections could be detected between the mating types of two other species which did not elaborate proteolytic enzymes *Nannizzia incurvata* and *Nannizzia gypsea* (*Microsporum gypseum* complex).

The mechanisms of disease production have been studied extensively in bacteria, but little work has been done on the role of the specific factors involved in the initiation and maintenance of infection by the fungi. Previous work (1) has shown that collagenase is necessary for the pathogenicity of *Streptomyces madurae* in experimental infections. Elastase has been demonstrated in a number of dermatophytes associated with the more virulent forms of dermatophytosis (2) and in some species this enzyme is associated with only one mating type (3).

In the present study four species of dermatophytes were examined for their production of several proteolytic enzymes and the association of a number of other enzymes with mating type of the organism and severity of experimental infections.

MATERIAL AND METHODS

Organisms. Trichophyton mentagrophytes (*Arthroderma benhamii* 798 (a) and 797 (A)). *Microsporum gypseum* (*Nannizzia gypsea* 1317 (a) and 1315 (A)), *Microsporum gypseum* (*N. incurvata* 5912 (a) and 5913 (A)), and *Microsporum fulvum* (*N. fulva* 2181 (+) and 644 (−)) were maintained on Sabouraud Dextrose (SD) medium. For experimental infection, a standardized inoculum (1 ml) of spores was spread on three SD slants and inclubated for four weeks at room temperature. Spores were harvested with physiological saline, washed and resuspended to a turbidity of a No. 4 nepholometer standard. A Sterneedle gun, size 6, was dipped into the spore suspension and applied to the plucked flank of white 250 gm guinea pigs (4), using 10 animals for each strain or mating type of each species. The guinea pigs were examined weekly for the appearance of lesions, culture of fungus and KOH tests. Selected animals were biopsied and the fungal elastase content of the tissue was determined spectrophotometrically.

Enzyme assays. Hydrolysis of elastin and hydrolysis of azocoll were determined by previously described methods (3, 5). Elastase assay of tissue consisted of a 3 mm biopsy of skin and was taken from the outer edge of the lesion. The tissue was minced in saline, centrifuged and the supernate lyophilized. The powder was then analyzed in the usual manner for elastase activity.

Gelatinase activity was determined by a viscosimetric assay, using a Fenske-Ostwald viscosimeter. The reaction mixture contained 5 ml of a solution of 5% gelatin in O.IM phosphate buffer (pH 7.5) and 0.5 ml of solution of 20 mg powdered culture filtrate in 1 ml of distilled water. The increase in the flow-rate of the reaction mixture incubated at 36–37° C was determined at hourly intervals for four hours. Suitable controls of boiled enzyme preparation were used and an increase exceeding 10% was accepted as an indication of activity.

A reaction mixture containing 2 ml of a suspension containing 250 mg of casein (Difco) per 50 ml of 0.1 M phosphate buffer (pH 7.5) and 0.25 ml of a solution containing 20 mg powder culture filtrate in 1 ml of distilled water was incubated at 36–37° C for three hours. The absence of a clearing of the reaction mixture was assumed to indicate the absence of proteolytic activity for the substrate at these concentrations.

Assays for esterases, leucine aminopeptidases,

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and acid or alkaline phosphatases were obtained by using starch zone electrophoresis to separate such enzymes whose presence or absence was determined by immersing the gels in the appropriate reaction mixture. Squares (5 x 5 mm) of Whatman No. 1 filter paper was saturated with a solution of powdered culture filtrate (5 mg/1 ml) and then inserted into the gel. After development for 2.5 hours at 200 V, the gels were sliced horizontally and immersed in the appropriate reaction mixture (6).

A cup-plate method (7) as modified by Tseng and Bateman (8) was used to detect phosphatidase activity.

RESULTS

Experimental infection. In most cases infections with positive KOH and culture were obtained within one week after inoculation. Severity and length of infection, however, varied with the species and in some cases with the mating type. With *N. incurvata* the infected site which became reddened with scaling by the second week subsided by the third week. In most cases cultures were negative by the fourth week, and there was no evidence of scarring. No difference in severity between the mating types could be detected. With *N. gypsea* there were appreciable lesions by the second week and some crust formation during the third. The lesions resolved by the fourth week but some cultures remained positive. The infection was generally more severe and persistent than that of *N. incurvata*. No difference was noted between the mating types.

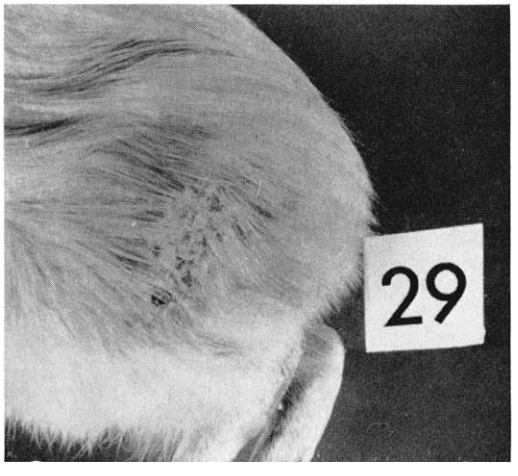

FIG. 1. Guinea pig infected with *A. benhamii* (A), 21 days, showing scaling, erythematous lesions.

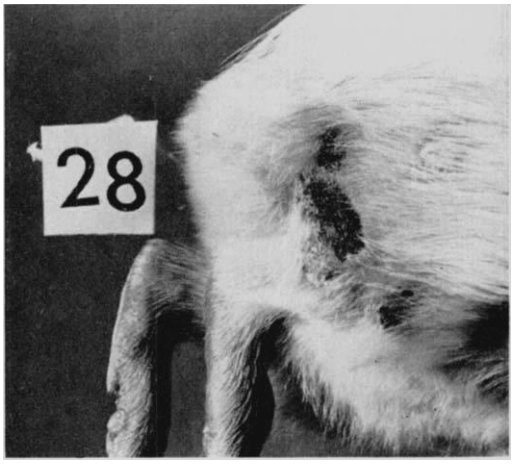

FIG. 2. Guinea pig infected with *A. benhamii* (a), 21 days, showing extensive, weeping, crusted lesions.

The infection with *N. fulva* took longer to become apparent (end of second or beginning of third week) than the infections with the other two species of *Nannizzia*. There was a marked difference between the mating types of this species. The plus strain (✻2181) produced an erythematous, crusted scaling lesion that peaked during the third week and began to resolve by the end of the fourth with some scarring and areas of permanent alopecia. The minus strain also produced reddened scaling lesions which did not crust and healed without scarring. In general, the cultures became negative by the third week with a few persisting through the fourth week. None of the lesions produced by the *Nannizzia* species extended beyond 4 cm and were usually smaller.

Infection with *T. mentagrophytes* was generally more severe with a greater tendency for spread and more persistent than infection with the *Nannizzia* species. Within the first week of inoculation, erythema was noted with a positive KOH test and culture. The lesion spread from the inoculation area during the second week and attained a size of greater than 7 cm (occasionally 12–15 cm). Severe inflammation with crusting and weeping lesions occurred during the third week in animals inoculated with *A. benhamii* (a) (Fig. 1). Resolution of these infections did not begin until the fourth and sometimes the fifth week. The crusting in some cases occurred over the entire flank and occasionally involved the legs and extended to

TABLE I

Comparative virulence of mating strains and species of dermatophytes

Species and mating type	Assessment of experimental infection in guinea pigs	
	Severity	Duration
N. incurvata (a)	Reddening and scaling second week	Three or four weeks
N. incurvata (A)	Same as above	Same as above
N. gypsea (a)	Lesion in second week, few crusts in third week resolved by fourth week	Cultures positive to fourth week, rarely to fifth
N. gypsea (A)	Same as above	Same as above
N. fulva (+)	Infection late to appear crusted scaling lesion by third week. Resolved rapidly by fourth	Cultures positive to fourth week. Very rarely to fifth
N. fulva (−)	Erythematous scaling. No crusts. Longer time to resolve than above	Cultures commonly positive to fifth week, but little or no evidence of disease in fourth week
A. benhamii (a)	Erythema first week. Crusting by third. Weeping lesions and extension by third or fourth week. Resolution begun in fifth week	Culture positive for eight weeks and rarely 10 weeks ($^2/_{10}$)
A. benhamii (A)	Erythema and spread by end of first week. Rare crusting ($^2/_{10}$). Resolution in fifth or sixth week	Cultures positive commonly into third and occasionally fourth month

the underside of the animals. When the lesions healed, scarred areas with permanent alopecia and some pitting were noted. Cultures were still positive as long as two and one-half months after inoculation.

Infection with *A. benhamii* (A) had a similar course and spread, but longer persistence. The host reaction was much less marked. Crusting occurred in very few animals, was not extensive and resolved quickly. Erythema and flaking were general and persistent (Fig. 2). When the infection resolved, there were no areas of scarring or of alopecia but a slightly reddened and furfuraceous condition remained for a longer time than with *A. benhamii* (a) infections. The infection generally persisted longer, commonly (seven out of 10) remaining culture positive, but not KOH positive, into the third and fourth months. The results are summarized in Table I, and tissue assay for elastase is given in Table II.

TABLE II

Elastase content of tissue

Source	OD 590 mμ
Standard enzyme (Worthington)	.036
Normal guinea pig	.001
A. benhamii (A) infected	.009
A. benhamii (a) infected	.026

The enzyme profiles of the examined species

TABLE III

Enzyme profiles of dermatophyte fungi showing sexual stage differences

	N. incurvata 5912 (a)	N. incurvata 5913 (A)	N. fulva 644 (−)	N. fulva 2181 (+)	N. gypsea 1316 (A)	N. gypsea 1317 (a)	A. benhamii 798 (a)	A. benhamii 797 (A)
Gelatinase	+	−	−	+	±	±	+	+
Alkaline phosphatase	−	−	−	+	−	+	+	+
Elastase	−	−	−	+	−	−	+	−
Azocoll	−	−	−	+	−	−	+	−

Other enzymes tested: Leucine amino peptidase, acid phosphatase, casein, phosphatidase—negative.

are summarized in Table III. In all cases at least one enzyme difference was noted between the mating types of the dermatophytes and in some several enzymes were present in one sexual stage and not in the other. In the two mating types showing the greater difference qualitatively between presence of enzymes, especially elastase, there was an association with the severity though not with the persistence of the infection.

DISCUSSION

Gordon *et al.* (9) and Weitzman *et al.* have compared the pathogenicity of the species of *Nannizzia* but not of the mating types and concluded that the *N. gypseum* complex (*N. incurvata* and *N. gypsea*) is more pathogenic than *N. fulva*. Although the plus strain of *N. fulva* produced a more inflammatory lesion than either of the other two species and *N. gypsea* was generally more persistent than *N. incurvata*, our results agree with those of Gordon *et al.* and Weitzman.

Several authors have examined the presence of enzymes present in dermatophytes (10) and other pathogenic fungi (11). Chataway *et al.* (10) found a complex of peptidases and keratinolytic enzymes in mycelial extracts of several dermatophytes. Montes and Wilborn (12) presented suggestive evidence for the lysis of tonofibrils during the invasion of *Candida*. An extracellular keratinase has been isolated from *T. mentagrophytes* (13). Elastase has been found in a number of species, usually associated with dermatophytes producing more inflammatory lesions (2). Rosenthal *et al.* (11) studied the enzymatic profile of various pathogenic fungi in growing cultures on media containing the specific substrate and found activity for casein in several species. In our work, culture extracts from organisms not grown in the presence of casein did not show caseinolytic activity.

A significant finding in our work was the association of several enzymes with one mating type of given species and their absence in the other mating type. These enzymes may be significant in the anastomosis of hyphal tips prior to the formation of the sexual stage fruiting body or in the severity of disease production in the process of infection. Although this observation was supported by the difference of severity and persistence of infection for mating types of some species, such differences were not found in other species. It would appear from our studies that the more severe the host-response, the less persistent is the infection. The relative antigenicity of the enzymes must also be considered. Some of the humoral antibodies in aspergillosis are directed against specific enzymes acting as antigens elaborated by the invading organism (14).

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