mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Review article

Mechanisms of skin adherence and invasion by dermatophytes

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Summary

Dermatophytes are keratinophilic fungi that can be pathogenic for humans and animals by infecting the *stratum corneum*, nails, claws or hair. The first infection step consists of adherence of arthroconidia to the *stratum corneum*. The mechanisms and the kinetics of adherence have been investigated using different *in vitro* and *ex vivo* experimental models, most notably showing the role of a secreted serine protease from *Microsporum canis* in fungal adherence to feline corneocytes. After germination of the arthroconidia, dermatophytes invade keratinised structures that have to be digested into short peptides and amino acids to be assimilated. Although many proteases, including keratinolytic ones, have been characterised, the understanding of dermatophyte invasion mechanisms remains speculative. To date, research on mechanisms of dermatophyte infection focused mainly on both secreted endoproteases and exoproteases, but their precise role in both fungal adherence and skin invasion should be further explored.

Key words: Adherence, dermatophytes, proteases, invasion.

Introduction

Dermatophytes are keratinolytic fungi. They have been classified as geophilic, zoophilic and anthropophilic species on the basis of their primary habitat associations. Geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves and horns, as a part of their decomposition process. Zoophilic and anthropophilic dermatophytes are adapted to the animal or human host and are the most frequent agents of superficial mycoses in animals and humans infecting the *stratum corneum*, hair, claws or nails. They have adapted to their environment using a variety of host proteins, particularly keratin as a nutrient and they notably secrete proteases degrading skin and hair proteins. Understanding the pathophysiological mechanisms involved in a dermatophyte infection is the basis for the rational development of new prophylactic and therapeutic strategies.[1,2] During the infection, arthroconidia adhere to host's skin and after germination, the fungal hyphae invade skin keratinised structures. During invasion, high keratinised tissues are digested into short peptides and amino acids to be assimilated via transporters.[3] The aim of this study was to briefly review the current knowledge of adherence and invasion mechanisms of dermatophytes that have been investigated both *in vitro* and *in vivo*.

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Accepted for publication 12 July 2011

Experimental models for study of kinetics of dermatophyte adherence

Adherence of microorganisms to host tissues is an important step in the establishment of most infections and a prerequisite for dermatophyte infections. Adherence to skin and nails was investigated in several

doi:10.1111/j.1439-0507.2011.02081.x

dermatophytes species using different experimental *in vitro* and *ex vivo* models.

These studies showed a time-dependent increase in the number of adhering spores, followed by their germination, and then the invasion of the *stratum corneum* by hyphae growing in multiple directions. Zurita and Hay [4] observed that maximum adherence of *Trichophyton* sp. arthroconidia to human keratinocytes in suspension occurred within 3–4 h. Aljabre *et al.* [5,6] used stripped sheets of *stratum corneum* or isolated human keratinocytes to demonstrate that adherence of *Trichophyton mentagrophytes* arthroconidia was the highest 6 h after contact and that germination of these spores began 4 h postexposure. In a nail plate model, adherence and germination of *T. mentagrophytes* arthroconidia were observed 6 h postinoculation, whereas growing hyphae were clearly identified 10 h later.[7] Adherence of *T. mentagrophytes* was also investigated using an *ex vivo* model made of human skin explants of full epidermis.[8] In this model, adherence was maximal after 12 h, germination started by 24 h and penetration of the *stratum corneum* occurred after 3 days.

Recently, a highly efficient adherence model of *Microsporum canis* arthroconidia was developed using a reconstructed interfollicular feline epidermis (RFE).[9] This model is very useful because both the cornified layer resulting from epidermal differentiation process[9] and skin permeability of RFE[10] were shown to be similar to the *in vivo* situation. Adherence of *M. canis* arthroconidia to RFE was found to be time-dependent, starting within 2 h and increasing up to 6 h postinoculation.[11]

Adherence mediators

Dermatophytosis is acquired from an exogenous source as causative agents are not part of the fungal flora of healthy humans or animals. Thus, initial contact between arthroconidia and *stratum corneum* is a crucial event in the initiation and establishment of the infection of the skin. The adherence of fungi to host cells is mediated through fungal adhesins and their interactions with host receptors. Little is known about the factors that mediate adherence of dermatophytes.

Trichophyton rubrum and *T. mentagrophytes* express carbohydrate-specific adhesins on microconidia surface that recognise mannose and galactose.[12,13] These adhesins could play a role on the adherence of the fungus during the infectious process, but this has not been investigated so far. Converse to other fungal species, sialic acids are absent from the cell surface of these fungi,[14] and therefore these components are not required for dermatophytosis.

Human skin explants inoculated with *T. mentagrophytes* arthroconidia revealed fibrils stretching out from fungal elements and connecting them to the skin tissue surface.[6,15] *Trichophyton mentagrophytes* arthroconidia produce long fibrils when it is on the surface of the *stratum corneum*, whereas short fibrils are produced inside the dense sublayers. Adherence occurs by the establishment of short and long fibrils that appear to anchor and connect the arthroconidia to the tissue surface and prevent the disconnection of the exposed fungal element under the rough conditions on the skin surface. The use of fibrils (also called fibrillar adhesins) for adherence was also observed in other pathogenic fungi such as *Candida albicans*,[16–18] but their precise composition remains currently unknown.

As shown for *C. albicans*, where secreted aspartic proteases (Saps) play a fundamental role in fungal adherence and invasion of skin and mucosal surfaces[19–21] the involvement of secreted proteases in the adherence process was also demonstrated for *M. canis*.[11,22] The implication of a family of secreted subtilisins (Subs) from *M. canis* in the adherence to feline corneocytes was demonstrated using chymostatin, a serine protease inhibitor, which significantly inhibits the adherence of *M. canis* arthroconidia to RFE.[11] The role of the secreted subtilisin Sub3 in *M. canis* adherence to feline epidermis was further demonstrated using a specific and stable RNA-silenced strain obtained by RNA interference.[22,23] However, this protease was found to be unnecessary for the invasion of keratinised tissues in experimentally infected guinea pigs.[22] The precise mechanisms by which secreted fungal proteases contribute to the adherence process are not clear. Two hypotheses are currently favoured and were investigated for *C. albicans*.[24,25] First, proteins could act as ligands for surface moieties on host cells, which do not necessarily require activity of these enzymes.[24] Second, secreted proteases may act as active enzymes to modify target ligands on the fungal surface or on epithelial cells, and this could lead to conformational changes, facilitating adherence of the fungus.[25]

Besides subtilisins, *M. canis* secretes many other endoproteases, some of them are metalloproteases (Meps) of the fungalysin family and have been characterised.[2,26] However, *MEP* genes were shown to be not transcripted in *M. canis* arthroconidia and during both adherence (i.e. 4 h after inoculation) and early invasion (i.e. 48 h after inoculation) of feline epidermis.[27] These results suggested that the proteolytic activity of fungalysins, including the keratinolytic activity of Mep3[28] is not related to the ability of *M. canis* to adhere to or to invade host tissues.[27] Fungalysins may later play a role

in the infection process, because they have been shown to be expressed in the hair of experimentally infected guinea pigs, 14 days after inoculation.[26]

The gene encoding a highly specific exoprotease, dipeptidyl peptidase removing X-Pro dipeptides from N-termini of peptides (DppIV)[29] was found to be transcribed in arthroconidia and in the early stages of feline skin invasion.[27] In dermatophytes, this exoprotease may be particularly important for virulence, as it could be involved in activation or inactivation of biological peptides, of which the N-terminus is X-Pro.[29] For instance, in *Histoplasma capsulatum*, DppIV was found to be active against the immunoregulatory peptide substance P.[30] Dipeptidyl peptidases IV also play an important role in the pathogenicity of various bacteria. In *Porphyromonas gingivalis*, a DppIV is responsible for the degradation of collagen and interactions between bacteria and extracellular matrix proteins of the host.[31–34] *Streptococcus suis* DppIV is able to link to fibronectin, and is involved in the adherence of bacteria to the surface of host cells.[35] In the protozoan *Trypanosoma cruzi*, DppIV is also able to degrade collagen types I and IV, and is required for invasion of the host cells.[36–38]

Invasion

The penetration of the *stratum corneum* starts with the emergence of germ tubes from the arthroconidia. On stripped sheets of *stratum corneum* from humans, hyphae from *T. mentagrophytes* penetrate longitudinally and perpendicularly in and through the thickness of the *stratum corneum*.[6] By 7 days of incubation, hyphae start to form arthroconidia, thereby completing the vegetative growth cycle of the fungus.[6]

In contrast to dermatophyte adherence, for which at least one well characterised protein (Sub3) was shown to be clearly involved, dermatophyte invasion remains rather speculative. As pathogenic dermatophytes exclusively infect the *stratum corneum*, nails, claws or hair, research on mechanisms of the fungal invasion primarily focused on their secreted proteases. The secreted endo and exoproteases predicted by genome inspection can be compared with those of the *Aspergillus* spp. and particularly with those of the opportunistic pathogen *A. fumigatus* of which many proteases were previously characterised.[3] Most *A. fumigatus* exoproteases in A1 (pepsins), M28 (leucine aminopeptidases), S9 (dipeptidyl peptidases), S10 (carboxypeptidases) and S53 (tripeptidyl peptidases) families have an orthologue in dermatophytes.[3] However, in contrast to *Aspergillus*, endoproteases of the subtilisin (S8), deuterolysin (M35) and fungalysin (M36) families have expanded in dermatophytes with 12, 5 and 5 members respectively.[39] The precise role of these endoproteases *in vivo* in skin invasion remains to be investigated.

In vitro, in a medium containing keratin or any other protein as a sole nitrogen source, dermatophytes secrete several endoproteases of the two large families of subtilisins (Sub3, Sub4) and fungalysins (Mep3, Mep4)[28,40–42] and various exoproteases including leucine aminopeptidases (Laps) that are non-specific aminopeptidases and Dpps.[3,29,41,43] In comparison, in a neutral protein medium, *A. fumigatus* secretes also Laps and Dpps as exoproteases, but only one subtilisin[44] and one fungalysin (Mep)[45] as endoproteases. Dermatophytes and *Aspergillus* spp. Subs, Meps, Laps, Dpps secreted at neutral pH revealed comparable substrate specificity.[43] Thus, protein degradation by dermatophytes at neutral pH occurs in a way similar to that of *Aspergillus* spp. Subtilisins and fungalysins digest proteins in large peptides, which are subsequently digested into amino acids and short peptides by the synergic action of Laps and DppIV. In this process, Laps degrade peptides from their N-terminus until an X-Pro sequence that acts as a stop for these non-specific aminopeptidases.[43,46] However, in a complementary manner, X-Pro can be removed by DppIV, which allows Laps access to the following residues for further sequential degradation.

In dermatophytes, efficient keratin degradation by endoproteases has to be accompanied by the simultaneous reduction of cystine disulphide bridges, which are mainly responsible for the resistant nature of hard keratin.[47] During keratin degradation, dermatophytes excrete sulphite as a reducing agent[48] able to directly cleave disulphide bonds of the keratin into cysteine and *S*-sulphocysteine. Once disulphide bridges are cleaved by sulphitolysis, reduced proteins become accessible for further digestion by various endo- and exoproteases secreted by the fungi. Sulphite is produced from cystein metabolism, and is secreted by dermatophytes and filamentous fungi using a sulphite efflux pump encoded by the gene *SSU1*.[47] The high expression of *SSU1* is characteristic of dermatophytes, which renders these fungi to be able to efficiently degrade the *stratum corneum*, hair and nails.[47] A model of keratinised tissue degradation pathway by the dermatophytes at neutral pH is given in Fig. 1.

Dermatophyte gene expression during infection

Among the multiple proteases isolated from culture supernatants, only *M. canis* Sub3 was identified so far by

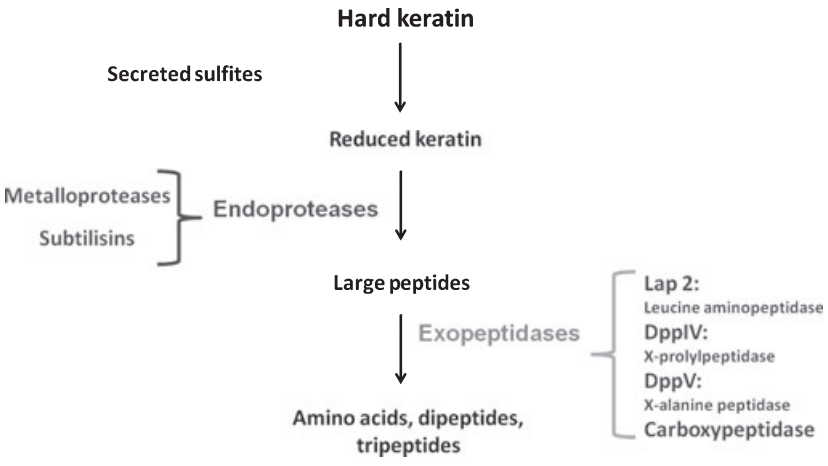

Figure 1 Representation of keratinised tissue degradation pathway by the dermatophytes at neutral pH.

a clear immunohistochemical signal in the intradermal and the intraepidermal portions of infected hair structures in cats using specific anti-Sub3 IgG.[42] Interestingly, Sub3 was also shown to have a role in adherence to feline epidermis.[22]

Using a guinea pig infection model with the zoophilic dermatophyte *Arthroderma benhamiae*, which causes highly inflammatory cutaneous infections in humans and rodents, cDNA microarray analysis revealed an *in vivo* protease gene expression profile totally different from the pattern elicited during *in vitro* growth on keratin.[49] None of the genes encoding the *in vitro* metalloproteases Mep1, Mep3 and Mep4, leucine aminopeptidases Lap1 and Lap2 or dipeptidyl peptidases DppIV and DppV was upregulated during infection. In contrast, we found the gene encoding the serine protease subtilisin Sub6 (orthologue of the major allergen Tri r2 in *T. rubrum*[50]) as the most upregulated *A. benhamiae* sequence, whereas this gene was not detectably activated during *in vitro* growth on keratin. Besides *SUB6*, the closely related *SUB7* as well as *SUB1* and *SUB2* were specifically upregulated *in vivo*. The strong expression of *MCPA* gene encoding a metallocarboxypeptidase also appears pathogenetically relevant. Secreted proteases are therefore suggested to fulfil important functions that are not exclusively associated with the degradation of keratin.

Moreover, this cDNA microarray analysis allowed the identification of genes encoding other candidate pathogenicity related factors in *A. benhamiae*, such as heat shock proteins, transporters, an opsin-related protein and metabolic enzymes.[49] Of particular interest was the strong upregulation of key enzymes of the glyoxylate cycle (i.e. isocitrate lyase and malate synthase). However, mutant analysis of specifically constructed *A. benhamiae* knock-out strains has excluded a pathogenic role of malate synthase in guinea pig dermatophytosis or during epidermal invasion in a model of reconstituted human epidermis.[51] This first broad transcriptional profiling approach during dermatophyte infection gives new molecular insights into pathogenicity associated mechanisms involved in dermatophytoses.

Conclusion

The knowledge of adherence and invasion mechanisms of dermatophytes remains currently limited. However, an improvement in this research field was achieved in the last few years due to the development of *in vitro* and *in vivo* experimental models, thanks to the availability of genome sequence information. Keratinolytic proteases secreted during *in vitro* growth on proteins were assumed to exert a major role during *in vivo* infection, as dermatophytes almost exclusively parasite cutaneous *stratum corneum*, hair and nails. However, this view is too simplistic, as the gene expression profile during infection was found to be remarkably different from the profile displayed by the fungus during *in vitro* growth in keratin medium. The development of genetic tools and the recent availability of seven dermatophytes genomes[39] (http://www.broadinstitute.org/annotation/genome/dermatophyte_comparative/MultiHome.html) will be of great importance to elucidate the mechanisms of host-dermatophyte interactions and invasion.

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