WALAILAK JOURNAL
http://wjst.wu.ac.th MiniReview

Virulence Factors Involved in Pathogenicity of Dermatophytes

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Received: 4 December 2014, Revised: 17 January 2015, Accepted: 10 February 2015

Abstract

Pathogenic dermatophytes are prevalent causes of a superficial cutaneous infection, which have the ability to invade keratinized structures such as skin, hairs and nails. Dermatophytes infection in the host involves 3 main steps: adherence to the host tissue, invading, and the development of a host response. In the first stage of infection, dermatophytes adhere to the surface of the keratinized tissue to reach the epidermis by using some factors that mediate adherence of dermatophytes. Various virulence factors are secreted from dermatophytes during the invading process in order to penetrate the host tissue, to obtain nutrients and survive. Antigens or metabolites from dermatophytes induce host cells to respond to pathogens by activating intracellular signaling pathways that induce the immune response against dermatophytes. Virulence factors involved in pathogenicity of dermatophytes are briefly described in this review that contribute to a better understanding of the function of virulence factors in the dermatophytes process.

Keywords: Dermatophytes, virulence factors, pathogenesis, *Trichophyton*, *Epidermophyton*, *Microsporum*

Introduction

Dermatophytes are keratinophilic fungi that belong to the genera *Trichophyton*, *Epidermophyton* and *Microsporum*, which exclusively infect the stratum corneum, nails or human hair. Groups of dermatophytes are divided into 3 groups according to their habitat: anthropophilic (human associated), zoophilic (animal associated) and geophilic (soil habitat) [1]. Dermatophytes produce virulence factors such as keratinases and cellulase to penetrate stratum corneum of host tissues and produce disease. This is a common opportunistic pathogen that uses several kinds of virulence factors for infection. The common virulence factors involved in pathogenicity of dermatophytes are briefly described here.

Adherence to the host tissue

The first step of dermatophytes infection involves contact and adherence of the infectious elements from dermatophytes. The transmission of dermatophytoses may occur by direct contact with infected animals and humans or by indirect contact with contaminated fomites [2]. Dermatophytes adhere to the surface of the keratinized tissue to reach the epidermis by germination of arthroconidium and then the hypha enters the stratum corneum. There is a time dependence to increase the number of adhering spores. Aljabre *et al.* [3] found that adherence of arthroconidia from *Trichophyton mentagrophytes* in stripped sheets of stratum corneum or separate keratinocytes requires approximately 6 h and germination of the conidia begins by 4 h. In the other experiment using layers of fingernail keratin, adherence and germination of *T. mentagrophytes* arthrospores were observed at 6 h and at 16 h, respectively [4]. Adherence of *Microsporum canis* arthroconidia to reconstructed interfollicular feline epidermis was also found to be time-dependent, starting at 2 h post-inoculation and still increasing at 6 h [5]. A skin cross-

sections experiment showed that *T. mentagrophytes* needs 12 h for adherence, 24 h for germination, and 3 days for invasion of the stratum corneum [6]. They not only showed a time dependent increase in the number of adhering spores to the skin surface but also showed polymeric material mediating between microconidia and stratum corneum cells that probably play an important role in the attachment of spores to skin [6].

At present, the knowledge of the factors that mediate adherence of dermatophytes is little known. However, it has been suggested that the mannose and galactose that are present on the skin surface are carbohydrate-specific adhesins recognized by *Trichophyton rubrum* and *T. mentagrophytes* [7,8]. These adhesins are probably involved in the first step of infection. Moreover, it has been suggested that dermatophytic-secreted proteases are necessary for the adherence process. They found that subtilisin (Sub) 3 of *M. canis* serine protease was involved in the adherence process by using reconstructed interfollicular feline epidermis as a model [5]. This result correlates with *in vivo* experimental infection in a guinea pig model. They found that a role in pathogenicity of *M. canis* could be attributed to a Sub3 protease, which is required for adherence to the epidermis [9].

Invasion

After dermatophytes adherence to keratinized tissue, the spores must germinate followed by penetration to the stratum corneum. The ability of dermatophytes to degrade keratin is considered a major virulence attribute [1]. During penetration, dermatophytes produce a variety of virulence factors for infection that include both enzymes and non-enzymes. Patterns of gene expression of dermatophyte virulence factors in the host or cultures have been studied.

Virulence enzymes

Dermatophytes secrete a variety of virulence enzymes that have different substrate specificities such as protease, lipase and cellulase. Dermatophytes secrete many enzymes to obtain the nutrients to develop and survive. The macromolecules that are present in the host tissue are used as a source of carbon, nitrogen, phosphorus and sulfur for dermatophytes [2]. Moreover, it had been suggested that released enzymes from dermatophytes also act an antigens and induce various degrees of inflammation [10].

Among the wide variety of enzymes secreted by dermatophytes, protease enzymes are the most studied and are the major type of the virulence factors from dermatophytes involved in invasion and utilization of the stratum corneum of the host [11]. Like other fungal pathogens, dermatophytes secrete proteases as virulence factors. It has been suggested that dermatophytes secrete proteases in response to the presence of the components of the skin during tissue invasion. Some authors suggest that dermatophytes secrete proteases to facilitate and are even necessary for an efficient adhesion of these pathogens to the host tissue. Furthermore, secreted proteases from dermatophytes also trigger immune response [2].

Previous studies have found that proteolytic digestion of hard keratin would not be possible without prior reduction of disulfide bridges [12]. Since keratin is composed of high disulfide bridges of cysteine that is necessary for the stability of protein, therefore, before dermatophytic keratinolytic proteases act, disulfide bridges of keratinized tissues are reduced within the compact protein network by sulfite [13]. In the presence of sulfite, cystine in keratin is cleaved to cysteine and *S*-sulphocysteine, and thereby, reduced proteins become accessible to hydrolysis by a variety of secreted proteases [2]. It has been suggested that this reduction in the dermatophytes depends on a sulfite efflux pump encoded by the *SSU1* gene, belonging to the tellurite-resistance/dicarboxylate transporter family [12].

The proteases are classified following their active sites: aspartic, cysteine, glutamic, metallo, serine and threonine proteases. In addition, proteases can be divided into endoprotease and exoprotease. Endoprotease cleaves peptide bonds within a polypeptide. Exoprotease cleaves peptide bonds only at the N- or the C-terminus of polypeptides [14]. Many fungal species of dermatophytes secrete endoproteases and exoproteases when cultured in a medium containing protein as a sole nitrogen source. The major endoproteases secreted from dermatophytes are serine protease and metalloprotease (**Table 1**).

Aminopeptidase, carboxypeptidase and dipeptidyl-peptidase are exoproteases isolated from dermatophytes culture supernatants. *T. rubrum* grown in soy protein liquid medium and keratin liquid medium secreted two leucine aminopeptidases (Lap), Lap1 and Lap2, and two dipeptidyl-peptidases (Dpp), DppIV and DppV. Lap1 and Lap2 are metalloproteases while DppIV and DppV are glycoproteins of approximately 90 kDa that classified as serine proteases with a Ser, Asp, His catalytic triad [15]. Dermatophytes also secreted a metallocarboxypeptidase A (McpA) and two serine carboxypeptidases (Scp), ScpA and ScpB [14]. Although protease enzymes from dermatophytes were initially studied, the other enzymes apart from proteases were also studied and have been identified as virulence factors for dermatophytes. Hellgren and Vincent [16] found that the capacity of dermatophytes to parasitize the host depends on the action of lipase and other enzymes required for keratin degradation.

Table 1 Secreted endoproteases purified from dermatophytes culture supernatants.

Dermatophyte species	Molecular mass (kDa)	Protease classes	Culture medium	References
T. rubrum	27	Serine protease	Sabouraud dextrose broth	[17]
T. rubrum	34.7	Serine protease	Glucose-peptone broth	[18]
T. rubrum	36	Serine protease	Sabouraud dextrose broth	[19]
T. rubrum	44	Serine protease	Sabouraud dextrose broth	[19]
T. mentagrophytes	38 - 41	Serine protease	Sabouraud dextrose broth	[20]
T. mentagrophytes	48	Serine protease	Keratin medium	[21]
T. mentagrophytes var. *erinacei*	33	Serine protease	Glucose-peptone broth	[22]
T. vanbreuseghemii	37	Serine protease	Modified Czapek-Dox liquid medium	[23]
M. canis	31.5	Serine protease	Medium containing cat keratin	[24]
M. canis	43.5	Metalloprotease	Medium containing feline keratin	[25]
M. canis	45	Serine protease	Medium with human hair	[26]
M. canis	31.5, 34 and 48 (Ekase)	31.5 and 34-kDa fragments are serine protease, 48-kDa fragment is metalloprotease	Medium with human hair	[27]

Non-enzyme virulence factors

Other virulence factors apart from virulence enzymes of dermatophytes have been studied. Knowledge of pathogenesis mechanisms of other fungi were used to predict virulence factors in dermatophytes. Xanthomegnin, a mutagenic mycotoxin best known as an agent of nephropathy and death in farm animals exposed to food-borne *Penicillium* and *Aspergillus* fungi, was predicted as a virulence factor of *T. rubrum* during human infection. It could be extracted from human nail and skin material infected by *T. rubrum* but not detected in uninfected nails [28]. Melanin or melanin-like compounds of dermatophytes was also predicted to play a role in the pathogenesis of dermatophytic diseases in infection of *Microsporum gypseum*, *Epidermophyton floccosum*, *T. mentagrophytes* and *T. rubrum* based on the known role of melanins in other pathogenic fungi [29].

Virulence genes

The recent sequencing of several genes from dermatophytes has been completed. Their sequence information is used for prediction about which genes are involved in virulence based on sequence similarity with other fungi genes where the pathogenesis mechanisms are known [1]. Any genes must be tested experimentally to confirm expression and role during infection before being identified as virulent. The protease gene is the one of the dermatophyte genes that has been identified as a virulence factor. For example, *SUB1*, *SUB2* and *SUB3* that encode a subtilisin family of serine protease are produced by *M. canis* during the invasion of keratin [30]. Moreover, 2 metalloprotease (*MEP*) genes, *MEP2* and *MEP3*, of *M. canis* are also produced during the infection of guinea pigs [31]. At least 22 distinct *T. rubrum* protease genes such as *SUB3*, *SUB4*, *LAP1* and *LAP2* are involved in protein digestion [31]. The *T. mentagrophytes 4 (Tri m 4)* protease gene increases transcription during growth of *T. mentagrophytes* with keratin [32]. Two single *M. canis* genes, *DppIV* and *DppV*, coding for secreted dipeptidyl peptidases of exoproteases could have specialized functions in the host-fungus relationship [33]. However, not all keratin-induced proteases play a role during infection. For example, the zoophilic dermatophyte *Arthroderma benhamiae* (a teleomorph of *T. mentagrophytes*) that causes inflammatory infection in humans expressed both endoprotease and exoprotease genes during growth on keratin-soy. Endoprotease genes encoding major keratinases and strong upregulated on keratin-soy from *A. benhamiae* were serine proteases (Sub3 and Sub4) and metalloproteases (Mep1, Mep3 and Mep4). A significant expression of exoproteases was also detected from *A. benhamiae* such as leucine aminopeptidases (Lap1 and Lap2), dipeptidyl peptidases (DppIV and DppV), metallocarboxypeptidase (McpA), and serine carboxypeptidase (ScpB) [34]. However, some of the keratin-induced genes of *A. benhamiae* were not upregulated during guinea pig infection. Only the *MCPA* gene was strongly induced during both infection and growth on keratin [34]. This finding correlates with research of Burmester *et al.* [35] who found that only some of the typically keratin-induced proteases secreted from *A. benhamiae* were strongly expressed during fungus-keratinocyte interaction.

Moreover, a previous study identified that non-protease genes were upregulated during infection such as genes encoding key enzymes of the glyoxylate cycle (the putative malate synthase and isocitrate lyase) and an opsin-related protein in *A. benhamiae* [34]. The glyoxylate cycle has been implicated in virulence of other microorganisms [36]. A fungal thioredoxin and cellulase homolog genes were identified as putative *T. mentagrophytes* virulence factors that had increased transcription during growth of *T. mentagrophytes* with keratin [32].

The expression mechanisms of dermatophyte virulence genes are little known. However, they suggested that keratinolytic activity of dermatophytes was probably induced [13]. The gene activation could be controlled by a transcription factor from the GATA family, zinc-finger transcription factors that induce the expression of a whole series of genes in response to a change in the nitrogen source [13]. A previous study found that some species of dermatophytes had different mechanisms of expression such as expression of endoproteases from *T. rubrum* that are upregulated by PACC, another zinc-finger transcription factor [37].

Development of a host response

When dermatophytes invade keratinized tissue, an innate immune response in the host tissue is induced by antigens or metabolites from dermatophytes. The components of the cell walls of dermatophytes such as chitin, glucan and glycopeptides represent the major antigens from these organisms. Therefore, antigenic substances from dermatophytes may be glycopeptides, peptides, or carbohydrates. Each type of antigens may induce different types of responses [38]. Moreover, secreted keratinases from dermatophytes may also influence immune defenses such as Sub3 and Mep3 from *M. canis* [13].

The main immune response is production of Th1-type adaptive immune response with the production of proinflammatory cytokines like interleukin (IL)-2 and interferon (IFN)-γ [39]. This response is induced to control the infection. The many soluble factors capable of regulating the immune response such as growth factors, interleukins (IL-1, IL-3, IL-6, IL-7, IL-8), and colony-stimulating factors are secreted from keratinocytes [2]. Antigens derived from dermatophytes can induce immediate (type I) or delayed (type IV) hypersensitivity skin test reactions. Immediate hypersensitivity responses are associated with chronic recurrent infections that produce high levels of immunoglobulin E, immunoglobulin G4 and Th2 cytokines by mononuclear leukocytes. Delayed-type hypersensitivity is associated with acute dermatophytosis [40]. The 29-kDa subtilase homologue, *T. rubrum* 2 (Tri r 2), can induce immediate hypersensitivity and delayed-type hypersensitivity skin tests [40]. An 83-kDa DppV, *Trichophyton tonsurans* 4 (Tri t 4), also induces delayed-type hypersensitivity and T lymphocyte cytokine profiles *in vitro* [41]. The degree of intenseness of immunologic response depends on the type of metabolites and enzymes released by the agent and immunosuppression, caused by the metabolites in anthropophilic dermatophytes [39]. Moreover, different species of dermatophytes cause different immunologic responses, zoophilic or geophilic dermatophytes cause intense immunologic response [39]. They found that several cytokines were secreted from keratinocytes in response to *A. benhamiae* infection but cytokine secretion from keratinocytes was limited in response to *T. tonsurans* infection that may induce a minimal inflammatory response in the skin [42].

Even though dermatophyte infections induce immune response in the host, some dermatophytes can avoid the immune response in chronically infected patients. They found that *T. rubrum* cell wall mannans involved in an immunosuppression. It can inhibit lymphoproliferative response of mononuclear leukocytes in response to several antigens, mitogens and stratum corneum turnover [43,44]. Furthermore, killing macrophages is probably another mechanism for *T. rubrum* to avoid the immune response [13]. The interaction of *T. rubrum* conidia with resident macrophages results in the production of tumor necrosis factor α (TNF-α) and IL-10 but not IL-12 and nitric oxide, that down regulates the expression of co-stimulatory molecules (CD80 and CD54) and decreases cells expressing major histocompatibility complex class II molecules and finally results in macrophage death [45]. Previous studies have found that IgG, IgA and IgM antibodies do not appear to protect in dermatophytes infections because uninfected humans have low levels of these antibodies [38,46].

Conclusions

Superficial dermatophytosis is a common fungal infection in humans. In pathogenicity, dermatophytic adhesion is begins with the use of mediate adherence factors. During penetration, dermatophytes secrete several kinds of virulence factors that are key factors in the invasion and utilization of the stratum corneum of the host. Therefore, an understanding of the specific virulence factors involved in pathogenicity of dermatophytes would assist in the development of new therapeutic approaches. However, the strategies dermatophytes use to avoid or inhibit the immune reaction also need to be investigated to design new therapeutics in the future.

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