

# Immunopathogenesis of Dermatophytoses and Factors Leading to Recalcitrant Infections

## Abstract

The pathogenesis of dermatophytic infections involves the interplay of three major factors: the dermatophyte, the inherent host defense, and the adaptive host immune response. The fungal virulence factors determine the adhesion and invasion of the skin while the immune response depends on an interaction of the pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMP) with pattern recognition receptors (PRRs) of the host, which lead to a differential Th (T helper) 1, Th2, Th17, and Treg response. While anthropophilic dermatophytes *Trichophyton rubrum* and now increasingly by *T. interdigitale* subvert the immune response via mannans, zoophilic species are eliminated due to a brisk immune response. Notably, delayed-type hypersensitivity (Th1) response of T lymphocytes causes the elimination of fungal infection, while chronic disease caused by anthropophilic species corresponds to toll-like receptor 2 mediated IL (interleukin)-10 release and generation of T-regulatory cells with immunosuppressive potential. Major steps that determine the ultimate clinical course and chronicity include genetic susceptibility factors, impaired epidermal and immunological barriers, variations in the composition of sebum and sweat, carbon dioxide tension, skin pH, and topical steroid abuse. It is important to understand these multifarious aspects to surmount the problem of recalcitrant dermatophytosis when the disorder fails conventional therapeutic agents.

**Keywords:** *Anthropophilic species, epidermal barrier, fungal virulence factors, immune responses, recalcitrant dermatophytosis, skin pH, steroid abuse, Th1 and Th 17 cells*

## Introduction

The pathogenesis of dermatophytic infections involves three major factors, and it is important to understand the interplay between them, in the era of recalcitrant dermatophyte infections.<sup>[1,2]</sup> These factors include the fungi, the inherent host factors including the skin barrier function, and the immune response mounted against the fungus. Among these, the type of fungi and immune response of the host play a major role, and these are crucial in causing recalcitrance and relapses. Dermatophytes belong to 3 genera -Trichophyton, Epidermophyton, and Microsporum. These are divided into anthropophilic, zoophilic, and geophilic according to their primary habitat. While possibly in other parts of the world, anthropophilic dermatophyte *Trichophyton rubrum* is a common cause, but this is now being increasingly replaced by *T. interdigitale* in some geographical locations.<sup>[1,3,4]</sup> *T. interdigitale* is currently

the prevalent strain in India and causes mild inflammation and chronic infections. There are known variations in the fungal virulence factors among different species, and it is likely to be playing a role in the current scenario the country is facing. For example, protease and lipase enzymes vary significantly between *T. tonsurans* and *Trichophyton equinum*.<sup>[5]</sup> This has been reinforced by a study where comparative proteomic analysis of *T. rubrum* and *Trichophyton violaceum* revealed differences in the amount and specificity of secreted proteins between them.<sup>[6]</sup>

Experimental models are required to transcend speciation and to assess the virulence factors and the immuno-pathogenetic pathways of diverse dermatophytes species. The immune responses elicited by zoophilic dermatophytes are studied using guinea pigs and mice. In contrast, in anthropophilic species, studies have been performed in ex-vivo models using epidermal tissues

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and keratinocyte co-cultures, which could have direct applicability to clinical situations.<sup>[7]</sup> In spite of this methodological issue of translation of research, we have attempted in this review to distill the vast amount of data to help understand the complex pathogenesis.

### *Dermatophyte specific factors*

The immune response stimulated by Dermatophytes vary depending on the species stimulate an immune response. *Trichophyton rubrum* causes chronic infections, possibly by the release of glycopeptides that can inhibit the proliferation of T lymphocytes in vitro, thereby suppressing host immunity.<sup>[8]</sup>

The dermatophytes' armamentaria mainly comprises of the surface molecules for adhesion, secreted enzymes to degrade and metabolise host molecules for nutrition, thermo-tolerance, and dimorphism (i.e., converting between mycelial and conidial forms depending on ambient conditions). It is important to emphasize that though certain factors related to the potential host predispose to dermatophytoses, it is the dermatophyte virulence factors which are primarily implicated for causing infection, regardless of the patient's immune status<sup>[9]</sup>

The major steps involved in the establishment and perpetuation of dermatophyte infection are detailed below:

### *Adhesion*

The initial step is the interaction of dermatophytes with the host tissues, and adhesion to the epidermis occurs within 1 hour. Adhesins present on the cell wall of fungi are crucial to the initial attachment. The molecules implicated include Sub3 secreted protease of the subtilisin family in *M. canis*, and *sowgp* gene and dipeptidyl-peptidase IV (DppIV) of the *Trichophyton* spp.<sup>[10,11]</sup> This is followed by germination, where arthroconidia germinate, and the hyphae quickly enter the stratum corneum to prevent elimination with cell shedding, which occurs within 3-4 hours.<sup>[12]</sup> Between 24 hours and 3 days, the hyphae spread through the skin. Fibril like structures have been demonstrated in the case of *Trichophyton mentagrophytes*, facilitating adhesion and thereby preventing elimination from the host tissue. The arthroconidium becomes flat, and the fibril-like structures become short and fine when it invades the deeper layers of the epidermis, leading to increased contact surface with the host tissue and thereby better adhesion and more nutrient acquisition.<sup>[13]</sup>

On the 4<sup>th</sup> day, the hyphae reach the granular layer, which coincides with a loss of the integrity of the epidermal barrier.<sup>[14]</sup> Consequently, the keratinocytes release antimicrobial peptides (AMP) and pro-inflammatory cytokines, resulting in control and, ultimately, the resolution of infection via the immune system's stimulation.<sup>[15]</sup> Further, there may be a role of biofilm formation by making connections between adjacent arthroconidia.<sup>[16]</sup>

### *Enzymatic activity and virulence factors*

A critical virulence factor of dermatophytes is best distinguished by its enzymatic activity. Dermatophytes produce several enzymes, including proteases, lipases, elastases, collagenases, phosphatases, and esterases, which have been implicated in host invasion and assimilation of nutrition.<sup>[16]</sup> The proteolytic enzymes, notably the keratinolytic proteases (keratinases), have a well-established role in the pathogenicity of dermatophytoses helping in invasion into tissues as well as providing oligopeptides and amino acids on keratin breakdown, which are important nutrients for the fungi. It was found that the *Trichophyton* spp. had the highest keratinase activity in a study that compared the keratolytic action of *T. rubrum*, *T. interdigitale*, *M. canis*, and *M. gypseum* using spectrophotometry.<sup>[17]</sup> This high keratinase activity of *Trichophyton* spp. has been implicated for adaptation of these dermatophytes to human skin and is referred to as "anthropization."<sup>[17]</sup>

Lipids are also degraded and utilized by the dermatophytes using relevant enzymes. Probably because of the destructive effects and the resulting alteration in epidermal differentiation by dermatophytes, the epidermal barrier of the glabrous skin is distinctly impaired in tinea.<sup>[18]</sup> However, in nails, before the action of the keratinolytic proteases can commence, the abundant disulphide bonds present within the hard keratin must be broken. This is achieved by a sulfite efflux pump, encoded by *TruSsu1* gene in *T. rubrum*. Cysteine dioxygenase (CDO) activity is also essential for the same, and hence *arthroderma benhamiae cdo1* and *ssu1* knockout mutants were found unable to grow on hair and nails.<sup>[19]</sup>

### *The role of ambient pH*

The initial environment encountered by the dermatophytes on skin and nail is acidic. The acidic pH is maintained by a complex balance between acidifying (products from glands and their breakdown by resident flora, filaggrin-histidine-urocanic acid pathway-related breakdown products) and alkalizing (ammonia, carbon-dioxide, and bicarbonates) factors.<sup>[1]</sup>

Proteases that function optimally at an acidic pH are unsuppressed, and keratin utilization begins. The use of keratin as the source of carbon generates metabolites which shift the ambient pH to alkaline. This is associated with the upregulation of proteases with optimal function at an alkaline pH. Thus, the fungus can obtain nutrition from proteins over a wide pH range.

This adaptive pH regulatory mechanism is an important virulence factor and depends on the PacC/Pal signal transduction pathway, which is highly conserved on pathogenic fungi, including dermatophytes. PacC is a transcriptional regulator regulating the pH-dependent gene expression<sup>[20]</sup> The proteolytic cleavage of PacC is triggered

by the signaling cascade which is composed of the six pal genes (palA, palB, palC, palF, palH, and palI) that senses the alkaline pH.<sup>[21-26]</sup> While the initiation of dermatophytic infection is promoted by the genes upregulated at acidic pH, those that function at alkaline pH are crucial for its maintenance. Also, the growth in keratin cultures containing hsp90 inhibitor lowered the pacC transcripts concentration, indicating an association between pacC and hsp90 that may affect fungal virulence.

### **The role of heat shock proteins (HSP)**

Various HSPs have been found to be over-expressed during dermatophyte invasion of host tissues. *T. rubrum* grown on human nails *in vitro* resulted in an increased expression of hsp60, hsp70, and hsp78 genes, while hsp70, hsp90, hsp related gene hsf1 and hspSSc1 are overexpressed when it is grown on skin.<sup>[20]</sup> Fungal virulence on the human nail was diminished when 17-allylamino-17-demethoxygeldanamycin (17-AAG, Hsp90 inhibitor) was used in an inhibitor concentration-dependent manner, suggesting the involvement of this HSP in pathogenicity.<sup>[27]</sup> Chemical inhibition of hsp 90 results in increased susceptibility of *T. rubrum* to itraconazole and micafungin, which can be used clinically as this is suggestive of a potential for combination therapy with existing antifungals.

### **Toxin secretion**

Toxin production has also been implicated, and the salient compounds are xanthomegnin released by *T. megninii*, *T. rubrum*, and *T. violaceum*, hemolysins released by *T. rubrum* and *T. interdigitale* and<sup>[28,29]</sup> lipophilic toxins, such as xanthomegnin and aflatoxin-like substances all of which are known to have immunosuppressive effects.<sup>[30]</sup>

### **Role of mannan**

Mannan, a glycoprotein component of the fungal cell wall, facilitates infection by inhibiting the proliferation of keratinocytes, thus preventing shedding, and suppression of the inflammatory response. *T. rubrum* produces mannan in larger amounts than *M. canis*.<sup>[16]</sup> Furthermore, mannan produced by *T. rubrum* inhibits cell proliferation and lympho-proliferation more effectively.

### **Biofilm formation**

Although many dermatophyte strains have been shown to form biofilms under experimental conditions and on ex-vivo nail tissue, their existence on the skin in tinea corporis/cruris has not yet been demonstrated.<sup>[31]</sup>

### **Host Immune Response to Dermatophytes**

There are several host defense mechanisms that prevent the establishment of tinea infections, including the physical and chemical composition of skin, UV light exposure, lack of humidity and temperature, action of phagocytic cells, and commensal microbiota. The rapid

proliferation of keratinocytes also plays an important role in defense against dermatophyte infections by continual renewal and epithelial shedding. While there is a suggestion that commensal skin fungi may play a role in host-dependent immunity and the various fungi isolated including *Malassezia*, *Penicillium* and *Aspergillus* amongst others (*Alternaria*, *Candida*, *Rhodotorula*, *Cladosporium* and *Mucor*) — their interaction with dermatophyte is as yet unknown though they may influence the Th17 cell and its pathway.<sup>[32]</sup> The various host defense mechanisms are listed in Table 1.

### **Dermatophyte- keratinocyte immune interaction**

Keratinocytes are the first cellular elements that come into contact with dermatophytes during infection and are capable of modulating the host immune response.<sup>[33]</sup> Upon exposure to dermatophytes or their antigenic determinants, the keratinocytes produce a wide range of cytokines, including interleukin (IL)-8 (a potent neutrophil chemotactic factor), the pro-inflammatory cytokine TNF (tumor necrosis factor)- $\alpha$ , IFN (interferon)-  $\gamma$ , IL-1 $\beta$ , IL-22 and IL-16 among others, to destroy dermatophytes [Figure 1].<sup>[34]</sup>

The ability to prompt the secretion of pro-inflammatory cytokines in the keratinocytes varies among the dermatophytes species. Zoophilic species like *Arthroderma benhamiae*, can lead to the expression of various cytokines by the keratinocytes, which lead to enhanced inflammation. On the contrary, *T. tonsurans*, an anthropophilic dermatophyte, has a limited ability to induce cytokines, thus leading to less inflammation.<sup>[35,36]</sup>

A study comparing the cytokine profile of *T. tonsurans* and *A. benhamiae* found that *T. tonsurans* triggered eotaxin-2, IL-16, and IL-8 secretion from the infected keratinocytes, whereas *A. benhamiae* produced a wide variety of pro-inflammatory and immunomodulatory cytokines/chemokines.<sup>[36]</sup> Complementary DNA microarray analysis shows that the genes encoding IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-10, IL-13, IL-15, IL-16, IL-17, and IFN-  $\gamma$  were upregulated by *A. benhamiae*, while *T. tonsurans* only upregulated genes encoding for IL-1 $\beta$  and IL-6. Also, both dermatophytes enhanced the IL-8 mRNA expression in keratinocytes. It was also found that *T. mentagrophytes* induces higher expression of IL-1 $\alpha$  by keratinocytes than *T. rubrum*.<sup>[37]</sup> Another recent study conducted to distinguish the change in the gene

**Table 1: Summary of the various host defense mechanisms against Dermatophyte Infection**

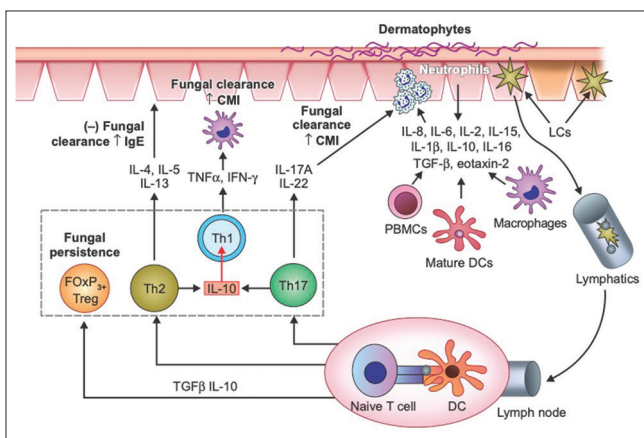
1. An increase in cell proliferation rate
2. An increase in anti-microbial peptides (AMP) including beta defensins 2 and 3, RNase7 and psoriasin
3. Fungal phagocytosis by neutrophils and macrophages
4. Complement mediated mechanisms
5. A complex immune response which helps to eliminate the infection

expression of HaCaT (Cultured human keratinocyte) cell line following dermatophytic infection found that pro-inflammatory cytokines and chemokines triggered the infiltration of neutrophils and other immune cell to the infection site following zoophilic *M. gypseum* infection due to the upregulated expression of genes related to immune response, while with *T. rubrum*, metabolic pathway genes were upregulated instead immune response-related genes.<sup>[38]</sup> This corroborates with the clinical observations that in acute infections provoked by the zoophilic fungi *T. mentagrophytes* and *M. gypseum*, marked neutrophils are seen in the epidermis, whereas *T. rubrum* lead to chronic infections and are characterized by a mononuclear infiltrate.<sup>11</sup> Trichophyton-induced inflammation causes the proliferation of peripheral blood mononuclear cells (PBMC) and the production of IFN- $\gamma$  and IFN- $\gamma$ -positive CD4<sup>+</sup> cells leading to a Th1 response<sup>[39,40]</sup> [Figure 1].

In addition to the vigorous immune response, antimicrobial peptides (AMP) such as cathelicidins and defensins are secreted by the human keratinocytes with likely antifungal activity as well.<sup>[41]</sup> *In vitro* fungistatic and fungicidal activity of human-defensins and cathelicidin (LL-37), respectively against *T. rubrum*, has been shown by several authors, and also there is increased expression of AMP *in vivo* in tinea corporis caused by this trichophyte.<sup>[18,42]</sup>

### Innate immune response

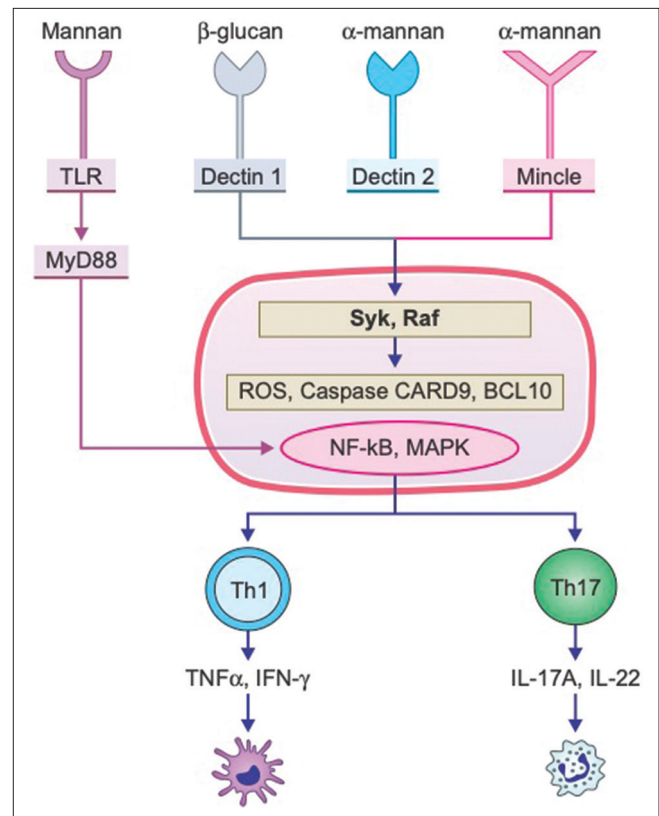
Epidermal dendritic cells (DC), especially Langerhans cells (LC), are crucial in initiating and modulating the adaptive immune responses against dermatophytes. A recent study has shown that a reduced number of LC in the epidermis increases the risk of dermatophyte infections.<sup>[43]</sup> These cells contain receptors for pathogen-associated molecular patterns (PAMPs) called



**Figure 1:** A depiction of the immune responses to dermatophyte infection. While Th1 and Th17 response leads to clearance of the dermatophytes, Th2 response inhibits fungal clearance and persistent Treg activation leads to chronic persistent infections. The immune response elicited also varies with the dermatophyte species involved. While zoophilic dermatophytes such as *Arthroderma benhamiae* induce a wide range of cytokines, anthropophilic dermatophytes such as *Trichophyton rubrum* induce the production of a limited array of mediators (highlighted in bold)

pattern recognition receptors (PRRs), such as, Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and the galectin family proteins, that sense the PAMPs and the damage-associated molecular patterns (DAMPs) that are present on fungi. [Figure 2] The major PAMPs for dermatophytes are the cell wall components, mainly glucans and mannans. Apart from TLRs, CLRs form the major receptors involved in recognition of the PAMPs of dermatophytes. These include Dectin-1, Dectin-2, Dectin-3, MR, Mincle, and dendritic cell-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin (DC-SIGN) [Figure 2].

DC-SIGN (CD209) is an important PRR and is structurally a type II transmembrane protein belonging to the CLR family and recognizes  $\beta$ -glucan on fungal cell walls.<sup>[44,45]</sup> Dectin-2, a CLR expressed in most DC, such as LC, recognizes and binds to *M. canis* and *T. rubrum* hyphae, causing the production of pro-inflammatory cytokines like IL-12, IL-10, and TNF $\alpha$  and help present the antigens to CD4<sup>+</sup> T cells, promoting their proliferation and release of IL-4, IL-10, and IFN- $\gamma$ .<sup>[46]</sup>



**Figure 2:** A depiction of the interaction of the pathogen-associated molecular patterns (PAMPs) and the pattern recognition receptors (PRR) for dermatophytes. The major PRRs for dermatophytes included the Toll-like receptors (TLR), dectin-1, minicle and dectin-2 which recognize the mannans and glucans on surface of dermatophytes. Their activation initiate a cascade of signals that induce the nuclear factor (NF)- $\kappa$ B and mitogen-activated protein kinase (MAPK) pathways, which in turn stimulate the T-helper (Th) 17 and Th1 cells which play important roles in the host immune response

Dectin-1 is another CLR that functions as a transmembrane PRR for fungal pathogens through its ability to bind  $\beta$ -glucan carbohydrates.<sup>[47]</sup> [Figure 2] Macrophages act as an intermediary of the keratinocyte and dendritic cell-mediated immune responses, and they either kill the fungus or undergo destruction. Toll-like receptor-2 located on the surface of monocytes mediates phagocytosis of conidia as well as promotes the a pro-inflammatory response with the secretion of cytokines such as TNF- $\alpha$ .<sup>[48]</sup> Downstream signaling and the resultant immune effects are depicted in Figure 1.

Besides keratinocytes and DC, neutrophils also play a vital role in innate immunity against dermatophytes that accumulate soon after the corneocyte adherence to the conidia during germination. Neutrophils, along with macrophages, are thought to be the final effector cells in eliminating dermatophytosis and the mediator of intra and extracellular lysis of dermatophytes both via the oxidative pathway and release of TNF- $\alpha$ .<sup>[40,49]</sup> A fungal hydrophobin, released following induction of the expression of hypA gene following interaction of *A. benhamiae* with keratinocytes, renders them less susceptible to neutrophil action.<sup>[50]</sup>

The contribution of innate immunity in clearing fungal infections was recently corroborated by a paper which showed that Rag2-/- mice, which lack T and B cells, can ultimately clear the dermatophyte infection.<sup>[51]</sup> Also, a study in immunocompetent wild-type mice showed that even there was only a modest reduction in the fungal load in a secondary infection versus a primary one, suggesting that innate immunity plays the major role in the immune responses against fungi.

Expression of IL-17 as early as three days after a dermatophyte infection means that there is a rapid activation of IL-17 is reminiscent of the innate Th-17 responses against fungal infections rather than adaptive immune response.<sup>[52]</sup>

### Acquired immune response

The comparative role of specific humoral and cellular immune responses against fungal infections has been a debatable conundrum in mycology. While cell-mediated immunity (CMI) is protective against many fungi, including dermatophytes, certain types of antibody responses may also provide protection. CMI also increases epidermal proliferation facilitating dermatophyte elimination.<sup>[1]</sup> The type of CMI response is critical in determining resistance or vulnerability to fungal infection even though the cytotoxic activity against dermatophytes is seen with both CD4 and CD8 T cells.<sup>[1]</sup>

Overall, the elimination of a fungal infection is mediated by Th1-type CMI, while Th2 response predisposes to infection or leads to allergic responses. Th1 cells produce cytokines such as IFN- $\gamma$  and trigger phagocyte stimulation.<sup>[1,40,53-55]</sup> In contrast, a Th2- response results

in enhanced production of antibodies and IL-4, IL-5, and IL-13.<sup>[1,56]</sup> IL-4 mediates IgE production by B cells while IL-5 helps in eosinophil recruitment via vascular cell adhesion molecule (VCAM)-very late antigen (VLA)-4 adhesion molecule pathway and enhances eosinophil production from the bone marrow.<sup>[1]</sup>

It has been shown that initially, IFN- $\gamma$  level is low along with high levels of IL-10, which inhibit Th1 response. Over time, IL-10 production decreases, while the levels of IFN- $\gamma$  increase. Thus, apart from inducing a Th2-type response, IL-10 plays a significant role in innate immunity and immune response regulation. IL-10 prevents a damaging inflammatory response by blocking excessive production of TNF- $\alpha$  and other cytotoxic metabolites, enabling the development of a specific immune response.<sup>[57]</sup> Interestingly, in a murine model study, the levels of IL-10 were notably higher during the early stage of the infection.<sup>[58]</sup>

Recently, the focus has shifted to the Th17 cell pathway, which promotes the Th1-type immune responses and inhibits Th2-type responses. IL-17A has been shown to mobilize neutrophils and stimulate defensins' secretion, contributing to the rapid and effective control of infection as an innate response.<sup>[59,60]</sup>

The earlier studies on cytokine profile at the dermatophyte infection site reveals TGF- $\beta$ , IL-1 $\beta$ , and IL-6, which are involved in the initiation and perpetuation of the Th17 pathway. Further, an increase in IL-22 mRNA was also observed in experimental models, both findings suggesting that the Th17 cell pathway may be involved in the immunopathogenesis of dermatophytic infections.<sup>[61]</sup>

In more recent studies, these hypotheses have been tested and validated, and the protective role of the Th17 pathway has now clearly emerged. Heinen *et al.* recently reported that with dermatophytic infections, the adaptive immunity is polarized to both Th1 and Th17 responses, with dermatophyte clearance mediated by the Th17 antifungal response and the Th1 response being involved in fungal clearance as well as the down-modulation of Th17 induced inflammation. Also, a study revealed that it may exert antifungal activity by enhancing the epidermal barrier function.<sup>[62]</sup> In fact, cytokines IFN- $\gamma$  and IL-17A, signature cytokines of Th1 and Th17 lineages, respectively, are needed for optimal protection to the disease. Moreover, the authors found that IL-17 and IFN- $\gamma$  show complementary immunological effects during the resolution of *T. benhamiae* infection.<sup>[63]</sup> However, in contrast to these findings, Rai *et al.* demonstrated high Th17 and T-reg expression in peripheral blood of patients with chronic skin dermatophytoses, suggesting a complex interplay of T cell lineages in causing disease persistence.<sup>[64]</sup> A summary of the role of Th cells is listed in Table 2. Here it is pertinent to point out that T Reg cells may effect a variety of sequels ranging from protective tolerance (defined as a host response that safeguards host's survival through a trade-off

**Table 2: Role of various T cells subtypes**

T Cells	Putative role
Th1 cells	<ul style="list-style-type: none"> <li>-Th 1 cell response correlates with protective immunity</li> <li>-Determined by DC response to the combination of TLR and CLR signals provided by fungi</li> <li>- Leads to increase in IFN <math>\gamma</math> , activates phagocytosis</li> <li>-Th 1 cell predicts asymptomatic and mild forms of the infection</li> <li>- Estradiol increases Th1 response</li> </ul>
Th2 cells	<ul style="list-style-type: none"> <li>-IL-4 and IL-13 reduce the protective Th 1 cell response</li> <li>- Favours fungal infections, fungus-associated allergic responses and disease relapse</li> <li>- Limiting IL-4 production restores antifungal resistance</li> <li>- Leads to Elevated levels of IgE, IgA and IgG</li> </ul>
Th17 cells	<ul style="list-style-type: none"> <li>-Activation via the SYK-CARD9, MYD88 and mannose receptor signaling pathways in DCs and macrophages</li> <li>-Promote Th 1-type immune responses and restrain Th 2-type responses</li> <li>-Enables neutrophil action</li> <li>-chronic infection is due to failure to restrain inflammation following IL-17A-dependent neutrophil recruitment, thereby preventing optimal protection and favoring fungal persistence.</li> </ul>
T reg Cells	<ul style="list-style-type: none"> <li>- inverse relationship between IFN <math>\gamma</math> and IL-10 production in patients with fungal infections</li> <li>-High levels of IL-10, which negatively affect IFN <math>\gamma</math> production, are detected in chronic candidal diseases</li> <li>-IL-10 acts as a homeostatic host-driven response to keep inflammation under control</li> <li>-T Reg cell response is to reduce damage &amp; also lead to fungal persistence and immunosuppression</li> </ul>

DC: Dendritic cell; TLR: Toll like receptor; CLR: C-type lectin receptors; IFN  $\gamma$ : Interferon gamma

between sterilizing immune responses and their negative regulation, which limits pathogen elimination) to overt immunosuppression.<sup>[65]</sup>

Interestingly, the severity of the inflammatory reaction depends on the depth of skin invasion, which is largely determined by proteases. It has been proposed that dermatophytes that are weakly invasive elude soluble or cellular components of the immune system by residing in the superficial non-living skin layers.<sup>[66]</sup> Specific immunogenic properties of the secreted proteases may also effect immune defenses. Notably, proteases 'subtilisin' from *T. rubrum* (Tri r2) and 'dipeptidyl-peptidase V' from *Trichophyton tonsurans* (Tri t 4) can modulate the immune responses.<sup>[1,67]</sup> Also, the surface antigen subtilisin Sub1 of *T. rubrum* has also been proposed to play a role in immune-modulation. Moreover, *Trichophyton rubrum* cell wall mannans may be a cause of dose-dependent immunosuppression and inhibit the *in vitro* lymphoproliferative response and stratum corneum turnover either directly or by altering lymphocyte function.

Acute dermatophytosis is associated with a delayed-type hypersensitivity (DTH) response to trichophyton and blastogenic response of T lymphocytes with progression to healing, while persistent disease corresponds to inadequate cellular immune responses with immediate hypersensitivity (IH) responses, high levels of IgE and IgG4 antibodies, and of Th2 cytokines released by mononuclear leukocytes.<sup>[68,69]</sup> Furthermore, chronic dermatophytosis, due to association with IH and Th2 cytokines, may underlie the pathogenesis of allergic diseases, mainly asthma.<sup>[70]</sup>

Though it is largely believed that the immune response to dermatophytes is DTH-dependent, there are contrarian findings. In AIDS, the incidence of invasive dermatophytoses is expected to be higher due to reduced CD4 + T lymphocyte counts. However, this is not seen in practice while it is more frequent in non-AIDS immunocompromised patients, suggesting different immunological mechanisms play a role.<sup>[1,71,72]</sup> Furthermore, patients with chronic dermatophytosis may have DTH against trichophyton.<sup>[73]</sup>

### Host Dependent Factors

Susceptibility to dermatophytosis is variable.<sup>[74]</sup> The risk factors favoring dermatophytosis include defective epidermal and immunological barriers.<sup>[18,67]</sup> Individual susceptibility factors are still unclear but include alteration in sebum fatty acids, presence of moisture or transferrin, and other inhibitors for the dermatophyte growth in sweat or serum and skin, including carbon dioxide concentration.<sup>[1,75]</sup>

An impaired epidermal barrier aggravated by scratching, atopy, ichthyosis, low level of sebum secretion before puberty, and possibly elevated environmental humidity, may promote dermatophytosis.<sup>[1,18,67]</sup> Impaired peripheral blood circulation with concomitant diminished nutrient availability, reduced oxygenation, and delay in the relocation of immune-competent cells or release of antimicrobial peptides (AMP) at infection site may also increase the susceptibility for infections. Diabetes predisposes to an almost three-fold increased chance of dermatophytosis, especially foot and nail tinea, due to altered peripheral blood circulation and nerve

endings.<sup>[76]</sup> Further, extensive and invasive infections have been reported in various underlying conditions such as patients with atopic dermatitis, leukemia or lymphoma, diabetes, hepatitis B and C related cirrhosis, on haemodialysis for renal failure, alcoholic liver disease, congenital adrenal hyperplasia, Cushing disease, patients on immunosuppressants/modulators for systemic lupus erythematosus, psoriasis, rheumatoid arthritis, Behcet's disease, autoimmune hepatitis and myasthenia gravis.<sup>[71,72,77]</sup>

It is important to note here that topical steroid use disturbs the local immune function and severely impairs the clearing of the fungi from the skin. The crucial effects on local immunity are detailed in Table 3.<sup>[78,79]</sup>

Lastly, various genetic predisposition factors have been identified, and certain HLA haplotypes predict increased risk of dermatophyte infections, particularly HLA-DR8 has been shown to increase susceptibility for onychomycosis.<sup>[80]</sup> Mutations in gene CLEC7A, which encodes protein dectin-1 that binds to fungal  $\beta$ -glucans, as well as mutations in the genes of signaling pathways such as CARD9 and STAT3, engaged in the antifungal immune response, are associated with an increased incidence of dermatophytosis.<sup>[81]</sup> Low copy numbers of gene DEFB4, encoding the antimicrobial peptide (AMP)  $\beta$ -defensin-2, also increase vulnerability to dermatophytosis.<sup>[82]</sup>

## Clinical Implications

It is obvious that the immunological interaction of the host immune response and the fungi determine the clinical

sequelae. Thus, understanding of the complex immune responses to *Trichophyton* spp is essential for developing adequate therapeutic strategies for treating chronic tinea infections.<sup>[54]</sup> It has been estimated that about a fifth of all *T. rubrum* infections are chronic. *T. mentagrophyte/interdigitale* complex, which is currently the prevalent strain causing dermatophytoses in India, also seems to subvert host immunity, though the precise mechanisms are not known at present. It is known that chronic infections with *T. rubrum* can invade the deeper levels of the epidermis, yet suppressing an immune response, and prolonging symptoms such as mild to intense itch, scaling, and development of plaques. These chronic infections often do not respond to treatment, having severe implications for the patients.

1. Chronic or relapsing dermatophytic infections in immunocompetent individuals are related to the prevalence of immediate hypersensitivity mediated by IgE (immunoglobulin E) to the fungus, as well as high serum levels of IgE and IgG4 (immunoglobulin G4).<sup>[83]</sup> *T. rubrum* produces substances, e.g., the mannans associated with glycoproteins that decrease the immune response, thus preventing complete eradication of the fungus.<sup>[84]</sup> Anthropophilic species induce immune-suppression through toll-like receptor 2 (TLR2) mediated IL-10 release, with consequent generation of CD4+ CD25+ T-regulatory cells with immunosuppressive potential.<sup>[85]</sup> Consequently, there is increased Th2-type responses that is inadequate to fight fungal infections leading to chronic and

**Table 3: The effect of corticosteroids on various steps involved in pathogenesis of dermatophytosis**

Immune mediators	Action
Effect on the APCs, neutrophils	<p>1. Glucocorticoids (GCs) act directly on APCs via cytoplasmic/nuclear receptors to suppress the production of IL-12. The inhibition of IL-12 production may represent a major mechanism by which GCs affect the Th1/Th2 balance since IL-12 is the main inducer of Th1 responses, enhancing production Th1 cytokines such as IFN-<math>\gamma</math> by antigen-primed CD4+ T cells and inhibiting the synthesis of Th2 cytokines such as IL-4 by T cells.</p> <p>2. Additionally, exposure of DC to GC induces the expression of TLR2 on their surface and stimulation of these cells with a TLR2 ligand initiates the secretion of IL-10, IL-6, and TNF<math>\alpha</math>, which inhibit Th1 cell activation.</p> <p>3. GC have also been shown to decrease the expression of IL-23 in DC, prohibiting Th17 polarization.</p> <p>4. GCs may decrease antigen presentation by APCs by decreasing MHC II expression</p> <p>5 GCs have been shown to reduce macrophage and neutrophil chemotaxis and decreases the IL-1 and IFN-<math>\gamma</math> release by macrophages with a small effect on the respiratory burst.</p>
Effect on adaptive immunity (T cells, cytokines)	<p>1. GCs suppress the Th1-cellular immunity axis and mediate a shift towards Th2 immune responses. This results from both a direct effect on T cells by downregulating the expression of IL-12 receptors on T and NK cells leading to a loss of IL-12 responsiveness and indirectly via inhibition of IL-12 production.</p> <p>2. GCs have a direct effect on Th2 cells by upregulating the production of IL-4, IL-10, and IL-13</p> <p>3. The effects of GC on Th17 cells are unclear; the existing data suggests that both Th17 differentiation and function may be affected by GC. GC administration has been shown to decrease the expression of IL-23 in DC and IL-6, TGF<math>\beta</math> as well as IL-17 in the joints of arthritic mice.</p> <p>4 In addition to affecting differentiation of specific T-cell subsets, GC directly or indirectly suppress the activation of proinflammatory cytokine genes such as TNF<math>\alpha</math> and IL-1<math>\beta</math>.</p>

APC: Antigen presenting cell; DC: Dendritic cell; TLR: Toll like receptor; IFN  $\gamma$ : Interferon gamma; NK cell: Natural killer cell; TGF $\beta$ : Transforming growth factor beta; TNF $\alpha$ : Tumour necrosis factor alpha

extensive infection.<sup>[86]</sup> Moreover, a study showed that macrophages and neutrophils have reduced *T. rubrum* phagocytosis and downstream cytokine release in patients with chronic dermatophytoses.

Also, the release of H<sub>2</sub>O<sub>2</sub> and NO and *T. rubrum* killing activity of these cells was reduced in these patients and was normal in patients with acute tinea pedis infections suggesting Trichophyton-specific functional defects in patients with chronic dermatophytoses.<sup>[87]</sup> The fungal cell wall mannans can inhibit DC-SIGN-dependent cell adhesion to ICAM-3 of wild-type T cells, thereby decreasing the initial interactions between DC and wild-type T cells, thus blocking antigen presentation and activation of T cells, favoring the development of invasive or disseminated infections caused by dermatophytes.<sup>[32]</sup> The clinical correlate of this is the leathery, lichenified lesions seen in some patients, and this is a surrogate clinical sign of chronicity.<sup>[1]</sup>

2. The use of immunosuppression via oral agents or topical steroid abuse can subvert almost all aspects of the host immune response. [Table 3] Dermatophytes can invade the dermal tissue, particularly after local trauma in patients with chronic infections.<sup>[88]</sup> In immune-deprived patients, dermatophytosis may involve the subcutaneous tissues and even deep organs, possibly becoming a life-threatening disease in the absence of appropriate treatment.<sup>[89]</sup>
3. A combination of impaired peripheral blood circulation, reduced oxygenation, and delay in migration of immune-competent cells or production of antimicrobial peptides (AMP) at the site of infection may favor the infectious process, which explains the 3X increased risk of dermatophytosis, especially foot and nail tinea in diabetes.<sup>[90]</sup>
4. A deranged barrier can lead to recurrences, hence epidermal barrier integrity is a crucial aspect that can determine a chronic infection.<sup>[1,18,67]</sup>
5. The pH of skin is important, and an alkaline pH can potentiate the virulence of the dermatophyte species.<sup>[21-26]</sup> Thus, the aggressive use of soaps can prove to be detrimental to the host defense, and thus the use of these products has no scientific rationale.<sup>[1]</sup> Moreover, providing sub-optimal concentrations of antifungals at the infection site can promote further resistance.<sup>[91]</sup> The acidic pH of skin is important to maintain the stratum corneum barrier function and thus acts as a resistance to invasion by dermatophytes.<sup>[92]</sup> It has been demonstrated that the skin pH at the site of infection in tinea pedis is higher than the normal skin pH.<sup>[93]</sup> A higher pH has also been demonstrated in the intertriginous regions of diabetic patients, providing a possible explanation for a higher incidence of candidal infections involving these sites.<sup>[94]</sup> The use of a syndet bar would help in maintaining the barrier function and a protective pH to withstand the infection with dermatophytes, and may thus be recommended.
6. The spread between family members, suggests (though it has not been scientifically proven) that the prevalent strain is transmitted through fomites. It is prudent to advise washing clothes at high temperatures (=60°C) as it is likely to be beneficial based on the results of *in vitro* experiments on various dermatophytes.<sup>[95]</sup>
7. A study on *T. rubrum* showed that the SLC11A1, RNASE7, and CSF2 genes were stimulated, and their products play a role in signaling and migration of immune cells and have potential antimicrobial activity. RNASE7 encodes for ribonuclease 7, which is part of the keratinocyte innate defense mechanisms. CSF2 encodes for granulocyte-macrophage colony-stimulating factor (GM-CSF), which is involved in recruiting immune cells to the site of infection and is part of the host innate immune response. SLC11A1 gene encodes an integral membrane protein involved in actuating macrophages and causing several effects on the signaling of the innate immune system such as TNF $\alpha$ , IL1- $\beta$ , among others. Genes that are involved in the maintenance of epithelial barrier integrity such as FLG and KRT1 were inhibited. This study revealed that modulation of the genes involved in *T. rubrum*-host interaction could signify possible targets for the management of dermatophytoses.<sup>[96]</sup> Also, products that target Heat shock protein 90 (Hsp90) can also be used to treat *T. rubrum* infections.<sup>[27]</sup>

## Conclusions

Studies on antifungal resistance in dermatophytes lag far behind those available for systemic fungi.<sup>[86]</sup> With increasing reports of resistance to terbinafine, the use of azoles (specifically itraconazole) for dermatophytoses is on an upward trend.<sup>[1,3]</sup> Even though Indian dermatologists have found that the western recommendations on the use of itraconazole for tinea corporis/cruris are proving inadequate to eradicate infections and thus higher doses and longer durations of treatment are being suggested, the MIC data and existing data on skin pK of antifungal drugs, reliably predicts efficacy to azoles.<sup>[1,97,98]</sup>

Almost all studies show sensitivity to itraconazole, implying that the solution to recalcitrant infection might need a synergism of antifungal drugs with the host immune response to clear the fungi.<sup>[3,4,91,99]</sup> Discovering the means by which the species evade the innate host defense and bypass the cellular immune response is the key to emerging effectual antifungal therapeutic modalities. More efficacious treatment for chronic dermatophytoses would therefore include aiming at the mechanisms by which the species restricts the immune response, such as the mechanism by which it inhibits TLRs, and finding a way to reestablish the cell-mediated immune response, such as reviving the macrophages phagocytic activity. Also, drugs that target the various virulence factors can help in treating dermatophytosis.



Here it is important to emphasize that although there is some experimental reasoning on the ability of *T.rubrum* to cause chronic minimally inflammatory disease, the presently prevalent species (*T.interdigitale*) barely has any such data to its credit. Thus, there is an emergent need to study the virulence factors of the prevalent strain. In essence, understanding the interaction of the immune response with the dermatophyte, can help supplant and supplement the existing pharmacological remedies that fail in recalcitrant dermatophytoses.

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### Conflicts of interest

There are no conflicts of interest.

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