Keratin Conquerors: The Unseen Battle of Dermatophytosis
An Epic Tale of Pathogenesis and Healing Arts

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Dermatophytes are a distinct group of keratinolytic and keratinophilic fungi, which possess a unique ability to digest keratin. This ability allows them to colonize and infect keratinized tissues. Dermatophytosis represents a major concern in veterinary dermatology due to its zoonotic potential, widespread prevalence, and impact on animal health and welfare.

Taxonomically, dermatophytes belong to the Ascomycota phylum, which is characterized by the formation of ascospores within specialized sac-like structures called asci. Within the Ascomycota phylum, dermatophytes fall in the Eurotiomycetes class, Onygenales order. The Onygenales order includes fungi that primarily infect keratinous substrates.

There are currently seven accepted dermatophyte genera: Trichophyton, Epidermophyton, Nannizzia, Paraphyton, Lophophyton, Microsporum, and Arthroderma. [4]
Additionally, the sexual form (teleomorph) and the asexual form (anamorph) were previously classified as two distinct species, each with its own name.[5] Recently, the names of teleomorphs and anamorphs have been united under the "One Fungus = One Name" system for species identification (Table 1).[6] Dermatophytes are further categorized into three groups based on their habitat: anthropophilic (living on humans), zoophilic (living on animals), and geophilic (living in the environment). With the evolution of mycological naming systems, molecular characterization has been incorporated alongside traditional methods to more accurately define dermatophyte species.[4,7]

Table 1. Species formerly known as *Trichophyton mentagrophytes* adapted from Monod *et al.* [3]

Sexual form (teleomorphic)	Asexual form (anamorphic)	Old nomenclature
A. benhamiae	*Trichophyton* sp.*	*T. mentagrophytes* (zoophilic strains) • *T. asteroides* • *Mentagrophyte microides*
-	*T. erinacei*	*T. mentagrophytes* var. *erinacei*
A. vanbreuseghemii	*Trichophyton* sp.*	*T. mentagrophytes* (zoophilic strains) • T. mentagrophytes var. mentagrophytes • T. asteroides Microides mentagrophytes
-	*T. interdigitale*	*T. mentagrophytes* (anthropophilic strains) • *T. mentagrophytes* var. *interdigitale* • *Microides interdigitalis*
A. simii	*T. simi*	• *T. mentagrophytes*

*A formal anamorphic name does not exist for *A. benhamiae* and *A. vanbreuseghemil*.

Each genus encompasses species with distinct morphological, ecological, and pathogenic characteristics:

Trichophyton: This genus includes species that infect both humans and animals, often causing infections of the skin, hair, and nails. Notable species include *Trichophyton mentagrophytes* and *Trichophyton verrucosum*.
Microsporum: Species within this genus primarily infect hair and skin, with *Microsporum canis* being a well-known pathogen in both cats and dogs. *Microsporum gypseum* is another species commonly found in soil and known to infect animals.
Epidermophyton: This genus is less diverse, with *Epidermophyton floccosum* being the primary species which predominantly infects human skin and nails. However, it can occasionally affect animals as well.

Dermatophytes can be categorized based on their ecological niches and preferred hosts into three main types (Table 2):
Zoophilic Dermatophytes: These species primarily infect animals but can also cause infections in humans through direct or indirect contact with infected animals. Examples include *Microsporum canis*, commonly associated with cats and dogs, and *Trichophyton verrucosum*, often found in cattle.
Anthropophilic Dermatophytes: These species primarily infect humans and are typically transmitted through human-to-human contact. They rarely infect animals. An example is *Trichophyton rubrum*, a common cause of athlete's foot and other human dermatophytoses.
Geophilic Dermatophytes: These species reside in soil and can infect both animals and humans upon contact with contaminated soil. They are often associated with infections in animals that frequently come into direct contact with soil. *Microsporum gypseum* is a well-known geophilic dermatophyte.

Table 2. Classification of dermatophytes into geophilic, zoophilic, and anthropophilic species

Geophilic species	Zoophilic species	Anthropophilic species
*M. gypseum**	*M. canis (A. otae)*	*M. audouinit*
(A. gypseum,	*• A benhamiae*	*• M. ferrugineum*
A. fulvum,	*T. erinacei*	*T. interdigitale*
A. incurvatum)	*• A vanbreuseghemii*	*T. tonsurans*
	T. quinckeanum	*T. rubrum*
	A simil	*T. violaceum*
	• T. verrucosum	*T. soudanense*
	T. equinum	*T. schoenleinii*
		T. concentricum
		E floccosum

The classification and identification of dermatophytes are traditionally based on morphological characteristics, such as the shape and size of conidia (asexual spores) and macroconidia. However, molecular techniques, including polymerase chain reaction (PCR) and sequencing of ribosomal DNA (rDNA), have enhanced the accuracy and resolution of dermatophyte taxonomy, leading to a better understanding of their phylogenetic relationships and epidemiology.

Dermatophytes are fungi that can exist in two forms throughout their life cycle: the sexual form (teleomorph or perfect state) and the asexual form (anamorph or imperfect state).
These two forms can be found in different environments and conditions.
Typically, the asexual form of dermatophytes, i.e., anamorph, is the most common and can be found in laboratory cultures and in dermatophyte infections. The anamorph stage represents the reproductive stage of these fungi where asexual spores, such as conidia or arthrospores, are produced. These spores are typically involved in the spread and transmission of dermatophyte infections. The anamorph form of dermatophytes is commonly encountered in laboratory cultures and in dermatophytic infections on the skin, hair, and nails of humans and animals.
In contrast, the sexual form of dermatophytes, i.e., teleomorph, is rarely encountered and is more commonly associated with natural environments such as soil. The teleomorph is responsible for sexual reproduction and the production of sexual spores. However, the sexual form is less common and may be difficult to observe in laboratory settings.

The term "perfect state" is used in mycology to refer to the sexual reproductive stage of fungi, which involves the production of sexual spores through the fusion of compatible mating types. Fungi in their sexual reproductive stage are considered "perfect" because they have achieved a full complement of genetic material through sexual recombination, resulting in the formation of sexual spores. These spores can give rise to new genetic variations and contribute to the genetic diversity of the fungal population.
In contrast, the asexual reproductive stage of fungi, known as the anamorph or simply, the asexual phase, typically involves the production of asexual spores without the fusion of mating types. While this stage is essential for rapid reproduction and dispersal, it does not contribute to genetic variation in the same way as the sexual stage.

The life cycle of dermatophytes involves both sexual (teleomorph) and asexual (anamorph) reproductive stages, although the teleomorph stage is less commonly observed in zoophilic organisms and it is not as studied as the anamorph stage.

Anamorph Stage (Asexual Reproduction):
The life cycle typically begins with the introduction of dermatophyte spores (arthroconidia or microconidia) onto the host's skin, hair, or nails, often through direct contact with infected individuals or contaminated environments.
Upon landing on the host's cutaneous surface, the spores germinate and form hyphae, which penetrate and invade the host's keratinized tissues (such as the stratum corneum of the skin, hair shafts, or claws).
Within the host tissue, the dermatophyte hyphae proliferate and produce enzymes that allow them to digest and utilize keratin as a nutrient source for growth.
As the hyphae continue to grow and spread within the host tissue, they may form characteristic lesions, such as alopecia, erhythema, scaly patches on the skin.
During this stage, the dermatophytes primarily reproduce asexually by producing asexual spores (arthroconidia or microconidia) through processes like budding or fragmentation of hyphae.
The asexual spores are shed from the infected tissue, contributing to the spread and transmission of dermatophytic infections to new hosts or environments.

Teleomorph Stage (Sexual Reproduction):

Sexual reproduction is readily observed among geophilic dermatophytes. The sexual reproductive stage of dermatophytes, known as the teleomorph stage, involves the fusion of compatible mating types and the formation of sexual spores.

The increased prevalence of sexual reproduction in geophilic organisms has led to the idea that the development of fruiting bodies is encouraged in moist soil that contains keratinous debris. Hence, while zoophilic dermatophytes linked to terrestrial animals exhibit sexual reproduction, species that have adapted to non-ground-dwelling animals and humans seem to predominantly reproduce asexually. Only some zoophilic species possess the ability to undergo sexual reproduction, while mating is not observed among anthropophilic species.[2]

During sexual reproduction, compatible mating types of dermatophytes come into contact, leading to the fusion of their hyphae and nuclei.

The fusion of nuclei results in genetic recombination and the formation of sexual spores.

These sexual spores may be released into the environment and serve to propagate the fungus, contributing to genetic diversity and adaptation over time. Ascospores, produced during the sexual phase, are more resistant than conidia to adverse environmental conditions (asexual spores), aiding in survival during dormancy in soil or litter. It is hypothesized that arthrodermataceous ascospores may be deposited on rodent fur and survive gut passage when these animals are eaten by predators, contributing to their presence in carnivore dung. Here are featuring tough, branching networks and helical appendages for protection. Sexual spore production, requires more time and protection than conidia thus offering ecological advantages.[8]

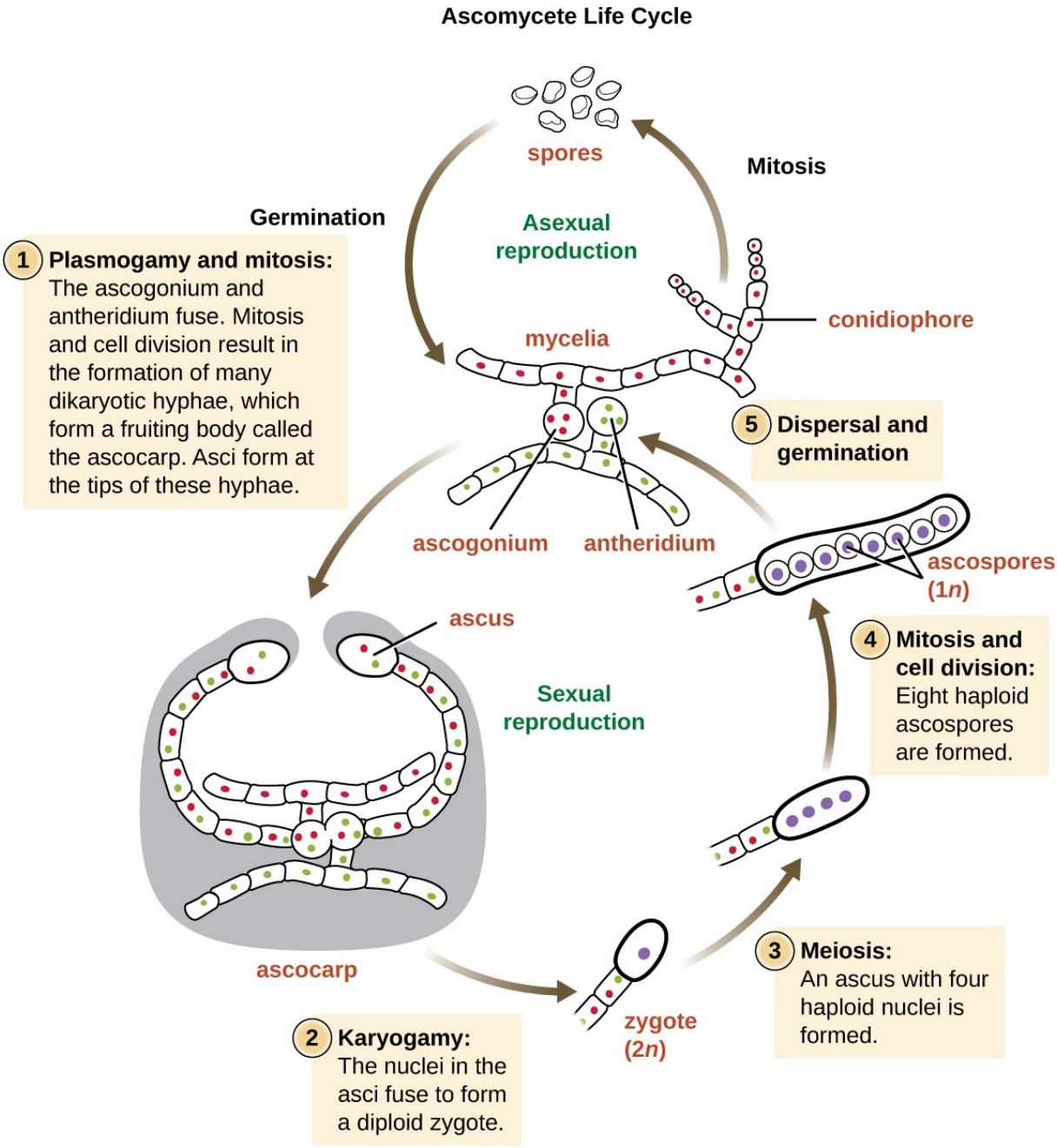

FIGURE 1. The life cycle of an ascomycete is characterized by the production of asci during the sexual phase. The haploid phase is the predominant phase of the life cycle.
https://courses.lumenlearning.com/suny-microbiology/chapter/fungi/

Risk Factors and Predispositions

Age:

Young Animals: Puppies and kittens are more susceptible due to their underdeveloped immune systems.

Older Animals: Geriatric animals may have weakened immune responses, making them more prone to infections.

Immune Status:

Immunocompromised Animals: Animals with compromised immune systems, due to either illness or treatments (e.g., corticosteroids), are at a higher risk. Animals with other

comorbidities (hypoadrenocorticism, leishmaniosis, ehrlichiosis, diabetes mellitus etc.) may have weakened immune defense leading to increased susceptibility

Environmental Factors:

Overcrowding: High-density living conditions, such as kennels or shelters, facilitate the spread of dermatophytes.

Poor Sanitation: Inadequate cleaning and disinfection can lead to a buildup of infectious spores.

Humidity and Temperature: Fungal spores thrive in warm and humid environments, which can promote the spread of dermatophytosis.

Breed Predisposition:

Certain Breeds: Some breeds, especially those with long or dense coats (e.g., Persian cats, Yorkshire Terriers), are more prone to dermatophytosis due to difficulties in maintaining a clean coat. They are also predisposed to developing subcutaneous dermatophytic infections. (*M. canis*).

Hunting and working dogs (German short-haired pointers, fox terrier, Labrador retriever, Belgian Groenendael, beagle, pointer, Jack Russell terrier, German shepherd dog and Jagdterrier) face several risk factors that increase their susceptibility to dermatophytosis. Frequent exposure to diverse and often harsh environments introduces them to various fungal spores present in soil and vegetation. The physical demands and stress associated with hunting can weaken their immune systems, making them more prone to infections. Close contact with other animals, both wild and domestic, facilitates the transmission of dermatophytes. Additionally, minor cuts and abrasions acquired during hunting can serve as entry points for fungal infections, further elevating their risk. (*M. persicolor* and *M. gypseum*) [5,9-10]

Infection: pathogenesis, virulence factors and host immune response

Dermatophyte infections can be transmitted by direct contact between infected and uninfected animals or through fomites such as grooming tools, bedding, collars, ectoparasites, and contaminated environments. Skin microtrauma significantly increases the risk of infection. *Microsporum canis* infections primarily occur through contact with infected animals, especially cats, while transmission from contaminated environments is less efficient. *Trichophyton* infections often result from contact with infected rodents or their nests, and *Microsporum gypseum* infections are typically due to contact with contaminated soil. Extensive skin trauma, humidity, and ectoparasites create optimal conditions for infection. Experimental infections require skin abrasion and moisture to establish clinical infection in laboratory settings, where normal grooming acts as a defense mechanism in cats.[5]

When dermatophytes infect the skin, they must first overcome physical, chemical, and morphological barriers. Abnormalities in the stratum corneum, such as macerations and occlusions, can promote fungal infection. Once these barriers are breached, colonization begins, involving processes such as adhesion, germination, and invasion (Figure 2).

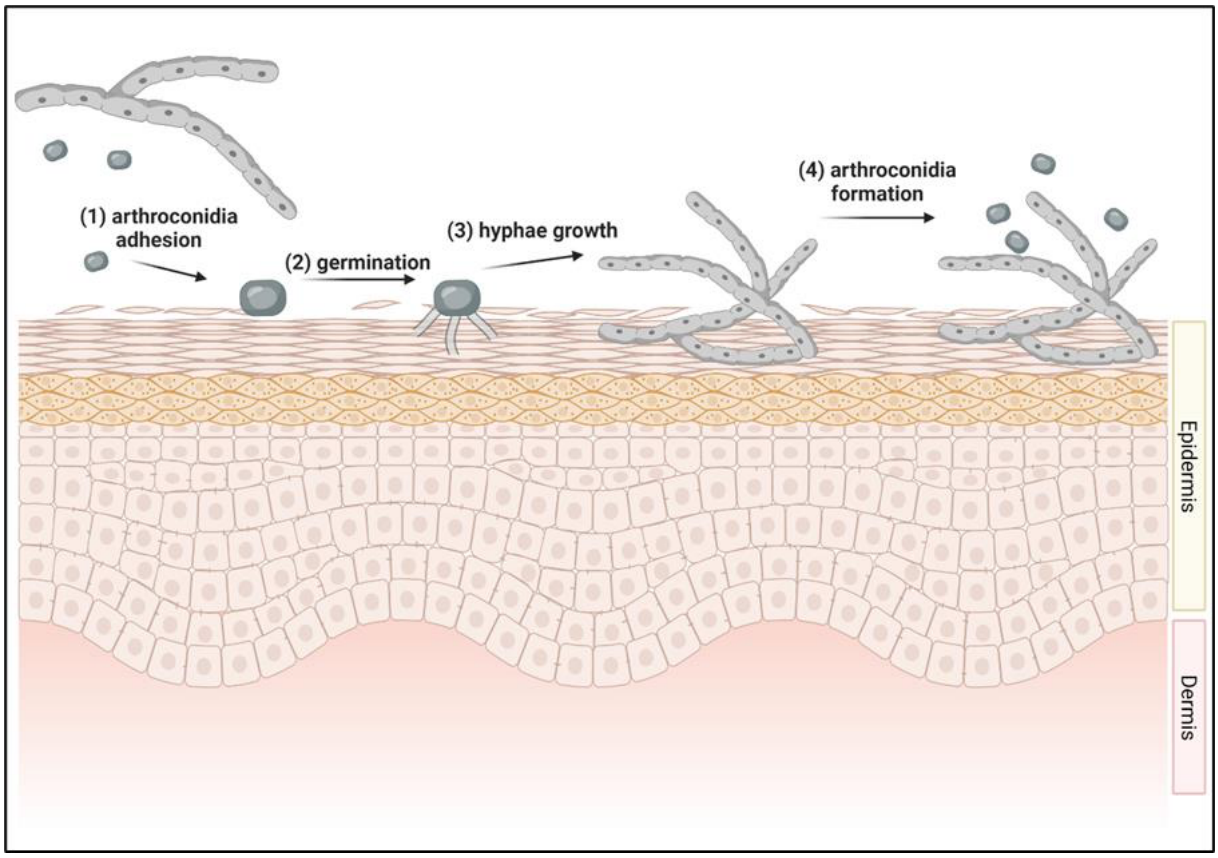

Figure 2. Initiation of dermatophyte infection in skin. (1) Arthroconidia from environment or 2–6 h after contact. (2) Another infected host contacts a new host's skin. Adhesion to skin occurs between 2–6 h after conthroconidia begin to germinate in the top layer of the epidermis, forming germ tubes. (3) Hyphae tact. (2) Arthroconidia begins to germinate in the top layer of the epidermis, forming germ tubes. continue to grow within the epidermis. (4) Within 7 days of infection, arthroconidia are formed, (3) Hyphae continue to grow within the epidermis. (4) Within 7 days of infection, arthroconidia are allowing for the cycle to repeat. (Alex E. Moskaluk 2022)

Adherance

The adherence step in the infection process caused by *Microsporum canis* is a critical phase that involves complex molecular interactions between the fungal arthroconidia and the host's keratinized tissues: specific adhesins, hydrophobic interactions, enzymatic degradation, and complex signal transduction pathways on skin. Adhesion to skin occurs between 2–6 h after contact.[11-14]

1. Surface Adhesins and Host Receptors: Arthroconidia of *M. canis* adhere to the host epidermis through specialized surface proteins known as adhesins. One of the primary adhesins identified in *M. canis* is subtilisin Sub3, which belongs to the serine protease family. Sub3 binds to glycoprotein receptors on the host's keratinocytes, facilitating initial attachment. This binding is facilitated by the enzyme's ability to degrade surface glycoproteins, exposing binding sites for stronger fungal attachment. This dual role of Sub3 in proteolysis and adhesion ensures efficient colonization by *M. canis. T. rubrum* binds to epithelial cells through carbohydrate-specific adhesins on the microconidial surface, while *T. mentagrophytes* protrudes fibrillar projections when it is in need of adherence capabilities.[17]

2. Hydrophobic Interactions: The surface of arthroconidia is hydrophobic, which enhances their ability to attach to the lipid-rich outer layer of the stratum corneum. This hydrophobic interaction is crucial for the stable adhesion of the spores to the host's skin.
3. Protein-Protein Interactions: Subtilisin Sub3 interacts with host proteins, such as keratin and collagen, through protein-protein interactions. These interactions are mediated by the binding of specific domains within the adhesin to complementary structures on the host cell surface.
4. Enzymatic Degradation: Once attached, Sub3, along with other proteolytic enzymes like Sub4 and metalloprotease MEP3, begins to degrade keratin and other structural proteins in the epidermis. This degradation process not only provides nutrients for the fungus but it also exposes more binding sites, reinforcing the adherence.
5. Signal Transduction Pathways: The binding of adhesins to host receptors activates intracellular signaling pathways in both the fungus and the host. *In M. canis*, this may involve the activation of pathways that regulate the expression of additional adhesion molecules and enzymes, enhancing the invasion process. In the host, signaling pathways may trigger an immune response aimed at fighting off the infection.
6. Cytoskeletal Rearrangements: The interaction between *M. canis* adhesins and host receptors can induce cytoskeletal rearrangements in keratinocytes, facilitating closer contact and potentially promoting endocytosis-like mechanisms that allow deeper fungal penetration.
Biofilm formation in dermatophytes, such as *Trichophyton rubrum, T. mentagrophytes and M canis*, involves the initial adhesion of conidia to keratinized tissues, followed by the production of a protective extracellular matrix and the development of a complex hyphal network, which enhances resistance to antifungal treatments and host immune responses.[15-16]

Germination
Penetration begins with germ tubes emerging from arthroconidia and attaching to corneocytes. Arthroconidia increase in size and produce germ tubes that extend horizontally and penetrate through the stratum corneum, leading to the formation of hyphae and subsequent tissue invasion, which in turn causes peripheral lesion expansion in dermatophytosis. This pattern of growth secures the fungal structure and facilitates infection.[18]
In vitro studies have demonstrated that *Trichophyton mentagrophytes* arthroconidia initiate germination within 4 hours on human stratum corneum. This process is enhanced by the presence of human corneocytes and requires specific humidity conditions. Germination of *T. mentagrophytes* arthroconidia occurs within 4-6 hours at 37°C. However, it is inhibited at extreme temperatures, such as 4°C and 45°C, but resumes when shifted back to optimal conditions (37°C). These findings highlight the critical role of temperature and environmental conditions in the germination and growth of dermatophytes.[18-19]

Invasion
Invasion of keratinized structures occurs when dermatophyte hyphae penetrate the stratum corneum and grow in various directions, including within hair follicles, which is common among dermatophytes infecting animals. Fungi begin producing arthrohyphae and invade keratinized tissues, forming arthroconidia within seven days of infection. This process allows the fungus to spread to other anatomical locations of the original host, to other hosts, or to contaminate the environment. Clinically, lesions usually appear one to three weeks after exposure.[5]

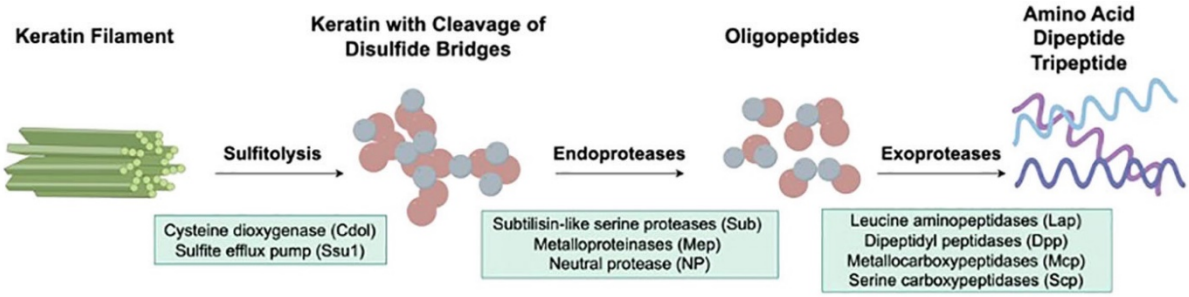

FIGURE 3. Virulence factors of dermatophytes involved in the keratinolysis process. The initial stage of native keratin biodegradation is sulfitolysis, conducted by key enzymes such as cysteine dioxygenase (Cdol) and sulfite efflux pump (Ssu1). Then, endoproteases degrade keratin to release free peptides, which are further cleaved into amino acids by exoproteases. 14

Dermatophytes infect keratin-rich structures such as hair, skin and nails. They rely on sulfite production to cleave keratin-stabilizing cysteine bonds, facilitating keratin degradation.
High environmental cysteine levels are toxic. Dermatophytes convert cysteine to sulfite via the enzyme cysteine dioxygenase (Cdo1), which is crucial for this process.
The sulfite efflux pump (Ssu1) helps secrete sulfite, reducing cysteine toxicity and aiding in keratin breakdown (Figure 4).
Mutants lacking either Cdo1 or Ssu1 show increased sensitivity to cysteine and sulfite, and have defective growth on keratinous materials such as hair and nails.

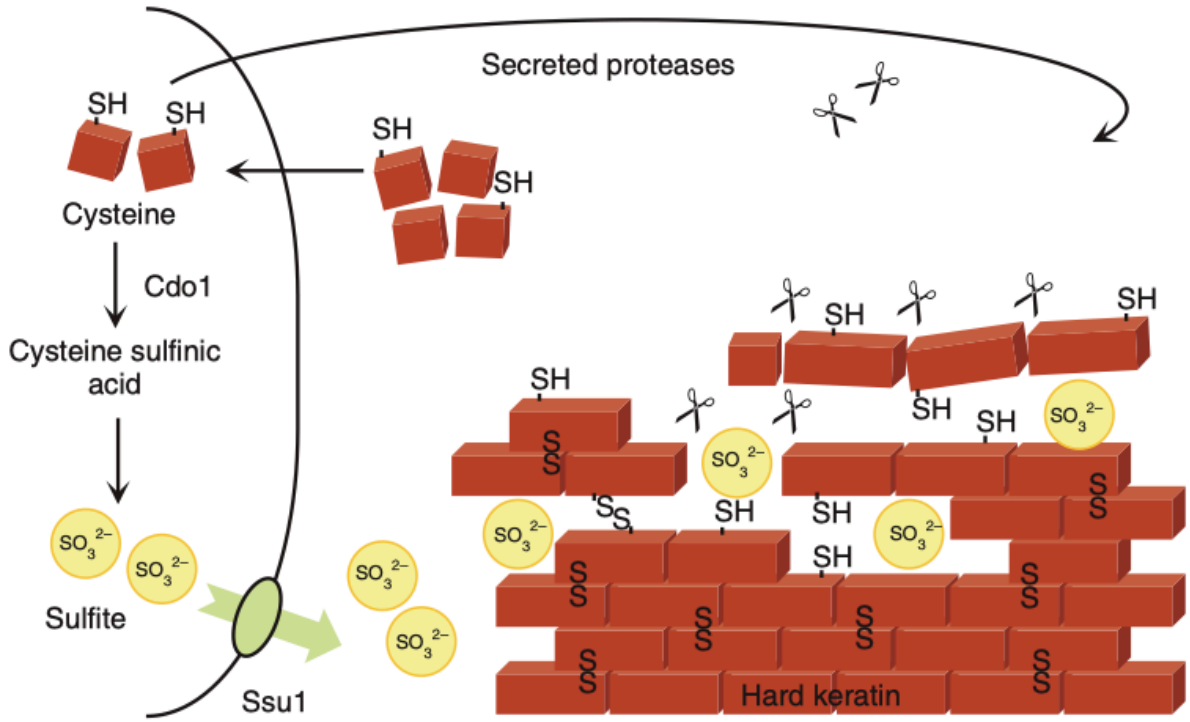

Figure 4. Proposed model of keratin degradation by dermatophytes. In hard keratin, peptide chains stick tightly together like bricks in a wall owing to disulfide bridges (S–S) formed between the highly abundant cysteine residues in the protein. Proteolytic digestion of keratin is not possible until the brick-like structure gets weakened by the reduction of disulfide bridges. Sulfite (yellow circles) excreted through the sulfite efflux pump Ssu1 can act as a reducing agent. The loosened structure of the keratin protein makes peptide bonds more accessible to digestion by secreted proteases. The joint action of proteases and reducing agents results in the formation of smaller peptides and amino acids that can be taken up by the cell. Elevated cysteine

levels are toxic but can be metabolized to sulfite via cysteine sulfinic acid mediated by the action of cysteine dioxygenase Cdo1. The formed sulfite is excreted again and further facilitates keratin degradation. Taken together, our findings reveal that sulfite formation from cysteine supports both keratin degradation and cysteine detoxification. It is reasonable to postulate that there is enough free cysteine in keratin to initiate the mechanism of keratin degradation by secretion of sulfite.[20]

Enzymatic Breakdown

Dermatophytes release various enzymes (virulence factors) to further degrade keratin into smaller, digestible pieces:

Endoproteases

Subtilisins: These serine proteases cleave peptide bonds within the keratin protein, breaking it into smaller fragments. Subtilisin Sub3 and Sub4 are particularly potent. (All discovered are numbered Sub1-7)

Fungalysins-type metalloproteases: These (Mep1, Mep3, Mep4) further break down the protein fragments, facilitating the digestion process. Fungalysins target the peptide bonds exposed after the initial attack by subtilisins. (all discovered are numbered Mep1-5)

Exoproteases

Leucine aminopeptidases (Lap1 and Lap2) and dipeptidyl peptidases (DppIV and DppV): These enzymes cleave individual amino acids or short peptides from the ends of the peptide chains. They include aminopeptidases and carboxypeptidases, which clip amino acids from the N-terminus and C-terminus of the protein fragments, respectively that can be effectively assimilated by hyphae (Figure 5).[21,27]

Adaptation to Skin pH Changes

Healthy skin and nails maintain a slightly acidic to neutral pH environment. During the breakdown of keratin by dermatophytes, amino acid metabolism causes an alkaline shift. Dermatophyte keratinases, exhibit optimal activity at different pH levels depending on the stage of infection:

Early Infection Stages: Keratinases are most active in a slightly acidic environment.

Later Infection Stages: Keratinases have maximal activity at higher, more alkaline pH levels.

This ability to adapt to changing pH environments is crucial for the pathogenicity of dermatophytes and is mediated by the conserved PacC/Pal signal transduction pathway. The PacC protein acts as a pH signaling transcription regulator and it is essential for dermatophyte growth on human tissues.

PacC Gene Disruption: Reduces secretion of keratinolytic proteases and impairs the ability of mutant strains to invade the stratum corneum.

This pH adaptation mechanism allows dermatophytes to efficiently invade and degrade human tissues, ensuring their survival and proliferation.[14]

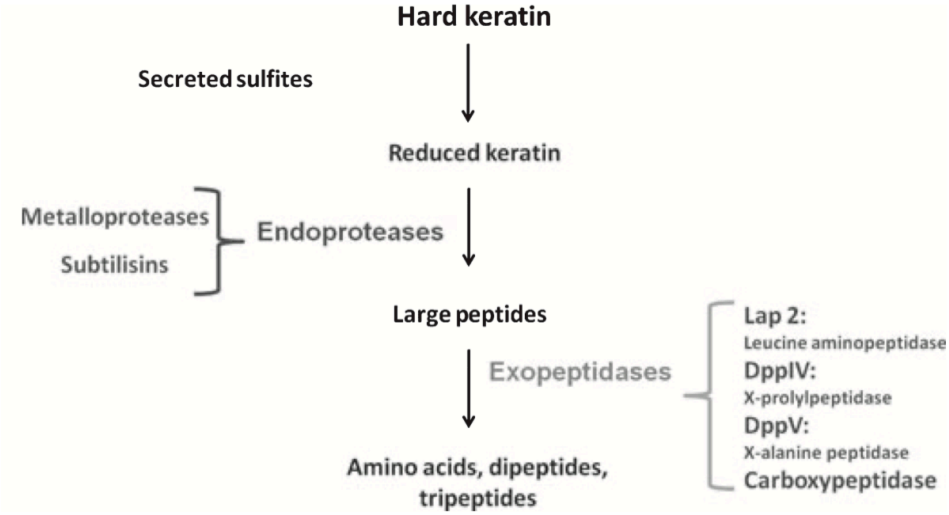

Figure 5. Representation of the keratinised tissue degradation pathway by dermatophytes at neutral pH. (Baldo 2011)

The experimental evidence also suggests that dermatophyte pathogenesis involves mechanisms beyond the fungal machinery used for keratin degradation, including virulence factors like cell wall components and products secreted into the extracellular space by extracellular vesicles. The following table summarizes the key virulence factors of dermatophytes, their functions, and the references supporting these findings. [27]

Virulence Factor	Description and Function	References
Subtilisin-like proteases (Sub)	Endoprotease activity in keratin digestion. Reported as allergens and involved in immune response induction.	Woodfolk et al. (13), Gao and Takashima (14), Burmester et al. (15), Eymann et al. (16)
Fungalysin-like Metalloproteases (Mep)	Endoprotease activity in keratin digestion.	Mehul et al. (18), Mercer and Stewart (9)
Leucinaminopeptidases (Lap)	Exoprotease activity in keratin digestion.	Burmester et al. (15), Eymann et al. (16)
Dipeptidyl peptidases (Dpp)	Exoprotease activity in keratin digestion.	Burmester et al. (15), Eymann et al. (16)
Secondary metabolite production-associated enzymes	Polyketide synthase and non-ribosomal peptide synthetase.	Burmester et al. (15)
Cysteine dioxygenases	Sulfitolysis of keratin. Involved in triggering humoral immune response during infection.	Grumbt et al. (20), Mercer and Stewart (9)
Hydrophobins	Hydrophobin rodlet layer on conidial surface. Related to avoiding immune recognition by neutrophils.	Heddergott et al. (21)
Extracellular vesicles	Unknown cargo, probably virulence factors. Related to modulation of the host response.	Eymann et al. (16)
LysM proteins	Protein domains related to binding to skin glycoproteins. Possibly involved in immune evasion.	Bitencourt et al. (22)
Heat shock proteins	Hsp 30, Hsp60, Hsp70. Associated with adaptation to human temperature, keratin digestion.	Martinez et al. (19), Kar et al. (23)

Virulence Factor	Description and Function	References
Other hydrolases and cell wall remodeling-associated enzymes	Lipases, glucanases, chitinases, beta-glucosidases, mannosyl transferases. Many involved in triggering humoral immune response during infection.	Martinez-Rossi et al. (24), Martins et al. (25)

Host Interaction and Immune Response

Immune and non-immune cells (keratinocytes) detect fungi through their cell wall components, secreted molecules, or intracellular contents via pattern recognition receptors (PRRs) (Figure 6).

PRRs include C-type Lectin Receptors (CLR), Toll-like Receptors (TLR), Nucleotide-binding and Oligomerization Domain (NOD)-like receptors (NLR), and Retinoic acid Inducible Gene (RIG)-like receptors (RLR).

These receptors transduce intracellular signals that promote fungal phagocytosis, respiratory burst and cytokine release, shaping immune responses. Signaling pathways mediated by CLR, TLR, and NLR are key factors for host antifungal immunity.

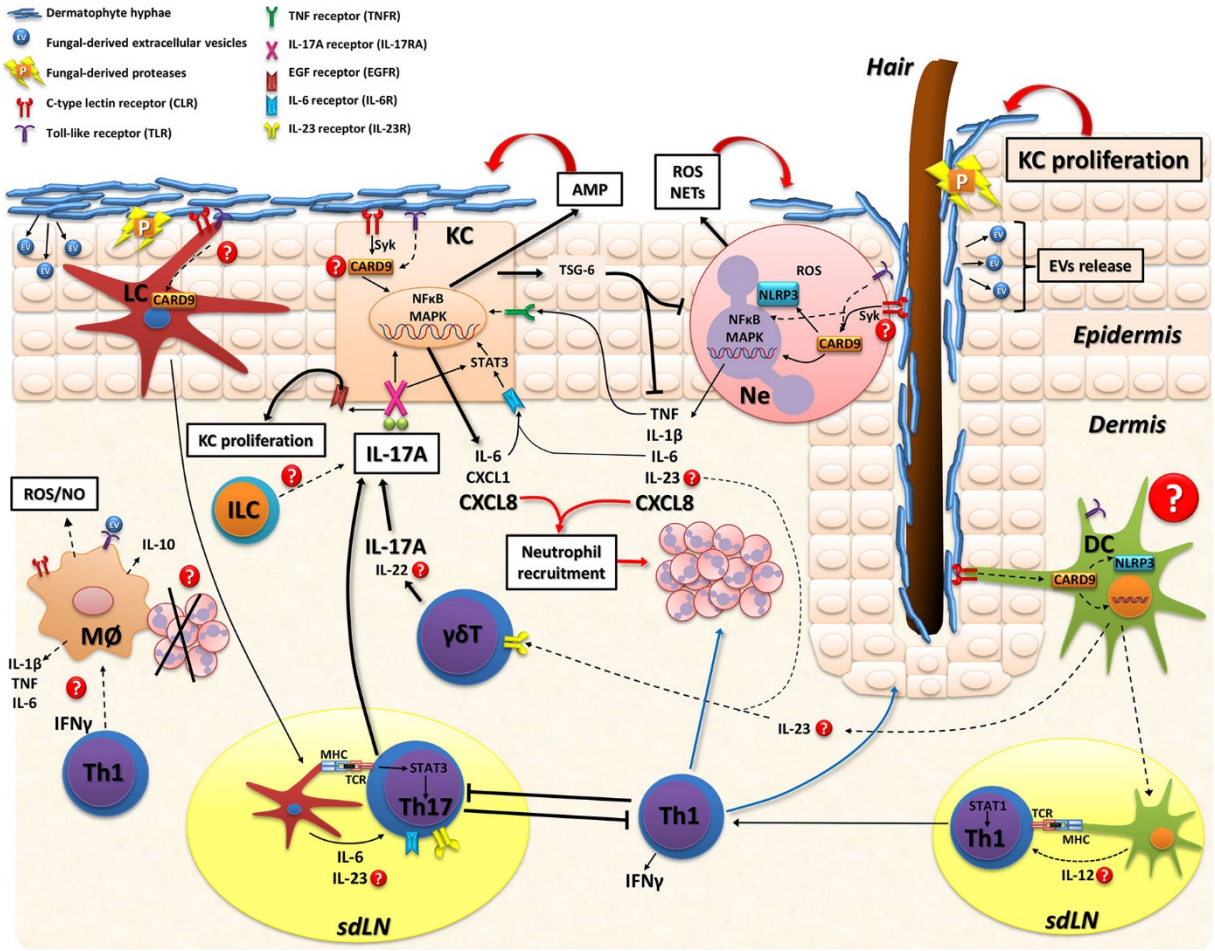

FIGURE 6. Model of skin immune response in dermatophytosis. Burstein 2020.

1. C-Type Lectin Receptors (CLR) in Antifungal Immunity

C-type Lectin Receptors (CLR) are proteins that can be soluble or membrane-bound, featuring at least one C-type lectin domain (CTLD). They recognize various fungal cell wall components

such as glycans, glycolipids, and glycoproteins. CLRs play a key role in antifungal immunity. CLR are primarily expressed in myeloid cells (monocytes, macrophages, neutrophils, dendritic cells), but they are also found in epithelial cells and keratinocytes.[27-31]

Dectin-1 (CLEC7a):
Recognition: Binds to β-glucans in fungal cell walls. It plays a role in the induction of trained immunity.
Signaling: Involves an immunoreceptor tyrosine-based activation motif (ITAM)-containing cytoplasmic domain, phosphorylated by Src family kinases, recruiting Syk kinase.[66]
Function: Promotes fungal phagocytosis, respiratory burst, and cytokine release (Figure 7).

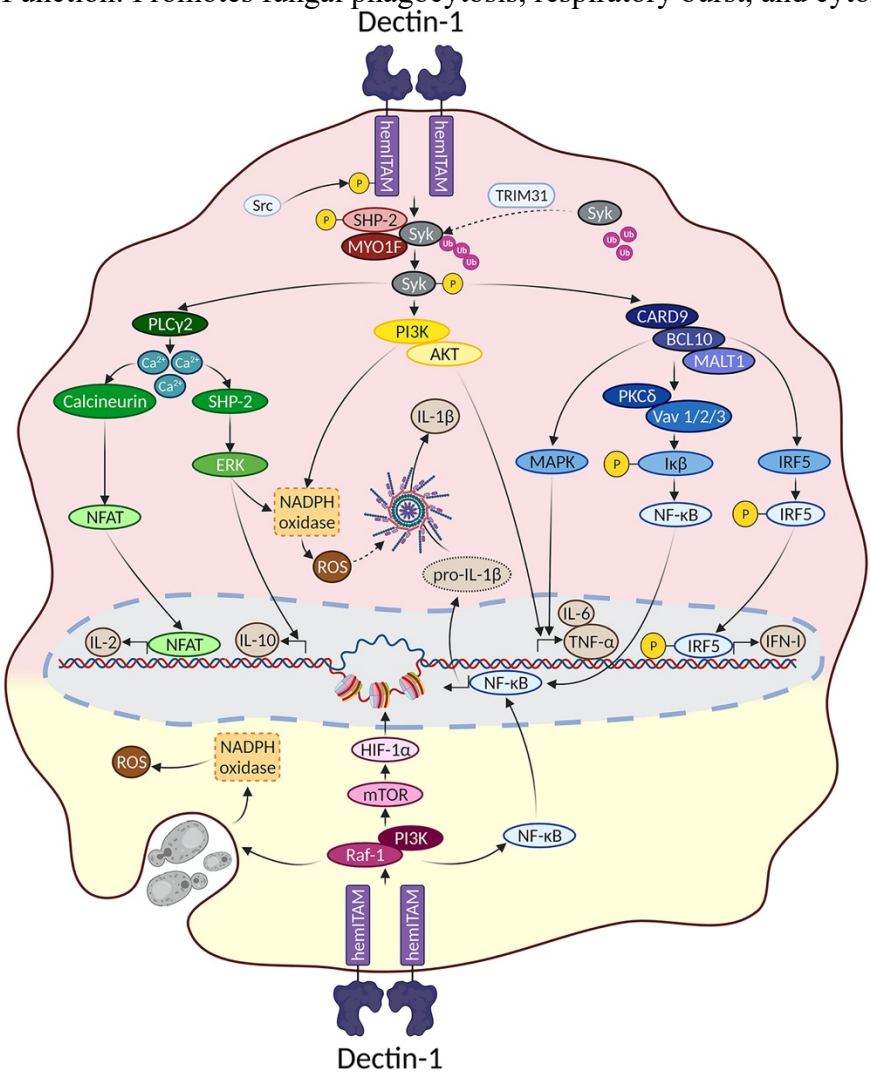

FIGURE 7. Main signaling pathways downstream Dectin-1. Main signaling pathways triggered downstream Dectin-1 ligation are depicted. In the upper reddish field, Syk-dependent pathways are represented. The lower yellowish field contains the main molecular routes triggered downstream Dectin-1 that do not depend on Syk. (Mata-Martı´nez *et al*.2022)

Dectin-2 (CLEC6a):
Recognition: Binds to α-mannans.
Signaling: Uses the ITAM-containing Fc receptor gamma (FcRγ) chain.
Function: Triggers innate immunity by activating the Syk pathway, involving CARD9, Bcl10, and MALT1, leading to NF-κB and MAP kinase activation.

Studies with mice show that Dectin-1 and Dectin-2 are essential for controlling systemic infections, as knock-out mice (lacking these receptors) cannot reduce fungal burdens effectively. In vitro studies demonstrate that blocking CLR pathways, such as using soluble α-mannans or mannose receptor blocking antibodies, inhibits fungal engulfment by macrophages. CARD9 deficiencies in humans also result in severe dermatophytosis due to impaired CLR signaling.

2. NLRP3 Inflammasome and IL-1β Production in Fungal Infections

IL-1β is a potent inflammatory cytokine produced mainly by macrophages and neutrophils, promoting cytokine production, phagocytosis, oxidative burst, and neutrophil degranulation. It is produced as an inactive precursor (pro-IL-1β) triggered by pattern recognition receptors (PRRs) recognizing microbial pathogen-associated (PAMPs) or damage-associated (DAMPs) molecular patterns. It is then activated into its biologically active form by caspase-dependent cleavage after the inflammasome assembly.

NLRP3 Inflammasome Activation:
First Signal: Provided by microbial binding to CLRs or TLRs, inducing pro-IL-1β synthesis and NLRP3 transduction via NF-κB-dependent activation.
Second Signal: Triggered by factors such as K+ efflux, extracellular ATP, ROS, fungal toxins, or particulate matter, promoting NLRP3 activation. This leads to the assembly of a multiprotein complex with NLRP3, ASC, and pro-caspase-1, facilitating pro-caspase-1 activation and the processing of pro-IL-1β to mature IL-1β.
Microsporum canis and *Trichophyton schoenleinii* induce IL-1β production in a NLRP3-dependent manner in both human monocytic cells and murine dendritic cells. Dectin-1-Syk-CARD9 signaling is critical for pro-IL-1β transcription, suggesting that dermatophyte glycan recognition by CLRs provides the first signal for NLRP3 and IL-1β synthesis. The second signal involves cathepsin B activity, K+ efflux, and ROS production.

3. Toll-Like Receptors (TLR) in Antifungal Immunity

Toll-like receptors (TLR) are membrane glycoproteins. In mammals, TLRs mediate host responses against microbial pathogens. Their intracellular signaling involves MyD88 and TRIF, which trigger inflammatory responses. Although fungal ligands for TLRs are not fully defined, evidence shows that TLRs cross-signal with CLRs to modulate antifungal defenses.

TLR-2 and TLR-4 Expression:
Interaction with dermatophytes increases TLR-2 and TLR-4 mRNA expression in myeloid cells, keratinocytes, and fibroblasts.
Feline neutrophils show increased TLR-2 and TLR-4 mRNA levels after stimulation with live and heat-killed *Microsporum canis*.
TLR-2's Role in Immune Response:
TLR-2 enhances CLR-mediated phagocytic activity, crucial for *Trichophyton rubrum* conidia phagocytosis and proinflammatory cytokine production.
Extracellular vesicles (EVs) from *Trichophyton interdigitale* induce proinflammatory mediators in a TLR-2-dependent manner.
Inflammatory Modulation by TLR-2:
In a deep dermatophytosis model, TLR-2 suppresses inflammatory responses and cytokine production (IL-17, IL-10, IFN-γ).
TLR-2 deficient mice show lower fungal burden and increased resistance to infections due to a stronger Dectin-1-mediated response.

TLR-2 expression appears to suppress Dectin-1-dependent CXCL8 production, important for neutrophil recruitment.

Dermatophytes and Skin Cells

The stratum corneum, the skin's outermost layer, is composed of:

- Dead Keratinocytes
- Keratin
- Hydrophobic Lipids
- Antimicrobial Peptides (AMPs)

Function: Acts as a barrier against environmental threats and potential pathogens.
Epidermis Role: Keratinocytes are essential for initiating the cutaneous immune response.
Innate Receptors:

- Toll-Like Receptors (TLRs)
- C-Type Lectin Receptors (CLRs)
- NOD-Like Receptors (NLRs)

Functions:

- Detect pathogens
- Induce cytokine, chemokine, and AMP synthesis
- Modulate recruitment and function of immune cells

Cytokine Receptors:

- IL-17R
- IL-22R
- TNFR

Immune Cell Subsets in Epidermis:

- Langerhans Cells (LC): Tissue immunosurveillance
- Resident Memory CD8+ T Cells

Immune Cells in Dermis:

- Dermal Dendritic Cells (DC): Various subsets
- Macrophages
- Mast Cells
- Innate Lymphoid Cells (ILCs)
- γδ T Cells
- Memory-Resident T Cells (CD4+ and CD8+)
- Regulatory T Cells (CD4+ and CD8+)

Additional Components:

- Nerve Terminals: Innervate the skin
- Lymphatic Vessels: Facilitate immune cell migration to lymphoid organs.[34]

Keratinocytes: Key Players in Cutaneous Immune Response

Keratinocytes are the first epidermal cells encountered during dermatophyte infection. Upon exposure to dermatophytes, they release pro-inflammatory mediators (IL-6, CXCL8, TNF) in order to stimulate inflammation and recruit neutrophils, and AMPs including cathelicidin and β-defensins witch promote fungal clearance.

Keratinocytes, even in the absence of other immune cells, besides forming a physical barrier against dermatophyte invasion, also actively contribute to early antifungal defense by triggering a skin-specific immune response. Notably, keratinocytes can also produce high levels of the immunosuppressive protein TSG-6 after *T. rubrum* infection, suggesting a role in inflammation control and tissue repair. IL-6, IL-17, and IL-22 further stimulate keratinocyte activation.

Role of Neutrophils in Dermatophytosis

In case of dermatophytosis, neutrophils are the first leukocytes to arrive at the infection site and are critical for fungal elimination from the skin. In both human infections and experimental models, neutrophils form epidermal microabscesses around the hyphae in the stratum corneum. Flow cytometry can detect CD11b+ Ly6G+ neutrophils in epidermal cell suspensions as early as two days after *Microsporum canis* infection in mice.

Dermatophyte-stimulated neutrophils can phagocytose conidia, activate MAPK and NF-κB pathways, leading to the release of proinflammatory cytokines/chemokines (CXCL8, IL-1β, IL-6, IL-8, TNF), enhancing keratinocyte activation, and recruiting more leukocytes. They also secrete reactive oxygen species (ROS) and neutrophil extracellular traps (NETs) to kill dermatophytes.

Despite this evidence, neutropenic patients (those with low neutrophil counts) are not particularly prone to extracutaneous invasive dermatophyte infections but often experience widespread superficial infections and dermal granulomas that resist antifungal treatments.

Impairments in neutrophil mobilization or fungal killing mechanisms, potentially linked to inherited CARD9 deficiency, may contribute to susceptibility to dermatophyte infections. However, the full extent of the role played by neutrophils in controlling cutaneous defense mechanisms and preventing extracutaneous fungal invasion remains unclear.[36-37]

Macrophage Role:

Macrophages might kill dermatophytes through IFN-γ-induced ROS and nitric oxide (NO) production or resolve inflammation by phagocytosing apoptotic neutrophils and producing anti-inflammatory cytokines like IL-10.[27]

Adaptive Immunity:

Langerhans Cells (LC): Located in the epidermis, they sense dermatophytes, migrate to lymph nodes, and promote Th17 differentiation.

Dendritic Cells (DC): In the dermis, they sense fungal molecules and produce cytokines driving IL-17 or IFN-γ-mediated immunity.

IL-17-Mediated Immunity in Dermatophytosis

Type 17 immunity plays a crucial role in both innate and adaptive immune responses in barrier tissues, including the skin. IL-17 cytokines are essential for maintaining local homeostasis with microbiota, protecting against infections, and mediating inflammatory diseases.

The IL-17 family consists of six related cytokines: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), IL-17F, and IL-17AF (a heterodimer). IL-17A is the most studied and is produced by various immune cells such as Th17 cells, Tc17 cells, γδ T cells, NK cells, ILC3, and natural Th17 cells.

IL-17 Function:

- Signaling: IL-17 cytokines act mainly on non-classical immune cells (epithelial, endothelial, and fibroblastic cells) through heterodimeric receptors like IL-17RA paired with IL-17RC, IL-17RE, or IL-17RB.

Effects on Keratinocytes: IL-17 stimulates the production of cytokines (GM-CSF, TNF, IL-6), chemokines (CXCL1, CXCL8), and vascular endothelial growth factor (VEGF). It also enhances the expression of AMPs (hBD-2, LL-37) and promotes keratinocyte proliferation, aiding in clearing fungal infections. IL-17A binding to IL-17RA/IL-17RC in keratinocytes activates Act1-TRAF6-NFκB/MAPK or STAT-3 pathways, inducing cytokine/chemokine and AMP production. This pathway also transactivates the epidermal growth factor receptor (EGFR), promoting keratinocyte proliferation.

Immune Activation in the Skin:

- Fungal Recognition: PRRs on myeloid cells (DCs, macrophages, neutrophils), keratinocytes, and fibroblasts detect fungal pathogens, triggering cytokine production (IL-23, IL-6, IL-1β, IL-21).
- Cytokine Signaling: These cytokines bind to receptors on lymphocytes, activating intracellular pathways (STAT3 phosphorylation, RORγt activation) that lead to Th17 cell differentiation and type 17 cytokine production (IL-17, GM-CSF, IL-22).

IL-17-mediated immunity is vital for protecting the skin against fungal infections, including dermatophytosis. Dysregulation or genetic defects in the IL-17 pathway can lead to increased susceptibility to chronic and severe fungal infections.[38-39]

IFN-γ-Mediated (Th 1) Immunity in Dermatophytosis

The role of IFN-γ-mediated response in protecting the skin against dermatophytes is less clear compared to IL-17-driven immunity. In a *Microsporum canis* infection model in mice, a shift to Th1 response was observed in IL-17-deficient mice, indicating a compensatory mechanism. However, neutralizing IFN-γ in IL-17RA KO mice increased Th17 cytokines and inhibited fungal growth, suggesting that deregulation of IFN-γ might inhibit IL-17 responses and contribute to superficial fungal overgrowth.[40-41]

In a *Trichophyton benhamiae* infection model, both Th1 and Th17 responses function complementarily. Only when both pathways are deficient do mice suffer from persistent infection.[42]

While IFN-γ may help clear infection by activating macrophages and promoting ROS production, its exact mechanisms remain unclear. In some models, IFN-γ contributes to fungal clearance and inflammation, whereas, in others, it inhibits protective type 17 responses.[43]

Mechanisms of immune evasion

Dermatophytes have developed several sophisticated strategies to evade the host's immune system and establish infection in keratinized tissues. Understanding these mechanisms is crucial for developing effective treatments and improving patient outcomes. Below are some key mechanisms through which dermatophytes evade the immune response:

1. Secretion of Immunosuppressive Substances

Mannans: Dermatophytes, such as *Trichophyton rubrum*, produce mannans that can inhibit cell-mediated immunity and keratinocyte proliferation, reducing the skin's ability to shed infected cells.[22-23]

2. Induction of Non-Effective Antibody Responses

Dermatophytes can stimulate the production of antibodies that do not contribute to fungal clearance. For instance, high levels of IgE against dermatophytes are observed in chronic infections, but these antibodies are ineffective at resolving the infection.[24]

3. Delayed-Type Hypersensitivity (DTH) Evasion

Chronic dermatophyte infections are often associated with defective DTH responses, which are crucial for fungal clearance. This immune evasion strategy helps the fungi persist in the host by avoiding the robust immune responses required for eradication. [25]

4. Activation of Host Signaling Pathways

Mitogen-Activated Protein Kinase (MAPK) Pathways: Dermatophytes can activate MAPK pathways in keratinocytes, leading to increased production of proinflammatory cytokines and tissue damage, which may help the fungi spread while diverting immune resources away from effective pathogen clearance. [26]

5.Th17 Cells and C-type Lectin Receptors:

Th17 cells are crucial for fungal defense, producing cytokines like IL-17 and IL-22, which help recruit neutrophils and produce antimicrobial peptides. C-type Lectin Receptors (CLRs) on

antigen-presenting cells recognize fungal components and activate immune responses. Dermatophytes modulate CLR signaling, reducing Th17 cell activation and immune response efficacy. By modulating the immune response, dermatophytes can establish chronic infections. This persistence is partly due to the reduced effectiveness of Th17 cell-mediated responses.[27] Other factors like keratin degradation enzymes, cysteine dioxygenze or cell wall components affecting complement inhibition are on the list of immune evasion.[28-29]

Hypersensitivity type IV and dermatophytosis

Altogether, the success of invasion strictly depends on a balance between virulence, responsiveness, and the ability to inhibit the host defense mechanisms.

Clinical signs

The clinical signs of dermatophytosis vary widely, reflecting the pathogenesis of the disease and the immune response of the host.

Alopecia: Often the first noticeable sign. It can occur in localized patches or be more widespread.
Papules and Scales: Small, raised bumps and flaky skin are common.
Crusts and Erythema: Scales and redness of the skin are frequently observed.
Follicular Plugging: Hair follicles may become blocked with kerato-seborrheic debris.
Hyperpigmentation: may occur in chronic cases.
Nail Changes: Onychogryphosis can affect one or multiple digits, leading to thickened and deformed nails.

Lesions typically exhibit an asymmetrical pattern and are minimally pruritic. However, when pruritus is present, it can lead to self-inflicted trauma, mimicking conditions such as pyotraumatic dermatitis or ulcerative eosinophilic lesions, particularly in cats.

Common Sites of Infection
In cats, lesions frequently appear on the face, ears, and muzzle, progressing to the paws and other body areas. This pattern has been corroborated by experimental co-habitant exposure studies.
In dogs, hunting breeds often present with lesions on the muzzle and paws due to their activities.

Nodular Lesions

Cats and dogs can develop nodular dermatophyte infections, diagnosed primarily via biopsy or cytological examination:

- **Kerion:** Presents as a single or multiple erythematous, alopecic, dome-shaped, exudative nodule(s) with granuloma or pyogranuloma formation, often containing fungal spores.
- **Pseudomycetoma and Mycetoma:** Characterized by nodules that fistulate, ulcerate, and drain serous to purulent debris. Diagnosis is through biopsy or cytology, with treatment often requiring surgical excision and systemic antifungal therapy.

Persian cats and Yorkshire terriers are among the most affected breeds. The Wood's lamp examination frequently yields negative results, particularly in animals that have had prior treatment. Tissue cultures are critical for accurate diagnosis, as the most commonly isolated pathogen, *M. canis*, is often identified from tissue samples rather than hair samples.

Prognosis for dogs with kerion reactions is generally positive. However, for cats and dogs with pseudomycetoma or mycetoma, prognosis is guarded due to short-term remissions and the severity of the condition, which may lead to euthanasia.[5, 44-46]

Diagnostic testing

No single diagnostic test is identified as the "gold standard".

Diagnostic testing for small animal skin diseases depends on various factors, including the stage of infection, whether treatment has been previously administered, the sampling technique used, site selection, clinician expertise, quality of diagnostic tools, and the ability to properly examine the animal.

Conventional Methods

- **Wood's Lamp Examination**: Many microorganisms produce phosphors that fluoresce under a Wood's lamp, aiding in infection detection. Dermatophytes of the *Microsporum* genus are known for this characteristic, with *Microsporum canis* being the primary fluorescing dermatophyte in veterinary practice. Other dermatophytes that show positivity in this test are *M. distorum*, *M. audouinii*, and *T. schoenleinii*. Species like *M. gypseum* and *T. mentagrophytes* typically do not fluoresce. The green fluorescence observed in *M. canis* infections is due to pteridine, a chemical metabolite in the cortex or medulla of the hair. In a recent study, the sensitivity of the Wood's lamp examination was 71% and the specificity was 92%. Fluorescence can develop as early as 5-7 days post-infection and is typically noticeable by 10-14 days. However, it can be obscured by crusts, so it is important to lift crusts to examine the infection sites for fluorescence.[5, 46-49]
- **Microscopic Examination**: Direct examination of hair and scales is a point-of-care technique used to confirm the presence of a dermatophyte infection. This identification technique requires prior training or at least reference images of positive cases to accurately compare and identify fungal elements under the microscope. Direct microscopy of hair and skin scrapings treated with potassium hydroxide (KOH), compounded chlorphenolac, lactophenol cotton blue or India Ink, or the basophilic stain from the Diff-Quik kit, helps visualize fungal elements. This method, while simple, is often less sensitive than culture-based methods. A study comparing hair plucking and skin scraping found that skin scrapings had higher positive results (78.4% in dogs and 80% in cats) than hair plucking (54.1% in dogs and 67.5% in cats). Combining both techniques yielded positive results in 83.7% of dogs and 87.5% of cats. [50]
- **Adhesive Tape Impression Cytology (ATI)**: ATI cytology is a simple, non-invasive diagnostic method that involves using adhesive tape to collect fungal elements from the skin. This method has been shown to be effective in diagnosing dermatophytosis and is comparable in sensitivity to fungal culture and hair plucks. It is particularly useful in cases where other methods are less effective, such as in dogs with kerion.[51]
- **Fungal Culture**: Culturing hair and skin scrapings on dermatophyte test media (DTM) or Sabouraud dextrose agar (SDA) is considered the gold standard. This method allows for the growth and identification of dermatophytes, although it requires a longer time to obtain results. The most common identification problem is raised by isolates not producing macroconidia on Sabouraud glucose agar: these isolates can almost always be stimulated to produce these conidia on potato dextrose and other sporulation media, and if subcultured to BCP milk solids-glucose medium. The isolates will do this while also producing a confirmatory negative reaction for alkalinization of the medium. The colour change in the medium from yellow to red is the result of a pH change triggered by fungal growth.[1,52]

Advanced Diagnostic Techniques

- **Polymerase Chain Reaction (PCR)**: PCR-based methods offer rapid and accurate detection of dermatophytes by amplifying specific DNA sequences. These techniques can significantly reduce the time required for diagnosis compared to traditional culture methods. Nested PCR and quantitative PCR (qPCR) have shown high sensitivity and specificity in detecting dermatophytes in clinical samples. The PCR protocol on paraffin-embedded tissues aids in diagnosing deep dermatophyte infections in cats. The results showed 100% concordance between PCR and culture. False positives were attributed to nonviable DNA on the hair coat, while false negatives were due to the limitations of fungal culture. Negative PCR in a treated cat is compatible with cure.[53]
- **CRISPR-based Assays**: Recent advancements in CRISPR technology have introduced the Cas12a-fluorescence assay, which combines recombinase polymerase amplification (RPA) for detecting dermatophyte DNA. This method is highly specific and allows for rapid, visual confirmation under blue light, making it suitable for point-of-care testing.[54]
- **Enzyme-Linked Immunosorbent Assay (ELISA):** The Enzyme-Linked Immunosorbent Assay (ELISA) is a valuable diagnostic tool developed to detect antibodies against *Microsporum canis* in dogs and cats. This assay is particularly advantageous because it provides a non-invasive method for identifying recent infections, which is crucial for the effective management and treatment of dermatophytosis. ELISA tests have demonstrated high sensitivity and specificity, with values of 92% and 95%, respectively. The test uses *Microsporum canis* glycoproteins as the antigen to capture specific antibodies present in the serum of infected animals.[55]
- **Dermoscopy**: Dermoscopy involves the use of a dermoscope to identify characteristic "comma hairs" indicative of dermatophyte infection, hyphae and spores on the shafts. The hairs are easier to identify in lighter coloured cats than black cats. While it is not a standalone diagnostic tool, it can help select hairs for culture or PCR analysis.[56]
- **Biopsy**: Histological examination of tissue is rarely used as a routine diagnostic method for small animal dermatophytosis. Skin biopsy diagnosis for dermatophytosis has been reported in the following situations: nonhealing wounds or nodules (kerion, pseudomycetoma, mycetoma), chronic facial lesions in dogs often investigated for suspected pemphigus, and unusual skin lesions with atypical presentations not easily attributed to other causes. Haematoxylin and eosin (H&E) staining may not always identify dermatophytes; thus, special stains like periodic acid Schiff (PAS) and Grocott methenamine silver (GMS) are required. Ideally, dermatophyte isolation from tissues should be done via biopsy samples in sterile saline for macerated tissue fungal culture, although false negatives can occur.[5]

Therapy and infection management.

Systemic Antifungal Therapy

Systemic antifungals are highly effective for treating dermatophytosis, especially in severe cases or when topical treatments alone are insufficient (Figure 8).

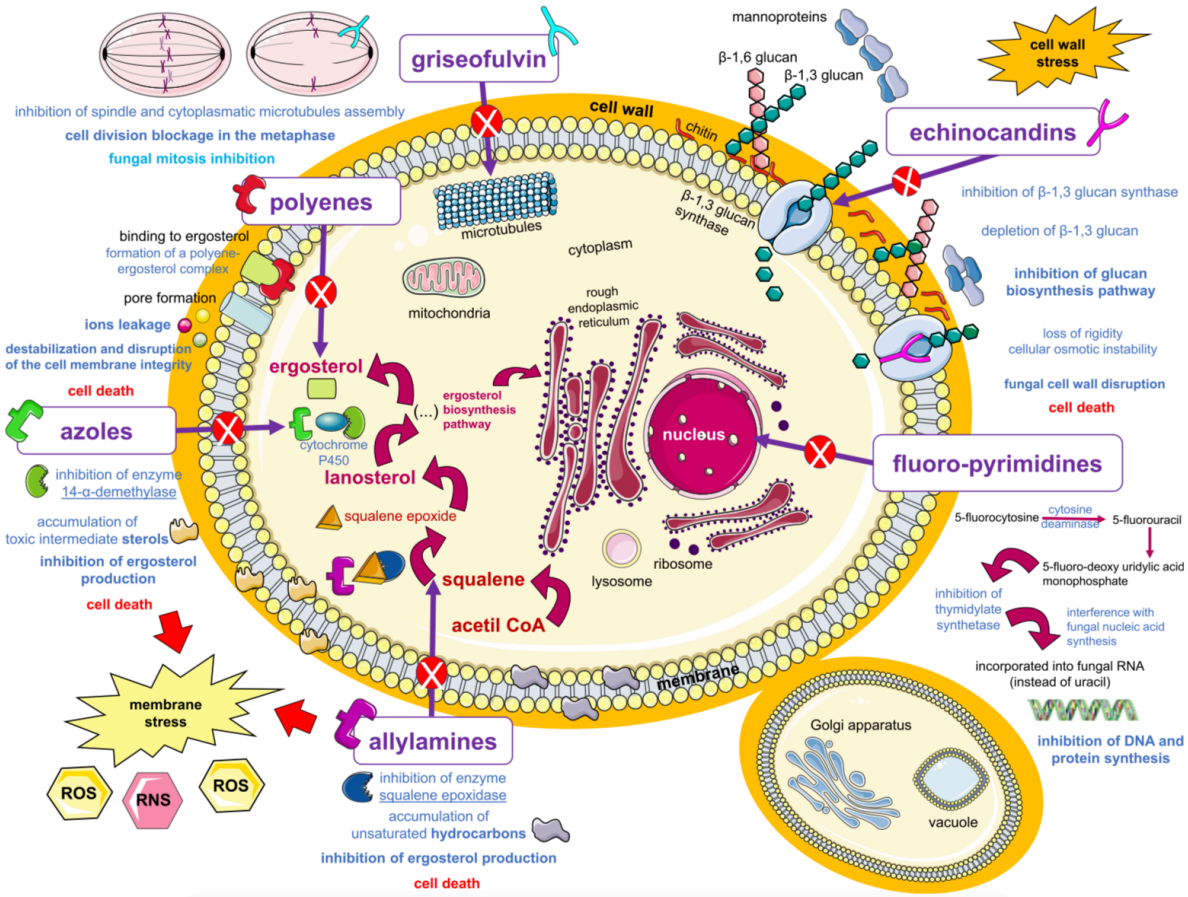

FIGURE 8. Action Mechanisms of Antifungal Drugs (F. Sousa, C. Nascimento, D. Ferreira *et al*. 2023)

Azoles

One of the main targets of small molecules effective in fighting off fungal infection is the cell membrane and the biosynthesis of ergosterol, a derivative of mammalian cholesterol. Ergosterol has several functions in fungi, the most important one being its role in cellular proliferation. Similar to mammalian cholesterol, it requires a specific structure with no methyl group at C14 and a double bond at C5-6. The key enzyme in this biosynthesis pathway is lanosterol 14α-demethylase (CYP51), part of the cytochrome P450 family, which catalyzes the oxidative removal of the 14-methyl group from lanosterol, leading to a double bond at C14-15 (Figure 9).

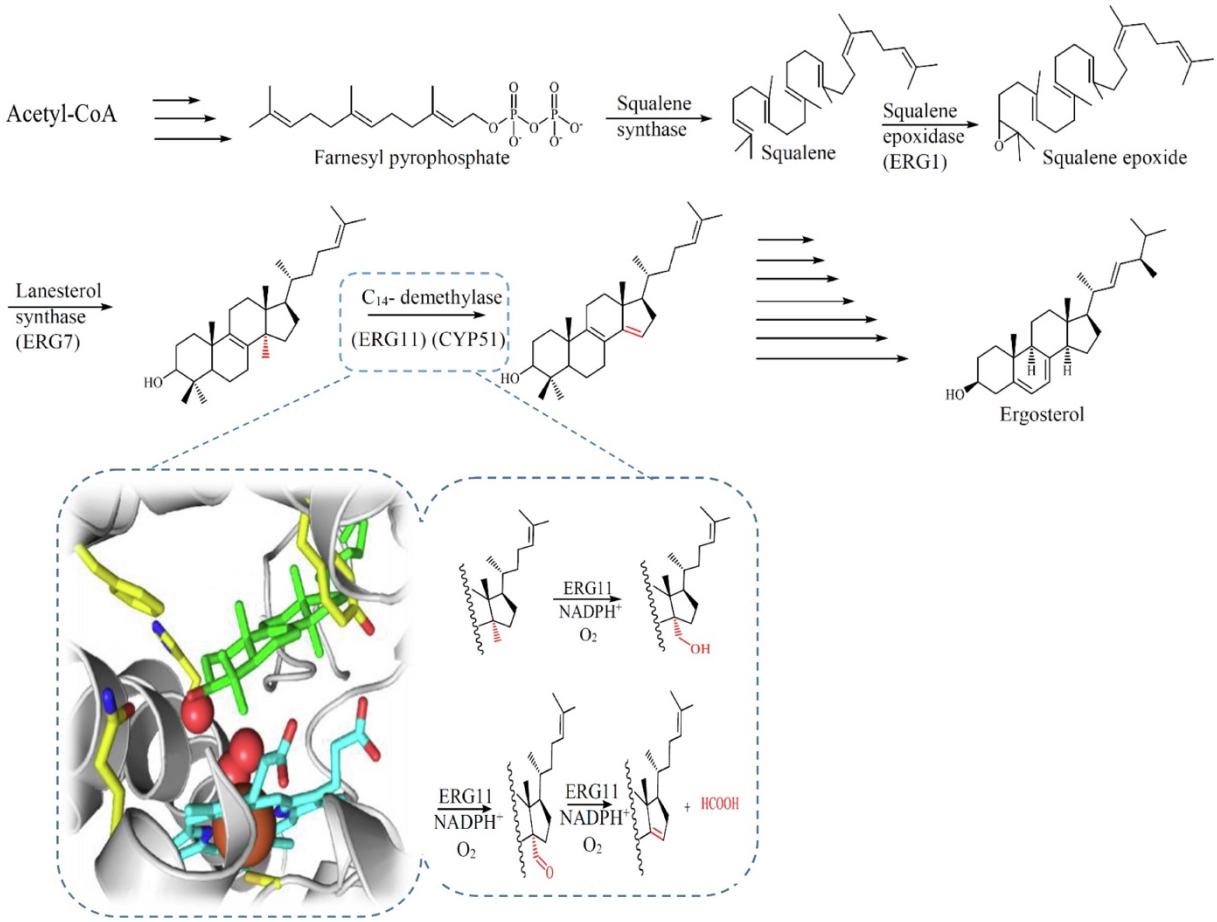

FIGURE 9. A. Sterol biosynthesis from Acetyl-CoA to ergosterol in fungi. (M. Shafiei, *et al.*2020)

Azole antifungals have a well-established role in the treatment of fungal infections due to their broad spectrum of activity and relatively low toxicity. By binding to the hem- iron of CYP51, azole drugs inhibit the enzyme through non-competitive, reversible interactions, preventing proton access to the active site. This inhibition stops the conversion of lanosterol to ergosterol, leading to the accumulation of toxic sterol intermediates and a depletion of ergosterol. As a result, the fungal cell membrane becomes more permeable and less functional, impairing cellular processes and leading to cell lysis. Specific interactions, such as the hydrogen bond between oteseconazole and His-377, are crucial for the drug's selectivity and potency.
There are primarily three main classes of azoles:
Imidazoles: This class includes antifungal agents such as clotrimazole and ketoconazole and are commonly used for topical applications and systemic treatments.
Triazoles: This class includes fluconazole, itraconazole, voriconazole, and posaconazole and are often used for systemic infections due to their broader spectrum of activity and better pharmacokinetic properties compared to imidazoles.
Thiazoles: Although less common, thiazoles like abafungin have unique structures and mechanisms that make them effective against certain fungal pathogens.

These classes are differentiated based on the number of nitrogen atoms in the azole ring and their specific molecular structures, which influence their antifungal activity and usage.

Azoles are also classified into four generations based on their structural characteristics and antifungal properties:

1. **First Generation**: Includes imidazoles such as clotrimazole and ketoconazole, primarily used for topical and systemic fungal infections.
2. **Second Generation**: Includes first-generation triazoles such as fluconazole and itraconazole, which have a broader spectrum of activity.
3. **Third Generation**: Includes second-generation triazoles such as voriconazole, posaconazole, and ravuconazole, with even broader antifungal spectra and improved pharmacokinetic profiles.
4. **Fourth Generation**: Includes next-generation triazoles still under development or in early clinical use, designed to overcome resistance issues and provide greater efficacy against emerging fungal pathogens.

Ketoconazole is a synthetic imidazole antifungal agent with a broad spectrum of activity, effective in treating both superficial and systemic fungal infections.
Forms and Administration: Available in various formulations including oral, topical (cream, shampoo, foam, gel), and as nanoparticles to enhance bioavailability and antifungal activity.
Spectrum of Activity: Ketoconazole is active against dermatophytes (e.g., *Trichophyton* spp.), yeasts (e.g., *Candida* spp.), dimorphic fungi (e.g. *Histoplasma capsulatum*), and other fungi.
Pharmacokinetics: Oral absorption is variable and can be affected by stomach pH. Antacids reduce absorption, while acidic conditions enhance it. Peak plasma levels are achieved 1-2 hours after administration. Ketoconazole is extensively metabolized in the liver and excreted primarily in feces.
Adverse Effects: Common side effects include gastrointestinal disturbances. Severe side effects can include hepatotoxicity and adrenal failure due to its inhibitory effect on steroidogenesis.

The non-antifungal effects of ketoconazole:
Liver Enzyme Inhibition: Ketoconazole inhibits cytochrome P450 3A (CYP3A) enzymes in the liver. These enzymes are responsible for the metabolism of cyclosporine. By inhibiting these enzymes, ketoconazole decreases the breakdown of cyclosporine, leading to higher blood levels of the drug even at lower doses.
5-Lipoxygenase (5-LOX) Inhibition: Ketoconazole inhibits 5-LOX, an enzyme involved in the biosynthesis of leukotrienes from arachidonic acid. Leukotrienes are potent mediators of inflammation, and their inhibition helps reduce inflammatory responses
Neutrophil Chemotaxis: It inhibits the migration of neutrophils, which are key players in the inflammatory response. This action helps in reducing the accumulation of neutrophils at inflammation sites and thereby diminishes tissue damage and inflammation.
Lymphocyte Blastogenesis: Ketoconazole suppresses lymphocyte proliferation (blastogenesis), which is crucial for the adaptive immune response.
CYP11B1 Inhibition: Ketoconazole inhibits CYP11B1, an enzyme involved in cortisol biosynthesis in the adrenal gland. By inhibiting this enzyme, ketoconazole reduces cortisol production, which is beneficial in conditions where cortisol suppression is desired, such as Cushing's syndrome.
Anti-inflammatory Effects: Ketoconazole activates the AhR-Nrf2 pathway, which enhances the cellular antioxidant response and reduces oxidative stress. This activation leads to the suppression of pro-inflammatory cytokine production and protects cells from oxidative damage. [58-62]

Itraconazole

It's a first generation triazole. At low doses it is fungistatic and at high doses it is fungicidal. The number of days to mycological cure, when reported, ranged from 36 to 112, with a combined continuous and pulse therapy regimen showing positive results.

Forms and Administration: Itraconazole is available in various formulations including oral capsules, oral solution, and intravenous form.

Spectrum of Activity

Itraconazole exhibits broad-spectrum antifungal activity. It is effective against dermatophytes, yeasts, and molds, including species of *Aspergillus, Candida, Histoplasma, Blastomyces*, and *Cryptococcus* sp. . Its wide range of efficacy makes it suitable for treating both superficial and systemic fungal infections.

Pharmacokinetics

Absorption: Itraconazole has variable absorption, which is enhanced in an acidic environment. The oral solution provides better bioavailability compared to capsules. Studies show that generic and brand-name itraconazole have similar bioavailability, but compounded formulations should be avoided due to inconsistent blood levels

Distribution: It achieves high and sustained levels in various tissues, including skin, nails, and deep organs. Itraconazole accumulates in adipose tissue and sebaceous glands and tissue concentrations are many times higher than plasma concentrations. It persist in the epidermis for up to four weeks after discontinuation of treatment.

Metabolism: Primarily metabolized in the liver via the cytochrome P450 enzyme system, specifically CYP3A4.

Elimination: Excreted mainly in the feces, with a small amount excreted in urine. It has a long half-life, allowing for once or twice daily dosing.

Adverse Effects

Common Side Effects: Gastrointestinal disturbances (nausea, vomiting).

Serious Side Effects: Rarely, it can cause liver toxicity. Monitoring of liver function tests is recommended during prolonged use. The drug is not recommended for use in pregnant or nursing dogs.

Drug Interactions: Due to its inhibition of CYP3A4, itraconazole can interact with various drugs, necessitating careful management and monitoring to avoid adverse interactions.[63]

Fluconazole

Fluconazole is a first generation triazole. Fluconazole is water-soluble and minimally protein-bound. Its absorption is not affected by the concurrent use of antacids and does not require food for optimal absorption. Half-Life: The mean half-life for both oral and intravenous administration is 12 to 14 hours in both dogs and cats.

Common Side Effects: Vomiting, diarrhea, and dose-dependent elevated serum ALT levels are the most common adverse effects.

Primary Use: Fluconazole is primarily used for the treatment of systemic mycoses.

Dermatophytosis: Fluconazole has poor antifungal efficacy against dermatophytes, showing the highest minimum inhibitory concentration (MIC) compared to itraconazole, terbinafine, ketoconazole, and griseofulvin for both *Microsporum* spp. and *Trichophyton* spp.

Fluconazole shows high bioavailability after oral administration. Studies indicate significant inter-dog variability in pharmacokinetics. Dosing adjustments and therapeutic drug monitoring are often recommended to achieve optimal therapeutic levels. Common dosing regimens for dogs include 5-10 mg/kg orally every 12 to 24 hours.[5,64-65]

Terbinafine

Terbinafine belongs to the allylamine class of antifungal agents. It is characterized by a tertiary allylamine functional group, which is essential for its antifungal activity.

Mechanism of Action: Terbinafine inhibits the enzyme squalene epoxidase, crucial for the biosynthesis of ergosterol in fungal cell membranes. This inhibition leads to the accumulation of squalene within fungal cells, which is toxic and disrupts cell membrane integrity. The resulting ergosterol depletion weakens the fungal cell membrane, making it more permeable and leading to cell death.

Pharmacokinetics: Terbinafine is well-absorbed orally, achieving peak plasma concentrations within 2 to 6 hours post-administration. It is lipophilic, allowing it to penetrate the skin, nails, and other tissues effectively. Terbinafine is metabolized in the liver, primarily via cytochrome P450 enzymes.

In dogs, the half-life is approximately 8.6 hours, allowing for once-daily dosing.

Clinical Applications in Dogs

Dermatophytosis Treatment:

Terbinafine is highly effective against dermatophytes such as *Microsporum canis* and *Trichophyton mentagrophytes*.

It is used at a dosage of 30 mg/kg orally in dogs, showing high cure rates and minimal side effects.

Studies have demonstrated significant efficacy in treating dermatophytosis with a typical treatment duration of 21 days in humans.

Interaction with Stratum Corneum Constituents:

Terbinafine interacts with the lipid and protein components of the stratum corneum, the outermost layer of the skin, enhancing its permeation and antifungal activity. This interaction supports the high efficacy of topical terbinafine formulations, allowing better penetration into the skin layers where dermatophytes reside.[67]

Selective Toxicity: Terbinafine selectively targets fungal cells, with minimal effects on mammalian cell cholesterol synthesis, contributing to its safety profile. Its mode of action does not affect mammalian cytochrome P450.

Adverse Effects: Common side effects include gastrointestinal disturbances (vomiting, diarrhea) and occasional liver enzyme elevation. Regular monitoring of liver function is recommended during prolonged treatment. Using an in vitro whole embryo culture system, ketoconazole and griseofulvin had relatively high teratogenic potential and terbinafine had none.[5,67-70]

Griseofulvin

Mechanism of Action: Griseofulvin works by interfering with fungal mitosis. It binds to the microtubules in the fungal cells, disrupting the mitotic spindle and inhibiting cell division. This process arrests cell division in metaphase, leading to the eventual death of the fungal cells. Additionally, griseofulvin may antagonize chitin synthesis in the fungal cell wall and cause morphological changes in fungal cells.

Griseofulvin has a high affinity for keratin, the main protein in the skin, hair, and nails. It accumulates in keratinocytes (skin cells), which allows it to act directly at the site of infection where dermatophytes thrive.

Griseofulvin is mildly water-soluble and poorly absorbed from the gastrointestinal tract. Its absorption is affected by dietary fat, drug formulation, and particle size. Nonmicrosized particles are better absorbed with a high-fat meal, while micronization improves absorption. In dogs, absorption improved when polyethylene glycol (PEG) was used as a dispersal carrier in ultramicrosized formulations.

After absorption, griseofulvin is widely distributed in the body, particularly in keratinized tissues. It is carried to the stratum corneum by diffusion, sweating, and transepidermal water loss.
Griseofulvin is metabolized in the liver and excreted in the urine. The drug and its metabolites can also be detected in sweat.

One study reported a 100% cure rate with an average time to cure of 41 days when using a dosage of 50 mg/kg once daily. Similarly, in cats, griseofulvin has proven effective. A study reported that griseofulvin-treated cats achieved a mycological cure within 70 days. Another study noted that griseofulvin was effective in 100% of treated cats with an average time to cure of 41 days
Combining griseofulvin with miconazole/chlorhexidine shampoo has been shown to accelerate the resolution of lesions and reduce environmental contamination in cats with dermatophytosis.
Treatment of *M. gypseum* infections in dogs and cats has shown varied results. Griseofulvin combined with topical enilconazole has been used, but treatment failures were noted in some cases due to poor sensitivity of the fungal isolates to griseofulvin.

Common Side Effects: Gastrointestinal disturbances such as vomiting and diarrhea are common. Anorexia and lethargy can also occur.
Serious Side Effects: Hepatotoxicity and bone marrow suppression are potential serious adverse effects. Regular monitoring of liver function and complete blood counts is recommended during prolonged treatment. In one report, seven cats developed lethargy, pyrexia, anorexia, depression, ataxia, upper respiratory infections, and in five of seven cases leukopenia or pancytopenia.

Griseofulvin is effective but has a higher potential for adverse effects compared to itraconazole and terbinafine.

Lufenuron
Lufenuron is a benzoylphenylurea drug that disrupts chitin synthesis, a critical component of the exoskeleton of arthropods and the outer cell wall of fungi. Its potential as an antifungal treatment was first suggested following observations that animals receiving lufenuron as a flea preventative did not develop dermatophytosis.

Mechanism of Action
Lufenuron inhibits chitin synthesis, which is essential for the structural integrity of fungal cell walls. By disrupting this process, lufenuron causes morphological changes in fungal cells, making it difficult for them to thrive.
Several field studies have yielded conflicting findings regarding the efficacy of lufenuron.
Lufenuron has no in vitro efficacy against dermatophytes, does not prevent or alter the course of dermatophyte infections, does not enhance the efficacy of systemic or topical antifungal treatments. This drug has limited utility in the treatment of dermatophytosis.[5]

Summary on Topical Antifungal Treatments
Effectiveness of Twice Weekly Application: Lime sulfur, enilconazole, and miconazole/chlorhexidine shampoos are recommended as effective topical therapies for treating generalized dermatophytosis in cats and dogs. These treatments help reduce the spread of infective material in the environment and minimize zoonotic risks. For instance, lime sulfur

has been shown to cure shelter cats with dermatophytosis within 18 to 49 days when combined with oral itraconazole.
Accelerated hydrogen peroxide products, climbazole, and terbinafine shampoos show potential for treating dermatophytosis. However, more in vivo studies are needed to conclusively document their efficacy before they can be definitively recommended. For example, terbinafine shampoo combined with chlorhexidine showed promise in a small study but requires further validation.
Miconazole shampoos have shown effectiveness in vitro and are most effective in vivo when used in combination with chlorhexidine. This combination helps achieve better results in treating dermatophytosis. A study found that miconazole/chlorhexidine shampoo was superior to miconazole or chlorhexidine alone in resolving infections.
Chlorhexidine as a single treatment is poorly effective against dermatophytosis and is not recommended for use as a standalone therapy. It is best used in combination with other antifungal agents. Studies have shown that chlorhexidine alone does not prevent infection or significantly reduce fungal loads.
Clotrimazole, miconazole, and enilconazole have demonstrated effectiveness in treating localized dermatophytosis. These antifungals are recommended as concurrent treatments alongside other therapies but should not be relied upon as sole treatments. Localized applications of enilconazole, for instance, were effective in treating focal lesions in cats.
Topical treatments, particularly lime sulfur, have been effective in preventing the spread of dermatophytosis in shelter environments. Studies have shown that cats in contact with infected animals do not develop lesions or become culture positive when treated with lime.
The use of topical antifungals, along with rigorous environmental cleaning, is crucial for controlling the spread of dermatophytosis. Studies indicate that environments housing infected animals treated with topical therapies can remain culture negative.
Lime sulfur is effective but can cause adverse effects such as drying of footpads and hair coat discoloration. Enilconazole is well tolerated, with minor side effects like drooling. Miconazole/chlorhexidine combinations have shown good tolerability and effectiveness, especially when used with systemic antifungal treatments.

Some studies suggest that certain breeds, such as Persian cats, may have different responses to treatment protocols. Additionally, concurrent conditions like upper respiratory infections can influence treatment outcomes.[5,52,57,73-74]

Conclusions on Fungal Vaccines for Dermatophytosis in Dogs and Cats

1. Dogs: Studies have shown mixed results regarding the efficacy of fungal vaccines in dogs. In a study where dogs were vaccinated with a live *T. verrucosum* or *M. canis* vaccine, those vaccinated against *M. canis* showed mild scaling instead of overt disease when challenged, suggesting some prophylactic effect.
2. Cats: Vaccination studies in cats have shown varying results. Some experimental vaccines, such as an adjuvanted killed vaccine and a combined live-inactivated vaccine, did not protect kittens from *M. canis* infection. Other studies indicated that commercial vaccines could reduce the severity of lesions in cats under one year of age and those with first-time infections. Field studies and case reports on the use of commercial vaccines for treating feline dermatophytosis have shown some promising results. For instance, one study reported clinical remission in 27 long-haired cats treated with an inactivated *M. canis* vaccine, with cats becoming culture negative within 28 days.

Another study using a pentavalent vaccine showed a slightly faster recovery in vaccinated cats with severe lesions compared to placebo-treated cats.

3. Individual case reports, such as the treatment of an 8-year-old cat with a commercial vaccine, have shown successful clinical and mycological cures by day 28.
4. Studies have indicated that both live and inactivated vaccines are generally safe, with only mild to moderate local reactions observed. These reactions typically resolved within a few days and did not result in severe adverse events. [57,75-80]

Conclusions on Environmental Disinfection for Dermatophytosis

1. **Minimizing Transmission:** Environmental disinfection aims to reduce disease transmission to humans and animals and to minimize fomite carriage on the hair coat of animals, which can complicate disease monitoring. This is crucial to shorten treatment duration by preventing false positive fungal culture or PCR results due to fomite carriage.
2. **Infection Sources:** Contact with a contaminated environment alone is a rare source of infection in people and animals. Dermatophyte transmission primarily occurs through direct animal-to-animal contact even in contaminated environments.
3. **Environmental Contamination:** Dermatophyte spores can contaminate both soft and hard surfaces in households with infected cats or dogs. Infected cats are more likely to contribute to environmental contamination than dogs.
4. **Misconceptions:** Common misconceptions include the belief that dermatophytes can cause respiratory infections or multiply in household environments. Dermatophytes require keratin from hosts to survive and do not cause fungal respiratory diseases or invade household structures like black mold.
5. **Veterinary Clinics:** Studies have found dermatophytes on the floors of veterinary clinics and teaching hospitals, indicating the importance of regular cleaning and disinfection routines to prevent contamination.
6. **Viability of Spores:** Dermatophyte spores can remain viable in the environment for extended periods under laboratory conditions, but their infectivity under natural conditions diminishes over time.
7. **Effective Disinfectants:** Sodium hypochlorite (household bleach), enilconazole, accelerated hydrogen peroxide, potassium peroxymonosulfate, and certain over-the-counter disinfectants have shown efficacy against dermatophytes. Essential oils are gaining popularity but require more evidence for widespread use.
8. **Disinfection Practices:** Effective disinfection involves mechanical removal of debris, washing with detergent, and applying disinfectants. Nonporous surfaces should be cleaned thoroughly, and washable textiles can be decontaminated through mechanical washing. Carpets and wooden floors require specific methods for effective disinfection.[5]
9. **Minimizing Shedding and Spread:** Strategies to minimize the shedding and spread of infective material include clipping the hair coat (with caution), using topical therapies, confining infected animals to easily cleaned areas, and frequent cleaning and disinfection. Clipping the hair coat can sometimes worsen the infection, particularly if not combined with systemic antifungals.
10. **Confinement and Cleaning:** Confining infected animals helps reduce the risk of transmission and allows for more effective environmental decontamination. Twice-weekly cleaning and disinfection are recommended to manage environmental contamination. [5]

Monitoring and Follow-Up

Continuous monitoring and follow-up are essential to ensure the infection has been fully eradicated. Techniques include:

1. **Wood's Lamp Examination**: This can be used to detect fluorescence from dermatophytes such as *Microsporum canis*.
2. **Fungal Cultures**: Regular fungal cultures should be performed to monitor the animal.

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