Cutaneous Protothecosis

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• Prototheca species are an achlorophyllic algae that cause infections primarily in immunocompromised individuals. At least one-half of infectious cases are cutaneous. Because protothecosis is seldom suspected clinically, patients may be subjected to various treatment modalities for extended periods without satisfactory results. Cutaneous protothecosis shares similar clinical and pathologic findings with deep tissue fungal mycoses. The typical presentation occurs most commonly on the face and extremities as erythematous plaques, nodules, or superficial ulcers. Prototheca spp are spherical, unicellular, nonbudding organisms that are sometimes noted on routine hematoxylin-eosin staining but are best visualized with periodic acid-Schiff and Gomori methenamine-silver histochemical stains. Although protothecosis can be diagnosed on biopsy, culture of the organism on a medium such as Sabouraud dextrose agar is required for definitive diagnosis. Treatment may require a combination of surgical excision and antifungal agents. Therefore, cutaneous protothecosis should be considered in a lesion that appears suspicious for the more-common fungal infections.

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Cutaneous protothecosis is an uncommon indolent condition occurring primarily in immunocompromised individuals. The infective organism, a Prototheca sp, is an achlorophyllic and ubiquitous algae. The first description of the Prototheca sp was made by Davies and colleagues in 1964.1–3 The organism has been isolated from a variety of reservoirs, including soil, water, animal, and food items. Protothecosis has been found on every continent, except Antarctica. Its prevalence in the United States is concentrated in the southeast. The 3 main clinical presentations of Prototheca sp infections are cutaneous lesions, olecranon bursitis, and disseminated systemic disease.4–6 This review will focus on the disease manifestations of cutaneous protothecosis and its similarities to deep tissue fungal infections. To our knowledge, at least 77 cases of cutaneous protothecosis have been described in the literature at the present time.1–2

CLINICAL FEATURES

Cutaneous protothecosis usually presents as an erythematous plaque that can be vesiculobullous or, less commonly, ulcerative, with crusting and purulent discharge (Figure 1).1,10–11 It can also manifest as verrucous, hypopigmented, and atrophic lesions.1–6 Most of these cutaneous infections occur in immunocompromised patients with risk factors, including human immunodeficiency virus/acquired immunodeficiency syndrome, diabetes mellitus, underlying malignancy, and prolonged use of systemic corticosteroids.1–4 Steroid use appears to be the most common risk factor for infection. Protothecosis can present in any adult but is most common in the elderly. Children are rarely infected.1 The presentation in immunocompetent patients is often localized papules and pustules. Lesions may be present for months to years, changing gradually in size and shape. The most commonly involved sites are the face and extremities.1–4

Prototheca wickerhamii is the Prototheca sp most commonly responsible for human infections.2 The postulated mode of cutaneous infection is through traumatic inoculation, and characteristically, the trauma is subtle and not noticed or remembered by the patient.1–2 The incubation period for disease presentation is not well documented and is speculated to range from a week to months.1,2 The typical clinical course of cutaneous protothecosis is chronic and indolent. Most patients described in the literature were initially suspected to have deep tissue infections, but the corrected diagnosis was rendered by skin biopsy and later, confirmed with tissue culture.1–2 Disseminated disease from cutaneous protothecosis is rare, occurring only in 8 patients with severe immunosuppression. The mortality rate is high, however, with a fatal outcome directly resulting from disseminated disease in 5 of those cases.2 The pathogenesis is not well understood because there are few reported cases. Neutrophilic response is thought to be the principle defense of the host through ingestion and eradication of the Prototheca organisms. It has been reported that a polymorphonuclear neutrophil can engulf P wickerhamii in about an hour.2 Neutropenia does not appear to alter infection rates. Rather, it alters defects in neutrophilic function. Result in the loss of neutrophil ability to engulf and kill the Prototheca organisms may be implicated in the development of protothecosis.1 Three cases of progressive, cutaneous disease have been reported in patients with neutrophilic defects.2

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LABORATORY AND MICROBIOLOGY FINDINGS

Prototheca spp are considered achlorophyllic mutants of the green alga genus Chlorella. They are spherical, unicellular, nonbudding organisms that range from 3 to 30 μm and consist of sporangia with thick, double-layer walls filled with multiple endospores. They reproduce asexually through internal septation and release the endospores when the parent cell ruptures. Although protothecosis can be diagnosed on biopsy, culture of the organism on a media such as Sabouraud dextrose agar is required for definitive diagnosis. Prototheca spp also grows on blood, Tween 80, eosin-methylene blue, and MacConkey agars.

One of the difficulties in identifying Prototheca spp is the overgrowth of bacteria and fungi on the culture medium. The metabolic demands of Prototheca spp are simple, and they can be grown on a wide variety of culture media. Some of these media, however, lack inhibitors to bacteria and fungi, which prevent Prototheca growth. The ideal method for growing Prototheca spp, it has been suggested, is through an isolation medium, with

Figure 1. Erythematous plaques and nodules on the dorsum of the hand.
Figure 2. A, Pseudop epitheliomatous hyperplasia and granulomatous inflammation. B, Numerous round or ovoid, refractile basophilic bodies forming morula-like aggregates (hematoxylin-eosin, original magnifications ×100 [A] and ×400 [B]).
Figure 3. Prototheca sporangia displaying morula-like septation (Gomori methenamine-silver, original magnification ×400).
fungal and bacterial inhibitors for selective cultivation.\textsuperscript{14} The optimum growth temperature is 30°C for colonization to thrive, although they will grow at temperatures up to 35°C.\textsuperscript{3} \textit{Prototheca} spp will usually present within 48 hours as soft, wet, yeastlike, white to light tan colonies.\textsuperscript{1,2} A wet mount of the culture can demonstrate the endosporulating sporangia if stained with lactophenol cotton blue or calcofluor white. These sporangia are the hallmark of the \textit{Prototheca} spp and tend to form morula-like structures that have an internal septation with a cartwheel-like appearance.\textsuperscript{1,2} The morula forms appear symmetrical in \textit{P. wickerhamii}, whereas in other species of the \textit{Prototheca} genus, the morula have a more random structure without the cartwheel-like appearance.\textsuperscript{1,14}

**HISTOPATHOLOGY**

Cutaneous biopsies demonstrate a pandermal, granulomatous, inflammatory infiltrate often admixed with lymphocytes, neutrophils, and eosinophils. Necrosis may be observed within the granulomas, but is not necessary to make the diagnosis.\textsuperscript{2,14} Multinucleated giant cells and plasma cells are usually present; however, the inflammatory response may be minimal in some cases.\textsuperscript{1,3-6} Other findings include hyperkeratosis and parakeratosis, pseudopodial hyperplasia of the epidermis, and hyperplastic lymphoid tissue.\textsuperscript{1,2,14} \textit{Prototheca} spp can be observed on hematoxylin-eosin–stained sections and are found in clusters, as solitary structures, within granulomas and giant cells, and in necrotic foci (Figure 2, A and B).\textsuperscript{1} They can also (rarely) be observed in the stratum corneum of the epidermis.\textsuperscript{4} The organisms are readily apparent with the use of special histochemical stains, such as Gridley fungus stain, periodic acid–Schiff (with or without diastase), and Gomori methenamine-silver stain (Figure 3).\textsuperscript{2,5,13}

**DIFFERENTIAL DIAGNOSIS**

The main consideration in the differential diagnosis is a deep, cutaneous mycosis, and common pathogens include \textit{Coccidioides immitis}, \textit{Blastomyces dermatitidis}, \textit{Paracoccidioides brasiliensis}, and \textit{Cryptococcus neoformans} (Table). The similarities in clinical presentation make it difficult to determine the causative agent of the infection. Thus, biopsy and tissue culture are necessary to identify the implicated organism. The size of the sporangia often aids in distinguishing \textit{Prototheca} spp from other cutaneous, nonsporulating fungi.\textsuperscript{1,2} Sporangia from \textit{Coccidioides} spp are typically larger than those of \textit{Prototheca} spp but have smaller endospores.\textsuperscript{2,14} They differ lacking reproductive budding and pseudomycelia. Protothecosis may also resemble green algae in tissue. In comparison to their mutant algal counterparts, a green algae or \textit{Chlorella} sp has chloroplasts and a triple-layered wall, in contrast to the double-layered wall and absence of chloroplasts of \textit{Prototheca} sp, as seen on electron microscopy.\textsuperscript{1,2} The \textit{Chlorella} sp also has numerous cytoplasmic starch granules that are negative for periodic acid–Schiff following diastase digestion, and thus, periodic acid–Schiff staining with diastase digestion can be beneficial in differentiating protothecosis from other green algae cells in tissue.

**CURRENT TREATMENT AND PROGNOSIS**

A standard treatment regimen has not been defined, and treatment may vary case by case. Aggressive therapy should be reserved for those patients with underlying comorbidities and immunosuppression.\textsuperscript{7} The most common pharmaceutical agents used are antifungal agents, such as ketoconazole, itraconazole, fluconazole, and conventional Amphoterin B. Amphoterin B appears to be the most effective of these agents against \textit{Prototheca} spp.\textsuperscript{1} Disseminated and visceral infections are usually treated with Amphoterin B, whereas theazole antifungal agents, especially itraconazole, are used for more localized infections.\textsuperscript{1,4,14} The antifungal agents target the \textit{Prototheca} sp cell wall, which is composed of 4% ergosterol.\textsuperscript{2}

Surgical excision of localized lesions has also proved beneficial and is especially effective against superficial cutaneous infections without deep dermal or subcutaneous involvement.\textsuperscript{7} With the deeper and more extensive infections, treatment usually requires a combination of surgical excision and antifungal agents.\textsuperscript{1,4} Difficult lesions may persist and can spread to other cutaneous sites if not treated effectively.

**CONCLUSION**

\textit{Prototheca} sp, albeit ubiquitous, is a rare cause of opportunistic cutaneous infections. Various forms of immunosuppression, such as human immunodeficiency virus/acquired immunodeficiency syndrome, diabetes mellitus, and systemic corticosteroid use, are predisposing factors. Microscopic findings on tissue biopsy and tissue culture are essential to the final diagnosis. Without proper treatment, such as antifungal agents and surgical excision, lesions may persist and spread to other sites. Thus, cutaneous protothecosis must be considered when

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Differential Diagnosis of Organisms Causing Soft Tissue Fungal Infections*  

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Size, µm</th>
<th>Histological Appearance</th>
<th>Clinical Forms</th>
<th>US Geographic Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Prototheca wickerhamii}</td>
<td>3–30</td>
<td>Spherical; nonbudding; sporangia with thick, double-layer wall; morula-like appearance; filled with multiple endospores</td>
<td>Chlorophyllic algae</td>
<td>Southeast</td>
</tr>
<tr>
<td>\textit{Coccidioides immitis}</td>
<td>10–200</td>
<td>Spheres vary in size; some contain nonbudding endospores</td>
<td>Spherules, endospores</td>
<td>Southwest</td>
</tr>
<tr>
<td>\textit{Blastomyces dermatitidis}</td>
<td>8–15</td>
<td>Large, double-refractile cells; buds are single, connected by a broad base</td>
<td>Yeast</td>
<td>South-central; Midwest</td>
</tr>
<tr>
<td>\textit{Paracoccidioides brasiliensis}</td>
<td>5–60</td>
<td>Cells have a mariners-wheel appearance (large cells with peripheral buds)</td>
<td>Yeast</td>
<td>Not typical in USA; Central and Latin America</td>
</tr>
<tr>
<td>\textit{Cryptococcus neoformans}</td>
<td>2–15</td>
<td>Cells are spherical to football shaped; polysaccharide capsule</td>
<td>Yeast</td>
<td>West</td>
</tr>
</tbody>
</table>

* Data modified from Baron and Finegold.\textsuperscript{15}
working up a lesion that is suspicious for the more common fungal infections.

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References