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I'VE GOT YOU UNDER MY SKIN – THE MOULDS OF MAN

There are thought to be over 1.5 million species of fungi. Of these, most live on decaying vegetation, in partnership with algae (lichens) or tree roots (mycorrhizas) or are parasites of plants or insects. Only a few tens of species cause us any direct harm but in forthcoming issues *Mycologist* is featuring a series of articles about the main species that do cause irritating, and in some cases life-threatening human infections. In this issue *Paracoccidioides brasiliensis*, a dimorphic fungal pathogen endemic in Central and Southern America, is discussed.

Paracoccidioides brasiliensis – the man-hater

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Some fungi are found only in specific geographical regions. The human pathogen *Paracoccidioides brasiliensis* has only rarely been isolated in nature and incidences of infections are restricted to Central and Southern America. Most commonly infected individuals are not urban dwellers, but rather farmers and people living in mountainous areas. In addition, it is almost invariably males who are infected. The gender bias has been reported as between 10 - 20:1 males over females! A possible explanation for this astonishing and unique phenomenon is given below.

P. brasiliensis is the causative agent of paracoccidioidomycosis, a human systemic mycosis for which the portal of entry of the fungus is via inhalation of airborne propagules into the respiratory tract. After entering the host, the fungus converts spontaneously to its yeast form, a fundamental step for the successful establishment of the infection. Although geographically confined, paracoccidioidomycosis constitutes one of the most prevalent deep mycoses in this region of the world. However, notification of its occurrence is not compulsory in the countries where it is endemic, hence accurate figures regarding the disease incidence and prevalence are difficult to determine. However, some figures suggest that around 10 million people are infected (San-Blas *et al.* 2001). The fungus has also been isolated from liver, spleen, and lungs of armadillos (*Dasypus novemcinctus*) and the geographical distribution of this animal superimposes very closely with the endemic area of paracoccidioidomycosis, from southern Mexico to Argentina. This suggests that the armadillo may well be a reservoir for the fungus in the wild (San-Blas & Nino-Vega, 2002).

Most infected individuals develop only mild non symptomatic or subclinical forms of paracoccidioidomycosis. However this may then progress into a disease classified into two more serious clinical forms, either as a rapidly developing widespread systemic mycosis (acute or subacute form) or as a persistent infection (chronic form). The type of disease depends on both host, fungal and environmental factors. The acute form is characterised by relatively short periods of incubation (from weeks to a few months) during which there is colonisation of the spleen, liver, lymph nodes and bone marrow, and severe depression of cell-mediated immune function. The form of disease represents only 3 to 5% of all cases and is normally found in children, young adults (up to 25 years of age), and immunosuppressed patients (Brummer *et al.*, 1993). Although there may be no sign of infections in x-rays of lungs in this clinical form, analysis of lung secretions is usually positive, indicating that the fungus is present. The chronic form represents around 90% of cases and affects mainly adult males over 30 years of age. It can be mild, moderate or severe, depending on the condition and immune status of the patient. This form progresses slowly and takes months or even years to become fully established, and unlike the acute form of disease, there is frequent involvement of the lungs as primary focus of infection (around 90% of cases) (Brummer *et al.*, 1993). In the chronic form of disease, the fungus may grow in the lung parenchyma, where

lesions may progress locally or disseminate to the oral and nasal mucous membrane, skin, lymph nodes and adrenals glands (Restrepo-Morano, 1993). This is a truly aggressive and serious type of infection requiring chemotherapuetic intervention. The symptoms of pulmonary paracoccidioidomycosis are non-specific and include a productive cough, chest pain, fever, weight loss and frequently, there is also some degree of breathlessness (Restrepo-Moreno, 1993). Despite colonisation of the lung and presence in expectorant there is no evidence to support the idea that paracoccidioidomycosis might be contagious.

Amphotericin B is one of the polyene macrolide antifungals and is used for the treatment of severe cases of disseminated disease, although follow-up therapy with sulphonamide is often used to prevent relapses. However, azoles have also been used lately in the treatment of the disease. Ketoconazole is effective in severe cases and relapses, although side gastrointestinal or endocrinal effects may appear. Itraconazole is ten times more active against the fungus than ketoconazole and does not interfere with endocrine metabolism, becoming the drug of choice for the treatment of paracoccidioidomycosis (San-Blas & Nino-Vega, 2002).

Recent studies in which various molecular typing methods such as RFLP and RAPDs have been used to type strains of *P. brasiliensis* give a surprising result. The molecular similarities between strains precisely mirror the geographical origin of the strains (Calcagno *et al.*, 1998; Nino-Vega *et al*, 2000). Given access to an unknown isolate it would be possible, in most cases, to tell exactly what Southern American country the infected patient came from by performing one of these molecular tests. This implies that there is little mobility of the patient acquiring the infection and also that there is little mixing of fungal strains.

Like many other fungi that cause infections in humans *P. brasiliensis* exhibits the phenomenon of structural dimorphism - that is it can grow either as a unicellular yeast or as a filamentous, branching mould. It is interesting to note that aside from *Candida albicans* most of the human dimorphic pathogens are pathogens in the yeast form. In the case of *P. brasiliensis* it is quite clear that the budding form is found in the human body and at 37°C in laboratory media. At lower temperatures, including presumably in natural environments, the organism grows as a branching mould. In the laboratory this is extremely convenient since temperature alone can be used to regulate the morphological form and this facilitates greatly the analysis of biochemical and molecular differences between the two forms. In other dimorphic fungi the yeast and hyphal forms often have to be grown in entirely different growth media which complicates analysis of how morphology is regulated.

One aspect of the regulation of the transition from hyphae to yeast is of particular note and this may relate directly to the aforementioned tendency for males to be infected much more often that females. In laboratory experiments the transition form the hyphal form to yeast growth is blocked by the presence of oestrogens (Restrepo *et al.*, 1984; Aristizábal *et al.*, 2002). Women, it seems, are naturally immune! So there is a serious case to be made that this fungus should only be investigated by female scientists.

The yeast form of the fungus has multiple buds (a useful feature for identification of the fungus from clinical samples), and multiple nuclei which limits the ability to obtain mutants using this form. Indeed although efforts are now being made to generate tools to understand this fascinating organism at the molecular level it has so far proved to be extremely difficult to study using molecular genetics. Nevertheless a full-scale genome project is now under way in Brazil and the availability of a genome database will hopefully catalyze interest in this important but relatively neglected man-hating fungus!

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