Host Parasite Relationship in Paracoccidioidomycosis

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Paracoccidioidomycosis (Pbmycosis) is a systemic disease confined to Latin America; its endemic areas extend from Mexico through Central and South America down to Argentina. *Paracoccidioides brasiliensis* (Pb), its agent, causes disease and mortality specially in rural populations being a major public health problem.

The disease was first recognized by Adolfo Lutz in 1908, in São Paulo. Its agent was identified by Floriano de Almeida and its clinical and pathological features described by Pupo and Cunha-Mota, the three of them from the today, University of São Paulo Medical School.

Eighty years after the first references to this mycosis we still do not know for sure the habitat of the fungus or how man is infected. As a consequence we also do not know the early manifestations of this disease that after an insidious onset and slow course end up compromising several organs in an unpredictable sequence.

The great majority of the knowledge on host parasite relationship in Pbmycosis is based on clinical and radiological descriptions of lesions and on the study of biopsies obtained when the infection is already well established; a few studies have included autopsies done almost always in patients who died in the final stages of the disease.

The result of these difficulties is that certain concepts about the pathogenesis of Pbmycosis have been mainly derived from animal experiments in which large number of *P. brasiliensis* in its yeast form have been inoculated in susceptible animals. Yeasts almost certainly are not the infecting forms of the fungus since yeasts only develop at temperatures well above the ones observed in the environment where the man-fungus interaction appears to occur. Experimental animal models are somewhat artificial but they have offered useful information and allowed a progressively better understanding of this mycosis.

Early investigators were of the opinion that the fungus invaded through the oropharingeal mucosa but latter clinical and radiological evidences, experimentally corroborated suggested that the lungs were the portal of entry. Nowadays the majority of the data favor the inhalatory route with early pulmonary lesions as the rule; exceptionally trauma and other routes have been reasonably well documented.

Once within the tissues the parasite can either be destroyed or allowed to multiply to produce a primary inoculation lesion. The fungus is then drained to the regional lymph nodes where a satellite lymphatic lesion is established. As described in tuberculosis, a Pbmycotic primary complex with possible lymphohematogenous dissemination is the accepted manifestations of the early infection phase.

The great majority of the infected persons, however, do not develop paracoccidioidomycosis disease. They become sensitized; up to 60% of the population in endemic areas are skin test positive, but only a very small proportion of them have clinical manifestations.

The initial foci of infection may: 1. regress with cicatrization of lesions; 2. regress with maintenance of viable fungi in the scars, that may in the future, give origin to disease and 3. progress with development of early signs and symptoms of paracoccidioidomycosis. The disease manifests itself in two different forms: one "acute-subacute" affecting young persons of both sexes and a "chronic" form that progress slowly and occur almost exclusively in males. The acute-subacute form is characterized by a severe progressive involvement of lymph nodes, spleen liver and bone marrow, that unless treated leads to death in months. The chronic form compromises slowly the lungs; the lesions remain localized, but, if not treated will progress with involvement of other organs and systems as skin, mucosae, bone, adrenals, CNS and urogenital system. When treated
the disease regres but may, at any time recur, recurrences being unpredictable in their site and severity 2).

**Host parasite relationship**

To understand paracoccidioidomycosis is essential to take in account a series of its peculiarities as: the large proportion of persons infected and small numbers that manifest disease; the marked differences on pattern and severity of the disease when it compromises youngs and adults; the marked male preponderance in the chronic form, in contrast with the even distribution of positive skin tests among sexes, as well as the lack of sexual differences in the acute-subacute form, the appearance of the disease decades after the patient abandoned endemic areas and also the frequency of unpredictable recurrences years after an apparent cure of the disease.

Even when we do not have definite explanations for these peculiarities it is known that they reflect variations of the fungus (virulence, antigenicity, infecting dose, portal of entry) as well as the mechanisms of natural and acquired resistence of the host.

**The fungus. P. brasiliensis** isolates vary in their growth curves, ultrastructural features, antigen composition and virulence, an indication that differences in clinical presentation of Pbmycosis may be caused by different strains of the Pb. Furthermore antigen composition may vary with in vitro passages of the fungus4-6).

Table 1 shows that different isolates inoculated in suscetible B10A mice differ in behavior; isolate Pb 18 besides its virulence leads to higher titers of antibodies; virulence does not appear to be related to growth in vitro or ultrastructural features of the fungus6-9). However there appears to be correlation between severity of the disease and virulence of the isolated fungus when inoculated in suscetible mice6). Even the pattern of granulomas changes in relation to the virulence of the inoculated isolate.

*P. brasiliensis* 43KDa glycoprotein is a proteinase capable of hydrolising casein, collagen and elastin and may facilitate host tissue invasion by the fungus. The 43 KDa glycoprotein has common epitopes in 2 virulent isolates that are not present in the avirulent isolates6). *P. brasiliensis* yeasts have laminin receptors and in the presence of antilaminin monoclonal antibodies there is a significant decrease in adhesion of fungal cells to epithelial monolayers; incubation of yeasts with laminin increases the infective capacity of the fungus in hamsters. *P. brasiliensis* activates complement by both, classic and alternate pathways; complement oponises the fungus enhancing macrophage phagocytosis and result in liberation of cytokines chimiotactic for polymorpho and mononuclear cells6).

Furthermore lipid and polysaccharide extracts of Pb inoculated into the peritoneal cavity induced an early aflux of PNMs followed by macrophages; the polysaccharide fraction induced epithelioid granulomas when inoculated subcutaneously10) and bentonite particles coated with Pb antigens injected i.v. leads to typical pulmonary epithelioid granuloma10).

**The host.** Latin American rural workers usually have low socio economic levels and malnutrition, alcoholism and smoking are frequent among them; the role of these factors in the pathogenesis and progress of the disease has been sugested. Alcohol per se, at least experimentally, and in rats does not appear to modify the evolution of peritoneal inoculation of Pb.

The primary host defenses against the inhaled propagules of Pb occur in the pulmonary alveoli and experimental evidences suggest that polymorphonuclear phagocytes (PNMs) are the first cells envolved. Complement apparently does not participate, since PNMs also accumulate in lesions of

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MRT (days)</th>
<th>DL50%*</th>
<th>No. Granulomas*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb 265</td>
<td>102.65</td>
<td>all survived</td>
<td>1</td>
</tr>
<tr>
<td>IVIC Pb 267</td>
<td>48.50</td>
<td>all survived</td>
<td>12</td>
</tr>
<tr>
<td>IVIC Pb 9</td>
<td>35.87</td>
<td>19.87×10⁶</td>
<td>18</td>
</tr>
<tr>
<td>Pb Sn</td>
<td>39.88</td>
<td>9.43×10⁶</td>
<td>34</td>
</tr>
<tr>
<td>Pb 2052</td>
<td>21.27</td>
<td>1.93×10⁶</td>
<td>169</td>
</tr>
<tr>
<td>Pb 18</td>
<td>58.38</td>
<td>0.38×10⁶</td>
<td>132</td>
</tr>
</tbody>
</table>

*DL50% calculated 200 days after infection
*cumulative number of granulomas of 3 groups of mice sacrificed 1, 2 and 3 months after inoculation
*Calich et al.6)
complement depleted animals. Peritoneal cells incubated with the fungus in vitro liberate a 15,000 KDa soluble factor that is capable of attracting PNMs when inoculated intraperitoneally in mice. It is suggested that once in the alveoli Pbs are phagocytized by resident macrophages that will secrete PNMs chemiotactic cytokines (IL8?). Early lesions in resistant mice (A/J) have much larger numbers of PNMs than in susceptible mice (B10A); furthermore phorbol myristate acetate activates better PNMs of resistant mice and Pbs yeast cells, in vitro, activates PNMs. One way speculate that resistant animals in the presence of Pb mount, from the beginning a more efficient PNM reaction when compared with their susceptible counter parts.

PNMs however are incapable of digesting Pb yeasts or conidia; since these cells are capable of digesting Candida albicans this may represent an important escape pathway for Pb. However, PNMs obtained from mice previously sensitized with killed Pbs were capable of killing Pb yeast cells, and killing was greater when the Pb tested was of low virulence. PNMs were incapable of killing conidia in vitro but when conidia were incubated in supernatant of antigen stimulated spleen cell cultures, conidia viability was reduced.

The second line of defense is represented by macrophages. Indeed, experimental evidences show that conidia, considered as the infective form of the fungus, end up phagocytized by alveolar macrophages and in vitro they are capable of killing 23% of them. If however alveolar macrophages are treated with lymphokines the rate of killing reaches 73%. Inside the macrophages, conidia transform into yeast cells and budding in these yeast cells increases when conidia were cultivated in serum containing media. These findings suggest that transformation to yeast and budding are stimulated inside the macrophages. Peritoneal macrophages treated with supernatants from antigen stimulated spleen cells limit intracellular transformation of ingested conidia. Iron transport by transferin receptors of macrophages seems to be essential for intramacrophage proliferation of Pb.

Transformation of mycelia or conidia into yeast is essential in the early phases of infection by Pb and there are in vitro evidences that estrogens (17 beta estradiol) inhibit this transformation. Pbs have specific receptors for estrogens and estrogens affect protein synthesis, blocking the synthesis of a 92KDa polypeptide essencial to mycelia to yeast transformation. These observations may be the explanation to the well known preponderance of chronic Pbmycosis in males; indeed chronic Pb mycosis is 13 to 87 times more common in males.

Murine natural killer cells limit the in vitro growth of Pb and lymphocytes from patients exhibit a decreased capacity to inhibit Pb growth in vitro when compared with lymphocytes of healthy controls.

Hamsters inoculated with a virulent strain of Pb have an initial increase in NK activity followed by a sharp decline after 4 weeks. This decrease in NK activity was associated with depression of cell mediated immunity and progression of the infection, indicating that impairment of NK functions also may play a role in the progression of Pb mycosis.

Clinical studies have suggested that susceptibility to Pbmycosis is dependent on several factors, including genetic background. Japanese living in Brazil have more severe forms of the disease; there are references to families in which father and sons or brothers are affected; patients wives have higher frequency of positive pracooccioidin skin tests than the female control population, but not an increase in disease.

The association of Pbmycosis with genetic markers (HLA antigens) have shown that HLA-A9 and HLA-B13 phenotypes were more frequent in Colombian patients. Controls of the same population had predominance of HLA-A1 and HLA-B40; HLA-A9 was frequent among patients with the progressive form of the disease.

It is difficult to evaluate this type of information, however, there are definite experimental data on the role of genetic background and susceptibility to Pbmycosis. Indeed, Calich and co-workers working with mice have shown that different inbred mice strains vary in susceptibility and resistance to Pb infection. They discribed 4 basic patterns of survival after inoculation: sensitive (B10.D2/oSn, B10.A, B10.D2) intermediate (BALB/c, C57B1/10, CBA, C3H/Fe), resistant (CBH/HeJ) and strongly resistant (A/Sn, AJ, DBA/2)20,21). These differences are not related to major histocompatibility complex, or to C5 since the more resistant mice present an hereditary C5 deficiency. Table 2 shows Calich results. Besides mortality, histological extension of infection, pattern of granulomas and number of fungi (CFU) where also different in susceptible and resistant mice.

Antibody response also differs among strains: susceptible strains produce high levels of IgM in the early stages of the infection and very high levels of IgG with progression of the disease; resistant mice present very low titers of IgG and only a delayed, smaller peak of IgM.

Characterization of antibodies by immunobloting
showed differences: IgM and IgG bands were also different in susceptible and resistant mice. The antibody response elicited in (nu/nu) athymic and euthymic (nu/+ ) BALBc mice showed that Pb also has T independent antigens capable of stimulating B cells. A polyclonal B lymphocyte activation leads to increased number of immune complexes and presence of antibodies to autoantigens; only susceptible mice had an increase in Ig-secreting cells, the highest increase being in IgG1 and IgG2b and IgG2a. Resistant mice showed increase in IgM and IgG3.

Recent studies have found that there are 2 subpopulations of T cells: Th1 related to cell mediated immunity associated with release of gamma interferon and Th2 cells responsible for antibody dominated immunity largely dominated by interleukin 4 (IL4)20).

The result of Calich et al.20) strongly suggest that in Pb mycosis susceptibility and resistance may be regulated by Th1 and Th2 subsets; susceptible mice may respond preferentially with Th2 predominant by IL4-activation with increase of IgG1 and IgG2 secreting cells and consequent high levels of IgG.

There are clinical and experimental evidences of the important role that cell mediated immunity (CMI) plays in paracoccidioidomycosis. Indeed, a direct correlation of the severity of the disease and the extent of CMI impairment has been observed; progressive or disseminated clinical forms have been associated with depressed CMI and high levels of specific antibodies7-9,23-26).

Experimental models confirmed that CMI response are important mechanisms in host defense to P. brasiliensis5,26).

Mota et al.27) studied results of 8 in vivo and in vitro tests for evaluation of CMI response in patients with different forms of Pb mycosis and showed that the more severe forms of the disease had the higher degrees of immunosuppression. Besides, experimental stimulation of CMI with Levamisole28) or dialysable leukocyte extracts delay the progress of immunosuppression, increases survival and decreases the dissemination of lesions29).

We have already discussed the possibility of the modulation of the immune response to Pb mycosis as related to changes in T cell regulation, susceptible mice responding with Th2 predominance and resistant ones with a Th1 predominant response.

Other factors have also been studied as the development in Pb mycosis of plasma factors with inhibitory T cell activity25). Immune complexes also may have a T cell inhibitory role and in hamsters models that permit the removal of excess antigen, by surgical removal of the inoculated tests, there is a decrease in antibody titers, circulating Ab-Ag complexes, and increased survival30). Cases of severe Pb mycosis are reported in AIDS patients31).

Conclusion

Failure of CMI whatever its cause is the main element responsible for progression and mortality in Pb mycosis. This failure has been considered as a consequence of the progressive involvement of lymph nodes by the disease, as due to the presence of plasma factors with inhibitory action on T cells (antibodies, antibody-antigen complexes, products of the fungus), as due to inherent susceptibility, as well as to host undernutrition or alcoholism. With treatment of Pb mycosis CMI depression is reverted and the patients may stay without symptoms for many years, an indication that the fungus or its products have an important role in the modulation of CMI. The role of stimulation of suppressor cells by fungal products has also been studied: treated patients have an increase in monocyte/null cells and low T helper/T supressor ratio. All of these information leads to the concept that the disease is the result of an imbalance of immune modulation in which besides the genetic profile of the host the virulence of the fungus also plays a part.

References


Table 2. Susceptibility of 10 inbred mouse strains to intraperitoneal infection with 5×10⁶ P. brasiliensis yeast cells (Pb18 strain). Comparison of survival time, H2 Haplotype, presence (+) or absence (−) of complement component C5*

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Mean survival Time in days</th>
<th>H2 haplotype</th>
<th>C5</th>
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<tbody>
<tr>
<td>DBA</td>
<td>316</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>A/J</td>
<td>299</td>
<td>a</td>
<td>-</td>
</tr>
<tr>
<td>A/Sn</td>
<td>283</td>
<td>a</td>
<td>-</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>200</td>
<td>k</td>
<td>+</td>
</tr>
<tr>
<td>CBA/J</td>
<td>162</td>
<td>k</td>
<td>+</td>
</tr>
<tr>
<td>C57B1/10</td>
<td>150</td>
<td>b</td>
<td>+</td>
</tr>
<tr>
<td>BALB/c</td>
<td>133</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>B10.A</td>
<td>124</td>
<td>a</td>
<td>+</td>
</tr>
<tr>
<td>B10D2/nSn</td>
<td>100</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>B10B2/nSn</td>
<td>97</td>
<td>d</td>
<td>-</td>
</tr>
</tbody>
</table>

*Calich et al.6)


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