Development of a chronic chromoblastomycosis model in immunocompetent mice

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An experimental model to study chromoblastomycosis caused by Fonsecaea pedrosoi was developed in immunocompetent BALB/c mice. Unlike previous models of chromoblastomycosis, in this model a chronic, progressive infection mimicking the infection in humans was developed. This model may be suitable for use in experimental studies of chromoblastomycosis.

Keywords chromoblastomycosis, murine model

Five different species of dematiaceous fungi cause chromoblastomycosis, a subcutaneous chronic infectious disease common in tropical and subtropical countries. Of these species, Fonsecaea pedrosoi is the most important in Colombia [1]. This disease is acquired by direct cutaneous inoculation of the fungus. Even though this infection is not lethal, it can cause incapacitating and deforming lesions [2]. Unfortunately, chromoblastomycosis responds poorly to available therapies [3].

Several animal models of the infection have been examined. Upon intraperitoneal inoculation of F. pedrosoi in mice, Borelli observed hyphae in some organs (lung, bone, brain, kidney and peritoneum), but never achieved progressive disease with transformation of the hyphae into Medlar bodies or sclerotic cells [4]. Ahrens et al. observed subcutaneous chromoblastomycosis lesions using immunosuppressed mice (BALB/c nu/+, nu/nu, and bg/bg), but reported handling difficulties with these immunosuppressed infected mice [5,6]. Acute but not chronic infection has been reported using subcutaneous and intravenous routes of inoculation [5,7].

We have developed a murine model of chromoblastomycosis using immunocompetent BALB/c mice inoculated intraperitoneally, with a strain of F. pedrosoi (23790 CIB) isolated from the leg of a patient with chromoblastomycosis. The strain was passaged four times through BALB/c mice (i.p. inoculation for each passage) to enhance the virulence of the fungus, then it was kept at 21–23 °C in Sabouraud glucose and Mycosel agar (BBL Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). The mycelium was scrapped from the surface in saline and vortexed to detach the conidia. Conidia and hyphal fragments were counted in a haemocytometer using trypan blue 0·3% and the suspension was adjusted to 10^6 or 10^8 colony forming units (cfu) ml^-1. These counts were confirmed by plating dilutions of the inoculum.

Two experiments were carried out to develop this animal model. In the first experiment, 68 BALB/c male mice between 4 and 8 weeks of age were used to evaluate the optimum route and size of the inoculum. The mice were inoculated by subcutaneous (s.c.) or intraperitoneal (i.p.) routes using an inoculum volume of 1 ml containing either 10^6 or 10^7 cfu. Twenty mice were infected s.c., 10 with 10^6 cfu and 11 mice with 10^7 cfu at various inoculation sites: base of the tail, pinna of the ear, loin, thigh, and footpads. Forty-seven mice were inoculated i.p. (27 with 10^6 cfu and 20 with 10^7 cfu) at various inoculation sites: base of the tail, pinna of the ear, loin, thigh, and footpads. Necropsy was performed in 17 mice (10 inoculated s.c. and seven inoculated i.p.) at 24 days; the remaining 51 mice were sacrificed at the end of the observation period (4 months). Histopathology specimens were taken from the inoculation site and from any organ and tissue with nodules or dark pigment; these specimens were stained with haematoxylin–eosin and silver–methenamine. In addition, tissue cultures were performed at each site, according to standard techniques. In brief, each tissue sample was
While the cutaneous lesions healed in an average of 24 days, tissue cultures and histopathological studies of deep tissues and organs from mice inoculated i.p. showed chromoblastomycotic lesions. Of the mice inoculated i.p., 11% of those who received 10^6 cfu and 34% of those given 10^8 cfu, had disease which was documented either by histopathology and/or by a positive tissue culture. In the histological cuts, granulomas, polymorphonuclear infiltrate, sclerotic bodies, muriform cells, hyphae and necrosis foci were observed (Fig. 1). Tissue cultures performed from necropsies at 24 days and 4 months after inoculation with 10^6 cfu i.p. showed 10^3–4 cfu g⁻¹ and the tissue cultures of mice inoculated with 10^8 cfu i.p., showed 10^5–7 cfu g⁻¹.

Based on these results, we undertook a second experiment. In this study, 122 male mice weighing 23 ± 2 g were inoculated with 1 ml of an inoculum of 10^6 cfu ml⁻¹, 0.2 ml in each of 5 abdominal sites by the i.p. route. Table 1 shows the percentage of positive tissue cultures and histopathology in this group of mice after 4 months of observation. As in experiment # 1, the tissue pathology demonstrated the classic features of chromoblastomycosis with granulomas, necrotic foci, sclerotic bodies, polymorphonuclear infiltrate, muriform cells and hyphae.

As noted by others [4,5,8] mice inoculated s.c. were resistant to infection. While others authors have examined i.p. inoculation [4,9], we have shown that a model of chronic chromoblastomycosis can be developed in immunocompetent mice. Our report is the first in which progressive and chronic disease with transformation of the hyphae into Medlar bodies and sclerotic cells in immunocompetent mice was shown (Table 2).

In this study we did not evaluate immune mechanisms of resistance to the infection. Other authors attributed this resistance to cell mediated immunity (CMI) [5–8]. In our experiments we found a clear difference in successful clearance of the fungus from the skin versus from the peritoneum. The inability of the mice to clear fungi inoculated i.p., may be due to the known inability of

Table 1 Results of tissue cultures and histopathology of mice inoculated with 10^6 cfu i.p.

<table>
<thead>
<tr>
<th>Test</th>
<th>cfu g⁻¹ ± SD (range)</th>
<th>No. positive/no. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>10^4 ± 10^4 (10^3–10^5)</td>
<td>13/122 (11)</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>10^5 ± 10^2 (10^3–10^5)</td>
<td>53/122 (43)</td>
</tr>
<tr>
<td>Other*</td>
<td>10^3 ± 10^2 (10^1–10^4)</td>
<td>18/122 (15)</td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>29/122 (24)</td>
<td></td>
</tr>
<tr>
<td>Peritoneum</td>
<td>57/122 (47)</td>
<td></td>
</tr>
<tr>
<td>Other*</td>
<td>10/122 (8)</td>
<td></td>
</tr>
<tr>
<td>Total positive by culture or pathology</td>
<td>101/122 (83)</td>
<td></td>
</tr>
</tbody>
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* Lymph nodes, liver, kidney, adrenal glands.
peritoneal macrophages, even activated macrophages, to kill *F. pedrosoi* [10]. Adherence of the fungi to the surface of activated macrophages triggers the respiratory burst as revealed by reduction of nitroblue tetrazolium. Activated macrophages failed to kill ingested *F. pedrosoi*, but they showed a fungistatic activity delaying hyphae formation [10].

This model can now be used for studies of other aspects of the infection, such as humoral and cellular immunological mechanisms during the evolution of the disease, host resistance, and antifungal agent efficiency.

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**References**