MYCOLOGICAL STUDIES ON THE STRAINS ISOLATED FROM A CASE OF CHROMOBLASTOMYCOSIS WITH A METASTASIS IN CENTRAL NERVOUS SYSTEM

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Chromoblastomycosis has been known as one of the superficial mycoses caused by Hormodendrum pedrosoi, Hormodendrum compactum, Phialophora verrucosa and other pigmented fungi, but the authors recently experienced one case in which this disease extended to a deep-seated infection. They identified the strains isolated from the original skin lesions and the metastasized brain lesions of a patient with chromoblastomycosis, as Hormodendrum pedrosoi.

The case was of a girl who died at the age of nine years and one month. In January, 1954, tinea capitis appeared on the head, extending to the face and arms. One year later, there appeared a small, brownish black pustule on the side of the nose and on the cheek of the right side, slowly growing larger. She was diagnosed as having chromoblastomycosis at the Department of Dermatology, Medical School Hospital, University of Tokyo, as a result of clinical and mycological tests. She received an antifungal agent, "Novex" (2,2'-thiobis-4-chlorophenol), daily, for about one month, but without any improvement. During this period, she was losing weight gradually. In the middle of June, nausea and vomiting began, lasting for one week, and then occurring intermittently. In the middle of July, her general condition improved, but she noted hemiplegia of the left side. In January, 1956, following its disappearance, hemiplegia on the right side and dysphasia appeared simultaneously. In February, she was admitted to the Department of Pediatrics of the same hospital and about a month later she died. At autopsy, at the Department of Pathology, School of Medicine, University of Tokyo, the lesions were found in the cerebra, cerebella and medulla oblongata, as well as in the skin. The existence of the etiologic agent was confirmed histopathologically in these lesions.

On the request of the Department of Pediatrics that the strains isolated from the lesions in the skin and brain be identified, the authors studied the morphological properties and pathogenicity of these strains in comparison with a stock culture of Hormodendrum pedrosoi, which was given by Dr. N. F. Conant of Duke University School of Medicine and designated as the "Duke" strain. The strains isolated from the lesions in the skin and brain were called the "skin" and "brain" strains, respectively and they were also generically called the "isolated" strains.
EXPERIMENTS AND RESULTS

**Morphological properties**

The results of the investigation on the morphological properties of the "isolated" strains and the "Duke" strain used as a control, are shown in table 1.

The giant colonies were grown on Sabouraud's glucose agar plate. The investigations on the appearance of the spores and hyphae and on the spore formation were carried out using Sabouraud's glucose agar slant and slide cell culture. All experiments were done at room temperature for two weeks.

As seen in table 1, the "skin" and "brain" strains were quite similar to the "Duke" strain. All the strains grew rather slowly, the diameter of the colonies reaching about 30 mm at the end of two weeks of cultivation. A dark brown or deep black pigment was produced into the surrounding medium as well as in the colony itself.

Concerning the growth on Littman oxgall agar, which was omitted from the table, the shapes of both strains were somewhat different; the "isolated" strains showed, after two weeks, a round colony with an irregular edge, covered with a heavily and coarsely furred surface with radial folds. The center of the surface was somewhat heaped. The "Duke" strain had a round colony with an irregular edge, covered with a relatively smooth and glistening surface without folds, which after two weeks was half globular and then, after three to four weeks, was heavily and closely furred. Both strains were also slow in growth, the colonies reaching about 25 mm in diameter after two weeks and pro-

<table>
<thead>
<tr>
<th>Organism</th>
<th>Spore formation</th>
<th>Spore size</th>
<th>Spore shape</th>
<th>Spore color</th>
<th>Hypha shape</th>
<th>Hypha width</th>
<th>Hypha color</th>
</tr>
</thead>
<tbody>
<tr>
<td>The &quot;skin&quot; strain</td>
<td>few</td>
<td>2-4 μm</td>
<td>oval</td>
<td>dark brown</td>
<td>branched,</td>
<td>1.5-3 μm</td>
<td>dark brown</td>
</tr>
<tr>
<td>The &quot;brain&quot; strain</td>
<td>none</td>
<td>same as the above</td>
<td>same as the above</td>
<td>same as the above</td>
<td>branched, septate</td>
<td>2.5-4 μm</td>
<td>brown</td>
</tr>
<tr>
<td>The &quot;Duke&quot; strain</td>
<td>none</td>
<td>large, round, flat, regular, flat, from the center, irregular, to the edge</td>
<td>oval</td>
<td>dark brown</td>
<td>branched, septate</td>
<td>1.5-4 μm</td>
<td>brown</td>
</tr>
</tbody>
</table>

* H, A, and P represent Hordomadenum, Arthrospora, and Phialophora types of sporulation, respectively.

Table 1. The Morphological Properties of the "isolated" Strains Compared with the "Duke" Strain
duced a brownish black soluble pigment, the base being black with a surrounding grey furry portion.

Fig. 1. Giant colonies of the "isolated" strains: Left. The "skin strain". Right. The "brain strain". Two-week growth on Sabouraud's glucose agar at room temperature.

Fig. 2. Hormodendrum type of conidiophore of the "brain" strain: Slide cell culture on Sabouraud's glucose agar at room temperature. Cotton blue preparation.

Fig. 3. Acrotheca type of conidiophore of the "brain" strain: Slide cell culture on Sabouraud's glucose agar at room temperature. Cotton blue preparation.
In both culture media described above, penicillin G potassium salt and dihydrostreptomycin sulfate in concentrations of 100 units per cc and 30 μg per cc, respectively, were added for the purpose of preventing bacterial contamination.

As to sporulation, the typical Hormodendrum type was observed in large numbers in the "isolated" strains as well as in the "Duke" strain (cf. Figure 2). The Acrotheca type of sporulation was relatively scarce and was observed more frequently in the "isolated" strains than in the "Duke" strain (cf. Figure 3). There were extremely few of the Phialophora type in either of them.

The surface of the conidia was, in general, smooth, and they were connected to one another with thick disjunctors in both strains. The chlamydospores were observed in both strains, too, but the primordia of the perithecia were recognized only in the "isolated" strains (cf. Figure 4). The cleistothecia-like bodies were not found in either strains.

The hyphae were septate and branched and the conidiophores varied in length, bearing the conidia terminally in branching formation.

Pathogenicity in experimental animals

The tests for the pathogenicity of these strains in experimental animals were carried out as follows: To the Sabouraud's glucose agar slant in a medium-sized test tube, on which the test organism was cultivated at room temperature for three weeks, was added 1 cc of a sterilized physiological saline solution containing a 0.1% solution of carboxy methyl-cellulose, the contents being streaked lightly with a sterilized wire loop. The culture suspension obtained was shaken well to make it homogeneous and 0.2 cc and 0.5 cc were injected intraperitoneally into a mouse weighing about 15 g and a guinea pig weighing about 200 g, respectively.

The mice and guinea pigs challenged like this had a fatal systemic infection within three to four weeks. The "isolated" strains were much more lethal and caused much severer lesions than the "Duke" strain. The difference between the "skin" and "brain" strains could not be recognized. The mice were more susceptible to both the "isolated" and "Duke" strains than the guinea pigs. The lesions were abscesses, both pigmented and non-pigmented, large and small. They were formed chiefly in the liver, spleen, mesentery and other internal organs, the kidney, however, being not so much affected.

When 0.1 cc of the above mentioned suspension was injected subcutaneously into the back of the mice, the abscesses were formed at the site of injection.
after about two weeks and gradually extended into the deeper tissue, even penetrating the thorax into the lung.

In both cases, the remarkable development of the mycelia and the spherical or oval pigmented spores was observed within these lesions.

DISCUSSION

It is obvious, as a result of the above experiments on the morphological properties and pathogenicity, that the "skin" and "brain" strains are both Hormodendrum pedrosoi and they are identical with one another.

According to Okudaira(1) the thick walled septate bodies were found inside the giant cells in the lesions of the skin and brain. This fact strengthens the evidence for a true infection with Hormodendrum pedrosoi.

The authors regret that they had no opportunity to collect immunological data on this case.

Thus, it seems that the infection in the brain was produced with the same strain of Hormodendrum pedrosoi as that found in the primary skin lesions, and this also correlates with the clinical picture of the course of the disease in the patient. That she had been ill of the chronic dermatomycosis, may be considered to be, more or less, closely associated with the induction or expansion of the secondary infection with Hormodendrum pedrosoi. The details of the clinical data were reported by Fukushiro(2), and the pathological data by Okudaida(1).

In Japan, only a few cases of chromoblastomycosis have been reported, all describing the superficial infections.(3, 4, 5, 6)

Chapman and his colleagues(7) described a case of the brain lesion with a pigmented strain, Cladosporium trichoides similar to Hormodendrum pedrosoi in many respects, but different in some.

Several cases of chromoblastomycosis showing metastases in other parts of the body have been observed, all of which were also with superficial infections.(8, 9, 10)

deAlmeida(11) reported a case of chromoblastomycosis involving the central nervous system, but the causative organism could not be isolated.

The authors have so far never seen any reference definitely describing a deep-seated type of this infectious disease. Thus, such a case reported here seems to be one of an exceptionally rare deep-seated infection with Hormodendrum pedrosoi, and is, therefore, of significance.

SUMMARY

The two strains isolated from the skin and brain lesions of a nine year old girl who died of chromoblastomycosis were both identified as Hormodendrum pedrosoi, and were identical with each other in their morphological properties and pathogenicity in experimental animals. Thus, it was confirmed that this was a rare case of the deep-seated type of chromoblastomycosis caused by Hormodendrum pedrosoi.

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