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In vitro Cultivation of Cells from Ovotestis Tissue of Pigmented Biomphalaria glabrata

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ABSTRACT—Cells derived from ovotestis tissue of pigmented Biomphalaria glabrata, Puerto Rican strain were cultured in double diluted GIT medium supplemented with modification of amino acids components of pigmented B. glabrata, ovotestis and mid-gut region and 3% inactivated fetal calf serum. As a result, two types of cells, epithelial and fibroblastic like cells increased in number during the cultivation. It seem that the medium used in this study is a suitable medium for cultivation of cells from ovotestis of pigmented B. glabrata. These two types of cells have been maintained by successive transplantation for over 3 passages.

Key words: Schistosoma mansoni, Biomphalaria glabrata, ovotestis, cell culture, GIT medium

INTRODUCTION

Cell and organ culture of invertebrates have been used to study the physiology proper of those species. Although primary tissue and cell cultures of mollusks have been frequently reported (Perkings and Manzel, 1963; Burch and Cuadros, 1965; Manaka, et al., 1980; Iwanaga et al., 1985), these reports have not maintained cells and tissues over 2 months, except reports for cell lines designated HA (Vago and Chastang, 1958) and Bge (Hansen, 1974).

It is well known that different geographic strains of Schistosoma mansoni have different rates of infection within different subspecies of Biomphalaria snails (Files and Cram, 1949; Paraense and Correa, 1963; Kagan and Geiger,1965; Iwanaga et al., 1992). This suggests that physiological and biochemical differences exist between Biomphalaria snails and that these differences affect the growth of Schistosoma mansoni larvae. In the course of development of S. mansoni larvae in the snail, sporocysts almost grow in near the reproductive organs especially, digestive gland and ovotestis region of the snail, and there start mature cercarial production (Iwanaga et al., 2000). Establishment of cell cultures from Biomphalaria snails will facilitate study of the interaction between the parasite and the intermediate hosts and permit characterization of the metabolic pathways of these cells. Cell cultures from embryo of B. glabrata have been reported to had been established successfully (Hansen, 1974; Hansen, 1976 a,b; Yoshino et al., 1999). However, that from the ovotestis have not yet to be grown in vitro.

In the present study, we have established long-term cell cultures from the ovotestis of pigmented B. glabrata, Puerto Rican strain.

MATERIALS AND METHODS

Juvenile snails (5–7mm in shell diameter) of pigmented Biomphalaria glabrata used in the present study originated from the Puerto Rican strain. The snail has been maintained in our laboratory for many generations.

A basal culture medium was based on GIT medium (Nihon Parmaceutical Co., Ltd.). GIT is a cell culture medium the development of which was enabled by the successful separation of the cell growth factor GFS from animal sera, and can be used in any mode of adherent or non-adherent cell cultivation. And so, it has been demonstrated that GIT is useful for culturing a wide variety of cells (Murakami et al., 1982; Sasai et al., 1985). In this study, 100ml of GIT was diluted 1 : 1 with double distilled water and supplemented with 10ml of amino acids components on empirical modification, as shown in Table 1, to 200ml of diluted GIT medium (medium A), and final concentration of amino acids in medium A was adjusted to the concentration of four times amount (400mg) of amino acids components (percentage by weight) of pigmented B. glabrata, ovotestis and mid-gut region as shown in Table 2. Two different media were prepared for this study; 1. Basal culture medium (medium A). 2. Basal culture medium supplemented with 3% fetal calf serum inactivated at 56°C for 30 minutes (medium B). These two media were prepared with penicillin G, 100 IU and streptomycine sulfate, 100 µg.
Components of basal culture medium (Medium A)
1. Diluted (x2) GIT medium
2. Amino acids solution

Table 1. Basal culture medium for cultivation of tissues and cells from pigmented *Biomphalaria glabrata*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine-HCl</td>
<td>4.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>20.3</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>53.1</td>
</tr>
<tr>
<td>Glycine</td>
<td>32.5</td>
</tr>
<tr>
<td>Cystine-HCl</td>
<td>2.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>4.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>25.7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 2. Contents of amino acids of pigmented *Biomphalaria glabrata*, ovotestis and mid-gut region

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine-HCl</td>
<td>4.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>567.0 (15.3)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>533.0 (14.4)</td>
</tr>
<tr>
<td>Alanine</td>
<td>287.0 (7.8)</td>
</tr>
<tr>
<td>Methionine</td>
<td>57.6 (1.6)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>91.2 (2.5)</td>
</tr>
<tr>
<td>Histidine</td>
<td>67.5 (1.8)</td>
</tr>
<tr>
<td>Threonine</td>
<td>238.0 (6.4)</td>
</tr>
<tr>
<td>Proline</td>
<td>227.0 (6.1)</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Amebocytic cells migrated out from explanted tissue 20 to 24 hr after the culture was started, and they formed a colony of cells near the tissue after 5 to 7 days (Fig. 1). The cells were variable in shape and size. Peak cell movement from the explants was observed at the end of the first two weeks, but the cells continued to migrate from the explants moved slowly during the end of culture period. The stage of proliferation of cells could not observed. At 10 to 14 days after initiation, the tissue culture yielded outgrowths of epithelial and/or fibroblastic like cells reported by Kitamura (1970) and Hansen (1976a). The growth rates of epithelial and fibroblastic like cells were faster in number than that of amebocytic cells until 7 weeks after culture (Fig. 2), but the number of epithelial like, fibroblastic like and amebocytic cells declined slowly and some cells failed to continue to develop and died and disappeared during the succeeding week, and so, it was exactly difficult to count the lived cells during cultivation.

The suspension of two types of cells, migrated out from explanted epithelial and fibroblastic like cells was placed in a T-flask containing 5 ml of medium B. In preparations of cells stained with Giemsa, one type, i.e., epithelial like cells were characterized by polygonal cell with a central, granular nucleus and appeared patch-like in the monolayer. The fibroblastic like cells exhibited different sizes, and contained large nucleus (Fig. 3). The growth rate of cells showed in Fig. 4. The cells increased in number to 3 to 4 fold at 30 days after initiation. It seemed that fibroblastic like cells relatively increased in numbers than that of the epithelial like cells.
Cultivation of *B. glabrata*
cells, but they then slowly decreased in number and became granular at 50 days after inoculation (data not shown).

Hansen (1976a) reported that primary cultures of cells from embryos of *B. glabrata* started with about $1 \times 10^4$ cells, and cells usually were well grown by 10 days, but they then slowly decreased in numbers and died, so the cells rapidly needed to transfer to new flak. In present study, inoculation started with $2 \times 10^2$ cells, and they activity lived by next inoculation. It seem that inoculation of cells number in primary culture is a debatable point.

In cultivation of embryonic cells from *B. glabrata* reported by Basch and Diconza (1973) and Hansen (1976a), they observed at least three types cells, that is, amebocytic, epithelial like and fibroblastic like cells, and a cell line designated Bge (*Biomphalaria* embryo) that has been maintained through many subcultures of colonies of epithelial and/or fibroblastic like cells. In our observations of the tissues culture in the present study, three types of cells, i.e., amebocytic, epithelial like and fibroblastic like cells were observed near margins of explanted tissue. In previous experiments, subculture of these three types of cells was made repeatedly at about 2 weeks intervals with medium A for 2 months, but the cells were not increased noticeably, especially amebocytic cells were not growth (not published).

Serial cultures were made using the same medium that was used initially for cell culture. There was at least 3-fold increase in number over the primary culture in the third passages of culture. Cells suspension obtained from every serial culture always contained two types of cells, and no morphological change were observed. Mitotic figures were usually seen, but chromosome number and karyotype of cells could not count. For long-lived culture of cell and/or tissues of invertebrate, Grace (1966) established cell line from the *Aedes* mosquitos using 900mg amino acid components. and also Hansen (1976a) maintained the cells form embryos of *B. glabrata* by Schneiders Drosophila medium diluted to 22% (included about 240mg amino acids). It seem that amount of amino acids components in medium is the important factor for long-term culture. Basch and Diconza (1973) maintained embryonic cells from *B. glabrata* with medium 199 and components from analyses of hemolymph from adult *B. glabrata*, but the cell could not maintained long-term. It was considerable that amount of total amino acid components of the hemolymph in medium was not enough during the culture period. This problem is need to reexamine. In the present study, we have been able to culture cells derived from ovotestis of *B. glabrata* by using GIT medium supplemented with 400mg of amino acids components of the snail and 3% inactivated fetal calf serum. These cells showed active growth, and the cells have been subculturing over 3
passages at the present culture, it seem to be possible to establish the cell line from ovotestis of pigmented *B. glabrata*. Although the medium used in the present paper was a suitable medium for cultivation of ovotestis, the significance nutrients of the medium for culture of ovotestis were not evaluated. Two types of cells have been subculturing over 3 passages at the present study using medium B (Fig. 5).

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