Culture and Phagocytic Characteristics of Schwann Cells In Vitro. A Possible Model Substrate for Cultivation of M. leprae

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Tissue culture offers a unique opportunity for obtaining insight into living processes of cells under controlled conditions. A tumor arising from Schwann cells, such as the acoustic schwannoma, provides ideal material to confirm and study the phagocytic activity and relationship between mycobacteria and Schwann cells in vitro. In these past two decades, both light and electron microscopy have clearly shown the predominant bacilIation of Schwann cells in leprous neuritis of any type (4,5,8,10,11,17,20,26,32). It also became obvious that the Schwann cell could serve as a reservoir for possible later dissemination of M. leprae (20). At the same time, acoustic schwannomas constitute a fairly common type of intracranial tumor, comprising 7.8% of intracranial space-occupying lesions verified histologically at our Unit (2). Cultivation and origin of Schwann cells from embryonal ganglionic nerve tissue has been known to tissue culturists and its behavior well documented, mainly by Lumsden and his colleagues (22,23,30). This source of Schwann cell has always been of limited experimental use. Acoustic schwannomas, on the other hand, are easy to grow and provide adequate cell culture for the study of morphology, behavior and experimental infection with M. leprae (26,27). The present paper reports the culture characteristics and phagocytic activity of Schwann cells in vitro. A somewhat fuller account has been given in a thesis by one of us (19).

MATERIALS AND METHODS

During neurosurgery on five patients with acoustic schwannomas and four others with spinal schwannoma, biopsies were collected aseptically in tissue culture medium with added antibiotics. The cultures were set up within four hours in plasma clots and on glass-slips, and transferred to flat Leighton tubes (20). The cultures were fed 1.5 ml growth medium consisting of Eagle's Minimum Essential Medium (MEM) plus 20% human serum, and penicillin (100 IU) and streptomycin (100 UG) per ml. The cultures were examined daily. When desired they were fixed in acetic alcohol (1:3) or 10% formal saline, and stained with hematoxylin and eosin.

When the Schwann cell cultures showed good spreading, generally between the fourth and ninth days, they were inoculated with acid-fast bacilli. The organisms constantly used were ICRC bacilli which were originally derived (at the Indian Cancer Research Centre) from lepromatous nodules (1,28). The ICRC bacilli served as a model for M. leprae. In two instances some of the culture preparations were inoculated with M. leprae obtained from fresh nodules of untreated lepromatosus patients. The inoculated cultures were incubated at 37° and fixed at 30 minutes, 1 hour, ½ hours and 2 hours in formal saline. These cultures were stained by the Ziehl-Neelsen method for demonstration of acid-fast bacilli (AFB). The cells containing bacilli were counted under oil immersion, and the phagocytic index of Schwann cells in vitro was calculated at ½, 1, 1½ and 2 hours. Gomori's method for acid-phosphatase reaction in cells (22) was carried out in three instances on Schwann cell cultures which were not inoculated with the bacilli.

In addition to the schwannoma cell cultures, the tissues from two spinal neurofibromas from patients with Von Recklinghausen's disease were cultured to serve as
<table>
<thead>
<tr>
<th>Serial No.</th>
<th>TC No.</th>
<th>NP No.</th>
<th>Histologic Picture (paraffin sections, H&amp;E stained)</th>
<th>Migration of Cells (living phase or fixed, H&amp;E stained)</th>
<th>Morphology of Cells in vitro</th>
<th>Period of maintenance (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>HBT - 4</td>
<td>G-714</td>
<td>Elongated Antoni type A cells forming bundles, and vacuolated Antoni type B cells forming sheets.</td>
<td>4th day</td>
<td>Spindle-shaped cells with nuclei bearing one or two nucleoli; round cells resembling microglia.</td>
<td>10</td>
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<tr>
<td>2.</td>
<td>HBT - 6</td>
<td>G-830</td>
<td>Both type A and type B cells. type A with large ovoid nuclei.</td>
<td>48 hours</td>
<td>Both spindle-shaped and round cells.</td>
<td>11</td>
</tr>
<tr>
<td>3.</td>
<td>HBT - 8</td>
<td>G-846</td>
<td>Predominantly type A cells in sheets, very vascular.</td>
<td>5th day</td>
<td>More of spindle-shaped cells.</td>
<td>12</td>
</tr>
<tr>
<td>4.</td>
<td>HBT - 15</td>
<td>G-937</td>
<td>Both type A and type B cells, latter vacuolated and in discrete groups.</td>
<td>48 hours</td>
<td>Both types, but more of spindle-shaped cells. Multinucleate cells by the 8th day.</td>
<td>16</td>
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<td>5.</td>
<td>HBT - 16</td>
<td>G-978</td>
<td>Both types of cells but predominantly type A, forming typical interweaving bundles.</td>
<td>48 hours</td>
<td>Both spindle-shaped and round cells. Multinucleate cells by the 8th day.</td>
<td>15</td>
</tr>
<tr>
<td>6.</td>
<td>HBT - 18</td>
<td>H-53</td>
<td>Predominantly type A cells, forming typical interweaving bundles.</td>
<td>3rd day</td>
<td>More of spindle-shaped cells.</td>
<td>12</td>
</tr>
<tr>
<td>7.</td>
<td>HBT - 19</td>
<td>H-72</td>
<td>Both type A and type B cells intimately mixed and bearing round nuclei.</td>
<td>48 hours</td>
<td>More of round cells.</td>
<td>22</td>
</tr>
<tr>
<td>8.</td>
<td>HBT - 21</td>
<td>H-146</td>
<td>Both types of cells, but more of type B in sheets.</td>
<td>48 hours</td>
<td>More of round cells.</td>
<td>11</td>
</tr>
<tr>
<td>9.</td>
<td>HBT - 23</td>
<td>H-525</td>
<td>Predominantly type A cells with typical interweaving bundles, hyalinized vessels.</td>
<td>3rd day</td>
<td>More of spindle-shaped cells.</td>
<td>15</td>
</tr>
</tbody>
</table>

TC No. = serial number of specimens grown in tissue culture.
NP No. = serial number of specimens received at the neuropathology unit.
H & E = hematoxylin and eosin.
controls. Similarly, human fetal muscle fibroblasts were cultured by the identical method also as controls. This too was infected with ICRC bacilli and the glass-slip preparations stained for AFB or for acid phosphatase activity.

RESULTS

As seen in Table 1, there was consistently good migration of cells from the explants of both acoustic and spinal schwannomas. The tumor cells began migrating within 48 hours. Two distinct types of cells were seen in all cases when examined in the unstained living phase (Fig. 1a). One type was an elongated bipolar cell (Fig. 1b), often with the nucleus bulging to one side (Fig. 2b) corresponding to Antoni type A cell in conventional histologic sections. The other type was a round cell (Fig. 1a), corresponding to Antoni type B cell. While the growth of Schwann cells from explants of spinal schwannomas was slightly slower than that of cells from acoustic schwannomas (Table 1), they too showed good migration by the third to fifth days, and with cell characteristics identical to those of the latter (Figs. 2a-b). By the end of eight days all the Schwann cell cultures showed large multinucleate cellular aggregates (Fig. 3).

Fig. 1. (HBT-6): a) Culture of acoustic schwannoma cells, showing two clearly distinguishable cell types: Antoni type A—spindle-shaped or elongated with a bulging central nucleus, and Antoni type B—circular or globoid with a central nucleus. (Unstained living phase, photographed while in Leighton tube, X 250); b) Closer view showing narrow elongated cells, each with a plump prominent nucleus. Fixed culture, hematoxylin and eosin (H & E), X 625.

Fig. 2. (HBT-8): a) Eight day old: Spinal schwannoma cell culture showing good growth and migration of fusiform cells; b) Another part of the same culture with more elongated and discrete cells with a central bulging nucleus, typical of Schwann cells. Both H & E: a) X 100; b) X 250.

Fig. 3. (HBT-16): Four day old culture of acoustic schwannoma; note A and B types of Schwann cells, the former predominating, and the multinucleate giant cells or syncytia (arrows). As in Figure 1a, X 100.
When the Schwann cell cultures were inoculated with mycobacteria (ICRC bacilli or separate isolates of M. leprae) the neoplastic Schwann cells exhibited avid phagocytosis (Figs. 4a-b, 5), at times with formation of globi. The control cultures of human fetal fibroblasts failed to show any phagocytic activity at the end of two hours. The phagocytic index at the end of two hours was seen to be over 90% (Fig. 7). The difference between the phagocytic index of
type A cells and that of type B cells was found to be negligible.

Acid phosphatase stain revealed intense granular activity in the cytoplasm of the neo-
plastic Schwann cells (Fig. 6), while the control cultures of human embryonic fibroblasts failed to show either acid phosphatase activity or any phagocytic capacity.

In contrast, in one of the two neurofibromas which were put up for culture, there was only scanty migration of cells after eight days. Most of the cells had morphologic characters of fibroblasts, and very few were slender and spindle-shaped like Schwann cells.

DISCUSSION

Sharenberg and Liss (9) described three types of cells in acoustic schwannomas, the first cell type being slender and spindle-shaped, the second arrow-headed and the third resembling fibroblasts. Cravioto and Lockwood (1) recorded the following four types of cells: 1) ameboid microglia-like cells, 2) slender, spindle-shaped cells, 3) racket-shaped cells, and 4) large kite-shaped cells. The first two types were Schwann cells and the last type were thought to be fibroblasts. They did not observe any transformation of one cell type to another. Lumsden (20-21) gives a detailed account of Antoni type B cells which are ameboid and a transitional type. In the present study two distinct types of cells were seen constantly, one being spindle-shaped and the other being round and resembling microglia, along with a few fibroblasts.

The vigorous phagocytic activity of schwannoma cells in vitro has been demonstrated by Lumsden (20-21) in his experiments on M. leprae and Schwann cells. He found that the uptake of bacilli by schwannoma cells was far greater than that by HeLa cells and human amnion cells. His observations did not indicate any special affinity of these cells for lepra bacilli and there was no evidence of intracellular bacillary multiplication. It is well known that Schwann cells are capable of engulfing any particulate matter such as myelin debris, hemosiderin, India ink particles injected subperineurally (22), and even melanin (23). Sharenberg and Liss (9) recorded a larger number of fibroblasts than Schwann cells in the cultures of neurofibromas of Von Recklinghausen’s disease, as seen also in two instances in the present study.

In the present study, ICRC bacilli were used because they serve as acid-fast bacilli and were easily available in the same laboratory at the Cancer Research Institute where the current tissue culture procedures were carried out. The schwannoma cells with a phagocytic index of 95% at the end of two hours were noticed to behave like macrophages. Deville (11) observed 80% of macrophages to contain bacilli within 24 hours of inoculation. Our control cultures of fetal fibroblasts did not show any phagocytic activity. This finding is at variance with that of Fildes (10), who reported the presence of M. leprae, predominantly in the cytoplasm of macrophages and fibroblasts in the infect-
ed organotypic cultures of dorsal root ganglia of rat and mouse, studied under the electron microscope. In our investigation, the period of maintenance of the cultures, up to 22 days, was too short for a consideration of multiplication of M. leprae in vitro. However, the avid phagocytosis and clarity for microscopic observation strongly suggests that schwannoma cells provide a useful substrate for cultivation of M. leprae in vitro, and for a sequential study if they can be maintained for a few months. Such cultures may also be useful in monitoring the effect of chemotherapeutic agents on M. leprae. It is relevant to recall here the in vitro cultivation of leprosy bacilli on a hyalu-
ronic acid-based medium (24), the rich content of β-glucuronidase (another lysosomal enzyme) in or around these bacilli and the control of infection by the administration of vitamin C which inhibits this enzyme (25).

It is difficult to comment on the lysosomal activity evidenced by the strong acid phos-
phatase reaction (12) invariably observed in the Schwann cells grown in vitro. The granu-
lar appearance and intracytoplasmic location of the reaction product was identical to that seen in Schwann cells and macrophages, in nerves and skin lesions of untreated lepro-
matous and tuberculoid patients (15). The two negative features that have now clearly emerged are: a) the acid phosphatase reaction was not related to the presence of bacilli since glass slips unexposed to mycobacteria were stained for this enzyme, which b) was also not detected even in rapidly multiplying
fetal fibroblasts in similar cultures. Similarly in human leprous tissue, fibroblasts at sites of active collagenosis fail to show this enzyme activity. One is led to postulate that cells with a phagocytic potentiality are endowed with lysosomal enzymes, which the marker reaction (acid phosphatase) detects when these cells are metabolically active and even when they have nothing in their milieu requiring engulfment or digestion (3).

**SUMMARY**

Tissue cultures of five acoustic and four spinal schwannomas demonstrated good growth and migration of Schwann cells within two to four days. Two types of cells corresponding to Antoni type A tissue and Antoni type B tissue were clearly recognized. Both these cell types showed avid phagocytosis when the cultures were inoculated with mycobacteria, either ICRC bacilli or M. leprae. The phagocytic index was 95% at the end of two hours.

The Schwann cells grown in vitro also showed intense acid phosphatase reaction with Gomori's stain, suggesting lysosomal activity. Neither this nor any phagocytosis was evidenced by fetal fibroblasts cultured similarly. Cells from two spinal neurofibromas grew and migrated slowly in vitro and were mainly fibroblasts.

**REFERENCES**


