Note

High IgM Production by Human-human Hybridoma HB4C5 Cells Cultured in Microtubes

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HB4C5 cells, a human-human hybridoma, were cultured in microtubes. After 24 h of cultivation, growth of the cells cultured in microtubes was 1.2- to 1.5-fold that in culture dishes or 96-well culture plates. The production of IgM was 2.6- to 3.3-fold that in the 96-well culture plates.

Key words: cell culture vessel; human-human hybridoma; immunoglobulin production; physical stimulation

The productivity of cultured animal cells used in the preparation of bioactive substances is important. There are three basic methods for the production of bioactive proteins in large amounts: (1) large-scale culture, (2) high-density culture, and (3) enhancement of cellular productivity. In our study of improvement of immunoglobulin production in serum-free media, we screened for immunoglobulin production stimulating factors (IPSFs). Lysozyme and glyceraldehyde-3-phosphate dehydrogenase (EC1.2.1.12) were identified as the IPSF. Another way to improve productivity is to try culture vessels of various shapes. Here, monoclonal human IgM production of human-human hybridoma HB4C5 cells cultured in various culture vessels was investigated. ERDF medium supplemented with 10 μg/ml insulin, 20 μg/ml transferrin, 20 μM ethanolamine, and 25 nM sodium selenite was used for the assay. Vessels were inoculated with 7.5 × 10⁴ cells/ml and incubated for 24 h at 37°C. The vessels used were 96-well flat-bottomed culture plates (Corning, NY, USA), 35-mm tissue culture dishes (Becton-Dickinson, Franklin lakes, NJ), and microtubes with capacities of 0.5, 1.5, and 2.0 ml (Eppendorf, Germany). The bottoms of the 0.5-ml and 1.5-ml microtubes were V-shaped, and the bottoms of 2.0-ml microtube were U-shaped. The culture volume for all vessels was 200 μl, except for the 35-mm tissue culture dishes, with a culture volume of 2 ml. During cultivation, some 0.5-ml and 1.5-ml microtubes were inverted (Fig. 1). At that time, the culture medium did not move from the V-shaped end of the tube. At the end of cultivation, the amount of IgM in each culture medium was measured by an enzyme-linked immunosorbent assay (ELISA) with anti-human IgM antibodies. Growth of HB4C5 cells cultured in microtubes of any size or in either orientation was 1.2- to 1.5-fold than that in 96-well flat-bottomed culture plates or 35-mm culture dishes (Table 1). The IgM production of HB4C5 cells cultured in microtubes was greater than that of cells cultured in 96-well culture plates or 35-mm dishes. The IgM production per 10⁴ HB4C5 cells cultured for 24 h in microtubes was 2.6- to 3.3-fold that in 96-well culture plates.

HB4C5 cells were used to inoculate flat- and V-bottomed culture plates with 96 wells at 7.5 × 10⁴ cells/ml, and plates were cultured for 3 d. IgM production of the cells cultured in the V-bottomed culture plate was 3- to 5-fold that in flat-bottomed culture plate (Fig. 2). The shape of the culture vessel was, as expected, important for immunoglobulin production. The difference in IgM produced by cells grown in microtubes (made from polypropylene) did not arise from the difference in the materials of the vessels being compared, because culture plates of...
Table 1. Cell Growth and IgM Production of HB4C5 Cells in Different Vessels

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Viable cell density (cells/ml)</th>
<th>IgM production (ng/ml)</th>
<th>IgM productivity (ng/10^4 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well flat-bottomed culture plate</td>
<td>8.2 × 10^4</td>
<td>25.0 ± 1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>35-mm culture dish</td>
<td>8.1 × 10^4</td>
<td>30.8 ± 1.2</td>
<td>3.8</td>
</tr>
<tr>
<td>0.5-ml microtube, upright</td>
<td>1.2 × 10^3</td>
<td>106.0 ± 7.6</td>
<td>9.0</td>
</tr>
<tr>
<td>0.5-ml microtube, inverted</td>
<td>1.0 × 10^3</td>
<td>101.8 ± 2.3</td>
<td>10.0</td>
</tr>
<tr>
<td>1.5-ml microtube, upright</td>
<td>1.1 × 10^3</td>
<td>101.8 ± 5.4</td>
<td>9.0</td>
</tr>
<tr>
<td>1.5-ml microtube, inverted</td>
<td>1.0 × 10^3</td>
<td>106.3 ± 6.4</td>
<td>10.3</td>
</tr>
<tr>
<td>2.0-ml microtube, upright</td>
<td>1.1 × 10^3</td>
<td>90.0 ± 2.0</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Human-human hybridoma HB4C5 cells were used to inoculate vessels at 7.5 × 10^4 cells/ml and cultured for 24 h. The IgM in vessels was determined by ELISA. IgM production is given as the mean ± SD of three independent measurements.

Both shapes (flat- and V-bottomed) were made from the same material, polystyrene. Culture in microtubes occurred without gas exchange; the microtubes were airtight. However, IgM production was high when HB4C5 cells were cultured in uncapped microtubes for 24 h (not shown). The supply of O2 gas and the discharge of CO2 gas did not affect IgM production in microtubes, at least during the first 24 h of culture. The shape of the culture vessels affected IgM production at various cell densities; the culture in the 1.5-ml microtubes was more productive than that in 96-well flat-bottomed culture plates at all cell densities tested (Fig. 3). How does the shape of the vessel affect immunoglobulin production by hybridomas? Probably, cells fell to the bottom of the tube during cultivation in the microtubes, increasing the cell density there. At a higher density, cells may interact affecting productivity. In fact, during cultivation in upright microtubes of 0.5- or 1.5-ml capacities, more than 98% of cells were at the bottom of the tubes. On the contrary, HB4C5 cells cultured in inverted tubes were fully dispersed throughout the culture medium during cultivation (not shown). Therefore, the high cell density at the bottom during cultivation was not the reason for the high productivity in microtubes.

Mechanical forces have a critical role in tissue remodeling and cellular homeostasis. There are many reports about the regulation of cell metabolism and productivity by physical forces. The concentration of transforming growth factor-β1 mRNA in rat tracheal epithelial cells is increased when there is constant pressure on their apical surfaces (transmembrane pressure).\(^5\) The mechanical stretching of neonatal rat cardiac myocytes cultured in serum-free medium increased the concentration of adrenomedullin mRNA in the myocytes by 88% compared with the base line in nonstretched cells.\(^6\) These findings show that transcription could be up-regulated by mechanical forces.
Ultrasound also affects biological systems. One of the most remarkable effects of ultrasound is causing cells to come to facilitate molecular transport through cell membranes. This effect may be useful for the delivery of foreign molecules into living cells, and for the release and collection of intracellular products.7)

Cells in mechanically dynamic environments often cause stress adaptation in the surrounding tissue if the mechanical environment deviates from normal. Gene expression in the cells is up-regulated by many physical forces as an adaptation to environmental stresses.8,9) We showed here that cultivation of HB4C5 cells in microtubes allows them to produce much IgM. These results imply that physical forces affected by the shape of the vessel, such as surface tension, contribute to the metabolism of the hybridoma. In addition to production of large amount of IgM, the cell growth of the hybridoma was also improved by cultivation in microtubes. These findings suggest that gene expression in hybridoma cells cultured in microtubes is facilitated by physical forces affected by the shape of vessel, and increased expression heightened cell proliferation and productivity. The facilitation of metabolism of this hybridoma may involve adaptation to environmental stress. Surface tension acting on HB4C5 cells during cultivation in microtubes may mechanically stimulate stress responses of the cells in suspension cultures. This phenomenon would be applicable to bioreactors with capillary culture vessels, which can create surface tension. The application of adaptative skills of cells against physical stresses could be used for the development of new kinds of bioreactors.

References