Note

Development of a New Protein- and Hormone-free Medium for Hybridoma Cultivation

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Monoclonal antibodies (MAbs), which have been used widely for diagnostic and therapeutic purposes, can be produced by in vitro and in vivo cultivation of hybridomas. Today, in vitro cultivation of hybridomas can be done in serum-free medium because this makes it easier to purify MAbs to high purity by simple procedures than the conventional cultivation in serum-supplemented medium. However, serum-free media are generally made up with some proteins such as transferrin, albumin, insulin, and lipoproteins,1,2) and sometimes it is difficult to purify MAbs to high purity because of these protein additives. Furthermore, these media are relatively expensive, especially for large scale production of MAbs in comparison with the production in mouse ascites. Protein-free media which contain no high molecular weight components could be more suitable for MAb production, if they could be supplied in large quantities at an appropriate price. Recently, some protein-free media have been reported.3-5) However, they are not good for the cultivation of human-human hybridomas, and also they have fairly complex compositions containing many kinds of trace elements and hormones. We have developed a new protein- and hormone-free (PHF) medium. This medium was designed to contain low molecular weight components alone and to be useful for the cultivation of both mouse-mouse hybridomas and human-human hybridomas.

The hybridomas tested are listed in Table I. Mouse-mouse hybridomas were all established in our laboratory by fusing spleen cells from antigen-immunized BALB/c mice with parental cells.6) HB4C5 cells7) were kindly given to us by Professor H. Murakami, Kyushu University. E-RDF medium8) purchased from Kyokuto Seiyaku

Table I. Immunoglobulins Produced by Hybridomas

<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>Parental cell*</th>
<th>Specific antigena</th>
<th>Isotypec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse-Mouse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>X63.653</td>
<td>AFP</td>
<td>IgG1</td>
</tr>
<tr>
<td>C7</td>
<td>NS-1</td>
<td>CEA</td>
<td>IgG1</td>
</tr>
<tr>
<td>I7</td>
<td>NS-1</td>
<td>IgE</td>
<td>IgG1</td>
</tr>
<tr>
<td>Human-Human</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB4C5</td>
<td>NAT-30</td>
<td>Lung cancer</td>
<td>IgM</td>
</tr>
</tbody>
</table>

* Parental cell line used as a fusion partner in preparation of hybridoma.

a AFP, α-fetoprotein; CEA, carcinoembryonic antigen.

Fig. 1. Effects of Ferric Citrate and Linoleic Acid on Cell Proliferation of Hybridomas.
A2 ( ), C7 ( ) and I7 ( ) cells in media with various concentrations of ferric citrate (left) or linoleic acid (right).

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Kogyo Co., Tokyo, was used as the basal medium.

The effects of ferric citrate on the growth of hybridoma cells were tested. Cells were inoculated at the density of $1 \times 10^5$ cells/ml in the basal medium supplemented with 0–500 $\mu$M ferric citrate. After culture for 3 days, the cells were counted. As shown in Fig. 1, the optimal growth was observed at the concentration of 50 $\mu$M for A2 and 17 cells, giving 4–5 times enhancement in the cell growth. Kovar and Franek described how ferric citrate at 500 $\mu$M gave the best results of the growth of hybridomas. On the contrary, we found that ferric citrate at concentrations over 100 $\mu$M inhibited the growth of two of the three lines tested, though not for one line. The effects of linoleic acid, taurine, and ethanolamine were examined in the same way, except for using the basal medium containing 50 $\mu$M ferric citrate. The optimal concentration of linoleic acid was shown to be 0.5 $\mu$M (Fig. 1). When its concentrations were over 1 $\mu$M, the cell growth was inhibited drastically. Taurine, an essential amino acid for newborn babies, was effective in concentrations over 5 $\mu$M, and showed no inhibition up to 1 $\mu$M (data not shown). Ethanolamine is usually used at 10–20 $\mu$M, but the optimal concentration in the PHF medium was found to be 100 $\mu$M, and the inhibition was not observed up to 500 $\mu$M (data not shown). Judging from the results described above, the composition of the PHF medium was decided on as follows: E-RDF medium supplemented with 50 $\mu$M ferric citrate, 0.5 $\mu$M linoleic acid, 10 $\mu$M taurine, 100 $\mu$M ethanolamine, and 25 $\mu$M sodium selenite.

Since hybridomas grew well in PHF medium for 3 days, we tried to culture two hybridomas using this medium for a long term, and cultured them for 38 days (Fig. 2). Although C7 mouse–mouse hybridomas inoculated in PHF medium showed an initial lag, they grew at a rate similar to those obtained in 10% fetal calf serum (FCS, S-PHF).

**Fig. 2.** Long-term Cultivation of Hybridomas in PHF and 10% Fetal Calf Serum Supplemented PHF (S-PHF) Media.

Five $\times 10^5$ cells were plated into 5 ml of fresh medium in 60 mm dishes at Day 0, and the culture was done for 5 days (1st passage). At Day 3, $5 \times 10^5$ cells were transferred to 5 ml of fresh medium, followed by culture for 5 days (2nd passage). Continuing the passages every 3 days likewise, the final culture was done in Days 33–38 (12th passage). C7 (upper) and HB4C5 (lower) cells in PHF (○) and S-PHF (●) media.

**Fig. 3.** Production of Immunoglobulins by Hybridomas in PHF and S-PHF Media.

C7 (left) and HB4C5 (right) cells in PHF (○) and S-PHF (●) media.
purchased from Gibco) supplemented PHF (S-PHF). During several passages, the growth rate decreased gradually, however, this cannot be a crucial point for the production of MAbS. Similarly, HB4C5 cells proliferated in PHF medium at a similar rate in the early period as those in S-PHF medium, but the growth rate decreased gradually, and in the final passage (days 33–38), the growth rate was almost half that in S-PHF medium. Although the growth of the human–human hybridomas was comparatively slow in PHF medium, they kept growing in the medium over a month. On the other hand, these cells could not be kept in the serum-free medium containing proteins of low concentrations, such as ITES medium. Therefore, the PHF medium could be useful for MAb production.

To identify the effects of the PHF medium of MAb production, immunoglobulin (Ig) G and IgM concentrations in the cultured supernatants of C7 (IgG producer) and HB4C5 (IgM producer) cells were measured by enzyme immunoassay (Fig. 3). The cells were cultured under the conditions shown in PHF or S-PHF media, and the supernatants were harvested daily from the 34th to 38th culture days. The amount of IgG produced by C7 cells in PHF medium was similar to that in S-PHF medium. On the other hand, the HB4C5 cells in PHF medium produced about 1/8 of the IgM of those in S-PHF medium. Although the productivity of HB4C5 cells in PHF medium was low, the amount of IgM produced by a single cell was not very different. Therefore, by using perfusion or hollow-fiber cultures, the human–human hybridomas may produce MAb in PHF medium like in S-PHF medium. Actually, the human–human hybridomas cultured by a hollow-fiber system (Acusist Jr., Endotronics) produced 8.9 mg of IgM in 39 days in the PHF medium and 8.3 mg in 55 days in the S-PHF medium. These productivities are comparable with that obtained in Dulbecco’s modified Eagle’s (DME) medium supplemented with 10% FCS.

The PHF medium developed in this study has a simple composition and contains no macromolecules. This medium is economically appropriate, and this makes it useful especially for the large scale production of MAbS not only by mouse–mouse hybridomas but also by human–human hybridomas.

References

12) K. Kondo, K. Maki and K. Inouye, Abstracts of Papers, the Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry, Fukuoka, April, 1990, p. 338.