Note

Selection of Fusion Partner Cell Lines for Generation of Human-Human Hybridomas that Secrete IgA

Hiroshi SHINMOTO, Shun'ichi DOSAKO
and Hiroki MURAKAMI*

*Technical Research Institute, Snow Brand Milk
Products Co., Ltd., 1-1-2 Minamidai,
Kawagoe 350, Japan*

**Institute of Food Science and Technology,
Faculty of Agriculture, Kyushu University,
Hakozaki, Higashi-ku, Fukuoka 812, Japan.*

Received February 1, 1989

In our previous paper,¹⁾ we aimed to fuse lymphocytes of lymph nodes from lung cancer patients with a fusion partner cell line, HO323, to produce IgA-secreting human-human hybridomas. However, we failed to produce hybridomas that secreted IgA alone, because all hybridomas that secreted IgA also secreted IgM. Limiting dilution analysis of hybridomas and reduction and carboxymethylation of the immunoglobulins revealed that a single hybridoma clone secreted complex immunoglobulins consisting of IgA (λ) originated from lymphocytes and IgM (κ) from HO323.^{1,2)} Although HO323 is not an immunoglobulin producer, it does synthesize a certain level of cytoplasmic IgM. Thus fusion partner cell lines without or with a very low level of cytoplasmic immunoglobulins are needed to obtain human-human hy-

bridomas that secrete IgA alone.

O-2 cells, an ouabain-resistant cell line from HO323,³⁾ were used as the origin of fusion partners. To obtain cell lines with a very low level of cytoplasmic IgM, O-2 cells were cloned in round-bottomed 96-well microculture plates (Corning 25850) without any feeder cells. The clones grown were transferred to 24-well culture plates (Corning 25820). After 4 to 7 days of culture, approximately half of the cells in the wells were harvested into micro-centrifuge tubes and washed with phosphate-buffered saline (PBS). Cell pellets were lysed overnight in 0.5 ml 1/10 diluted PBS containing 1% Tween 20 at 4 to 7°C, followed by centrifugation at $5,000 \times g$ for 10 min. The concentration of IgM in the supernatants was measured by enzyme-linked immunosorbent assay (ELISA).⁴⁾ Immunostaining of the clones with FITC-labeled anti-human IgM was insufficient to detect the clones that did not have cytoplasmic IgM, because of the low sensitivity of the staining method. Of 670 clones analyzed, lysates of four clones, O-22, O-23, O-241, and O-283, contained less than 1 ng/ml IgM. O-22 cells were recloned; lysates of six clones with less than 0.1 ng/ml IgM were obtained from 120 clones analyzed. Clones O-2227 and O-22114 were chosen for further analysis.

Six clones, O-22, O-23, O-241, O-283, O-2227, and O-22114, were fused with the IgA (λ)-secreting B-lymphoblastoid cell line 21-2³⁾ through polyethylene glycol. Hybridoma cells were selected with HAT medium containing $2 \mu\text{M}$ ouabain. Table I shows immunoglobulins secreted from the hybridomas. No hybridomas that secrete IgA alone were obtained from clones O-23, O-241, and O-283. With O-22 clones, IgA alone was detected in the culture supernatant of 31% of the wells analyzed. Furthermore, 64% of the wells with hybridomas from O-2227 and 82% of the wells with hybridomas from O-22114 contained IgA alone.

Immunoglobulin from SHH7, a hybridoma clone between O-22114 and 21-2, contained λ light chains and α

Table I. FUSIONS OF O-2 CLONES WITH 21-2 CELLS

Clone	Cell fused*	Wells seeded	Wells with cell growth	Wells analyzed	Wells with**		
					IgA +	IgM +	IgA + IgM -
O-22	1×10^7	288	288	96	96	66	30
O-23	1×10^7	288	288	96	96	96	0
O-241	7×10^6	192	168	168	168	168	0
O-283	7×10^6	192	152	152	152	152	0
O-2227	5×10^6	144	42	42	42	15	27
O-22114	5×10^6	144	38	38	38	7	31

* An equal number of O-2 derived cells and 21-2 cells were fused with polyethylene glycol. The cell mixture was seeded into 96-well micro-culture plates (Falcon 3072). Hybridomas were selected with RPMI 1640 medium containing 10% fetal calf serum (Flow laboratories), $2 \mu\text{M}$ ouabain, 0.1 mM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine (O-HAT medium).

** Wells with immunoglobulin concentration higher than 1 ng/ml were scored as positive.

Fig. 1. Gel Filtration Analysis of Culture Supernatant of Hybridoma SHH7 Obtained from Cell Fusion between O-22114 Parent Cells and 21-2 Cells.

The culture supernatant of SHH7 (200 μ l) was fractionated on Superose-6 (10/30) column using the Pharmacia FPLC system. Elution was done with PBS at a flow rate of 0.4 ml/min, collecting each 0.4 ml. Concentrations of immunoglobulins were measured by ELISA using anti- α and anti- μ antibodies.

heavy chains. This indicated that the immunoglobulin secreted from SHH7 originated from the immunoglobulin of the 21-2 cell line. However, a hybridoma, SHH1 (O-283 $\times$ 21-2) secreted complex immunoglobulin consisting of both κ and λ light chains and both α and μ heavy chains.

Figure 1 shows an elution pattern of the culture supernatant of SHH7 on a Superose-6 column (Pharmacia). The supernatant contained IgA with molecular weights of 160 kD (monomer) and 330 kD (dimer). No peaks resulting from μ chains were detected.

These findings suggested that clone O-22114 shows promise as a parent cell to produce IgA-secreting human-human hybridomas.

References

- 1) H. Shinmoto, H. Murakami, S. Dosako, K. Shinohara and H. Omura, *Agric. Biol. Chem.*, **50**, 2217 (1986).
- 2) H. Shinmoto, H. Murakami, S. Dosako, K. Yamada, and H. Omura, *In Vitro Cell. Develop. Biol.*, **24**, 505 (1988).
- 3) H. Shinmoto, H. Murakami, S. Dosako, K. Yamada and H. Omura, *Agric. Biol. Chem.*, **52**, 2653 (1988).
- 4) E. Engvall and P. Perlmann, *Immunochemistry*, **8**, 871 (1971).