Note

Production of Pentameric Hybrid Immunoglobulins Consisting of IgA and IgM

Hiroshi SHINMOTO, Hiroki MURAKAMI,*
Shun'ichi DOSAKO, Koji YAMADA*
and Hirohisa OMURA*

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

*Institute of Food Science and Technology,
Faculty of Agriculture, 46-09 Kyushu University,
Higashi-ku, Fukuoka 812, Japan

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Our previous papers^{1,2)} showed that cell fusion between an IgM-producing human B-lymphoblastoid cell line (BLCL) and lymph-node lymphocytes from cancer patients produced hybridomas that secrete hybrid immunoglobulins consisting of two monomeric IgA (mIgA) and three monomeric IgM (mIgM), with a molecular weight of 900k. Pentameric hybrid immunoglobulins would be much more effective as bifunctional antibodies than monomeric hybrid immunoglobulins such as IgG,³⁾ because they have ten antigen binding sites in each molecule. Hybridoma secreting pentameric hybrid immunoglobulins were also generated by the fusion of murine cell lines.⁴⁾ One way to develop bifunctional antibodies aimed at two separate antigens is to fuse hybridomas and/or BLCLs with known antigen specificity. However, we had not yet examined whether cell fusion between IgM-producing BLCL and IgA-producing BLCL results in hybridomas that secrete pentameric IgA/IgM hybrid immunoglobulins.

Two BLCL lines secreting IgM and IgA were used for fusion. Ouabain- and 6-thioguanine-resistant BLCL O-2, an IgM (κ) producer, was derived from HO323,⁵⁾ following the method of Kozbor *et al.*⁶⁾ An IgA (α) producer, 21-2, was obtained by transforming peripheral blood lymphocytes with Epstein-Barr virus.⁷⁾ An equal number (1×10^7) of both cells were fused as described elsewhere¹⁾ and plated in a 96-well microtiter plate. Hybridoma cells were selected with HAT medium containing $5 \mu\text{M}$ ouabain.⁶⁾ The fusion efficiency was 85.2%. Of 82 wells with cell growth, 56 wells (68%) contained both IgA and IgM; no immunoglobulin was found in the other wells.

A fast-growing hybridoma, H57, was chosen for further experiments. Figure 1 shows gel filtration patterns of immunoglobulins secreted from 21-2 and H57. The culture

supernatant of the parent 21-2 contained IgA with molecular weights of 330 k (dimeric IgA) and 160 k (mIgA). But both IgA and IgM from H57 were eluted into fractions with a molecular weight of 900 k. This suggested that IgA and IgM associated to form hybrid polymers. There were 170 ng/ml of IgA and 320 ng/ml of IgM in fraction #24. The molecular weight of mIgA is 160 k and that of mIgM is 180 k, so the molar concentration of mIgA in this fraction is 1.06×10^{-9} molar, and that of mIgM is 1.78×10^{-9} molar. We therefore estimate the ratio of mIgA to mIgM to be 2:3.

Table I shows the results of polypeptide chain composition analysis by ELISA. The immunoglobulin with a molecular weight of 900 k reacted with both anti- α - and μ -chain antibodies immobilized on the solid phase. The reacted immunoglobulin further bound to both peroxidase conjugated anti- α - and μ -antibodies. This indicated that the immunoglobulin molecule contained both α - and μ -heavy chains in the same molecule. In addition to heavy chains, the immunoglobulins had both λ and κ light chains. These results implied that α , μ , λ , and κ chains were associated to form a pentameric hybrid immunoglobulin, consisting of two mIgA and three mIgM.

FIG. 1. Gel Filtration of Immunoglobulins Secreted by 21-2 and H57.

Culture supernatants were precipitated by salting out with ammonium sulfate. Precipitates were dissolved with PBS and fractionated on a Sephacryl S-300 column (1.2×56 cm). Each fraction (1 ml) was collected and the concentration of IgA and IgM were measured by ELISA.

TABLE I. POLYPEPTIDE CHAIN COMPOSITION ANALYSIS OF H57 IMMUNOGLOBULIN BY ELISA

Antibodies		Reactivity
Solid phase ^a	Enzyme conjugated	
Anti α	Anti α	+
Anti α	Anti μ	+
Anti α	Anti λ	+
Anti α	Anti κ	+
Anti μ	Anti α	+
Anti μ	Anti μ	+
Anti μ	Anti λ	+
Anti μ	Anti κ	+

^a Anti- α or anti- μ (Cappel) were immobilized onto each well of a 96-well microtiter plate (NUNC). After blocking with Block Ace (Dainippon Seiyaku), H57 immunoglobulin was allowed to react for one hour at room temperature. Then peroxidase-conjugated anti- α , anti- μ , anti- λ or anti- κ (TAGO) were added. Peroxidase activity bound onto each well was detected by adding ABTS as substrate.

In conclusion, we demonstrated that the fusion between two established cell lines secreting IgA and IgM produced hybridomas secreting pentameric IgA/IgM hybrid immunoglobulins. We are now attempting to develop human pentameric immunoglobulins that can act as bifunctional antibodies.

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