Stimulation of Immunoglobulin Production in Human-human Hybridoma HB4C 5 Cells and Human Lymphocytes by Soymilk

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Soymilk was found to stimulate IgM production in human-human hybridoma HB4C 5 cells cultured in serum-free ERDF medium supplemented with insulin (I), transferrin (T), ethanolamine (E) and selenite (S) (ITES-ERDF). The activity for immunoglobulin production stimulating factor (IPSF) was recognized in the acid precipitable fraction. The activity was observed in a peak obtained by fractionation on DEAE-Toyopearl 650 M. Molecular size of the IPSF was estimated to be approximately 290 kDa by gel filtration on Superose 12 HR. The susceptibility of the IPSF to heat or trypsin treatment suggested that it was a proteinous substance. Soymilk also stimulated IgM and IgG production in human lymphocytes cultured in ITES-ERDF medium.

Production of human-human hybridomas producing monoclonal antibodies specific to human antigens has been developed recently¹⁾. *In vitro* system using the human-human hybridomas is useful for screening of various bioactive materials in foodstuffs. Using human-human hybridoma HB4C 5 cells which

produce IgM under culture in a serum-free medium, several proteinous immunoglobulin production stimulating factors (IPSFs) were found in milk and egg yolk²⁾³⁾. Egg yolk lipoprotein (YLP), one of the IPSFs, was found to stimulate both IgM and IgG production of human lymphocytes⁴⁾. This finding indicates that *in vitro* screening system using human-human hybridomas is useful for detecting some compounds which affect for human lymphocytes *in vivo*. This paper reports the results of the screening, using human-human hybridoma HB4C 5 cells, for IPSF in soymilk and its properties.

1. Materials and Methods

Soymilk: Soymilk was purchased from Nagoya Meiraku Co. (Nagoya, Japan). Preparation of soymilk involves heat sterilization process; first at 92°C for 1.5 min., and then at 145°C for 8 sec.

Cells and cell culture: Human-human hybridoma HB4C 5 cells, which produce IgM specific to human lung adenocarcinoma cells⁵⁾ and tissues⁶⁾, were used. The cells were cultured at 37°C for 2 days in ERDF medium⁷⁾ supplemented with 10 µg/ml insulin, 35 µg/ml transferrin, 10 µM ethanolamine, and 2.5 nM sodium selenite (ITES)⁸⁾ containing various test samples. The cell number was counted by a electric cell counter (Toa Iyou Denshi). IgM content in supernatant of the culture was measured by the ELISA method as described previously⁹⁾.

Isolation of IPSF in soymilk: An acid precipitable fraction was fractionated on DEAE-Toyopearl 650 M column by eluting with a linear gradient of NaCl from 0 to 1 M. Each fraction was assayed for IPSF activity.

Molecular size of the IPSF fractionated on DEAE-Toyopearl column was estimated by a Superose 12 HR column equilibrated with 10 mM phosphate buffer (pH 6.8).

Effect of soymilk on Ig production of human lymphocytes: Human lymphocytes were isolated from peripheral blood of a healthy volunteer. IPSF activity on lymphocytes was measured as described previously⁴⁾.

2. Results and Discussion

Effect of soymilk on IgM production of HB 4 C 5 Cells: Soymilk was diluted 10 to 10^7 -fold with phosphate buffered saline (PBS) and examined for its effects on proliferation and IgM production of HB 4 C 5 cells. Basically the same result was obtained in 3 separate experiments. Fig. 1 shows the typical dose-dependent stimulation of proliferation and IgM production of HB 4 C 5 cells by soymilk. The highest activity for stimulating IgM production (approximately 4-fold of control) was observed in the 10^2 -fold dilution.

Isolation of IPSF in soymilk: Five-fold increase in IPSF activity was observed by acid precipitation of soymilk. The acid precipitable fraction was fractionated on a DEAE-Toyopearl 650 M column. As shown in Fig. 2, the IPSF activity was observed in a peak eluted with 450 mM NaCl. Molecular size of the IPSF was estimated to be approximately 290 kDa from gel filtration profile on Superose 12 HR, as shown in Fig. 3.

Characterization of IPSF in soymilk: Fig. 4 shows the heat stability of the IPSF fraction-

Fig. 1 Dose-dependent stimulation of proliferation and IgM production of HB 4 C 5 cells by soymilk

HB 4 C 5 cells (0.5×10^5 cells/ml) were cultured in ITES-ERDF medium containing various dilution ratio of soymilk for 2 days.

Fig. 2 Ion exchange chromatographic profile of acid precipitable fraction of soymilk on DEAE-Toyopearl 650 M

Five-fold diluted soymilk was adjusted to pH 4.5 by the addition of acetic acid, and then it was centrifuged at $400 \times g$ for 30 min. The precipitate was dissolved in 10 mM sodium phosphate buffer (pH 7.0). The solution was used here as an acid precipitable fraction. This fraction showed the IPSF activity (5-fold increase).

Fig. 3 Gel filtration profile of the IPSF on Superose 12 HR

ated on DEAE-Toyopearl column and the susceptibility to trypsin. From the fact that the IPSF was not stable to heat and trypsin, we concluded that the factor was a proteinous substance.

Effect of soymilk on Ig production of human lymphocytes: Table 1 clearly demonstrates the enhancement of IgM (8-fold) and IgG (1.5-fold) levels by addition of the soymilk to the medium. This result, being similar to that of egg yolk lipoprotein (YLP)⁴⁾, indicates that soymilk has some function to affect the human immune system.

Fig. 4 Heat stability (A) and susceptibility to trypsin (B) of the IPSF fractionated on DEAE-Toyopearl 650 M

A: the IPSFs were heated at various temperatures for 30 min. B: the treatment of trypsin was described previously¹⁰⁾.

Table 1 Effects of soymilk and mitogens on Ig production of human peripheral blood lymphocytes^{a)}

Additives	Cell number ($\times 10^5$ cells/ml)	Ig concentration (ng/ml)	
		IgM	IgG
None	6.00	5	124
Soymilk ^{b)}	7.48	38	180

^{a)} Cells (9.5×10^5 cells/ml) were cultured in ITES-ERDF medium for 5 days.

^{b)} 500 times-diluted soymilk was added to the medium.

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豆乳によるヒト型ハイブリドーマ及びヒトリンパ球における抗体産生増強作用

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ヒト型ハイブリドーマを用いて、豆乳に抗体産生増強活性を見いだした。活性は、酸沈澱画分に認められた。さらに酸性沈澱画分を DEAE-Toyopearl 650 M で分離したところ、活性は 1 つのピークとして得られ、Superose 12 HR によるゲル濾過クロマトグラフィーで分子量約 290 kDa と推定された。また、本抗体産生増強因子 (IPSF) は、熱及びトリプシン消化に感受性が高く、他の食品中 IPSF 同様、タンパク質様物質であると示唆された。さらに、豆乳はヒトリンパ球の IgM 及び IgG 産生も増強した。