International Workshop
Summaries of Lectures
Presented at the 2004 General Meeting
of the BioBran Study Conference

Regulation of the Defense Mechanism with Food

- Relationship between Tumor Dormant Therapy and Food Function -

Date: March 14, 2004 (Sunday)

Place: Kokuyo Hall

Chemical Structure of Ingredients Involved in Immunoregulation

Tomisato Miura,[*1] Mitsuru Chiba,[*1] Yuko Miyazaki,[*1] Yoji Kato,[*2]

[*1] School of Health Sciences, Hirosaki University,

[*2] Dept. of Domestic Science, Faculty of Education, Hirosaki University,

[Purpose] BioBran, which is derived from rice bran, has been reported to have bioprotective actions such as increasing the activity of NK cells and the ability to activate macropharges. It has recently been shown to have antitumor activity, and clinical application as an antitumor agent is expected. However, the chemical structures of the ingredients involved in immunopotentiation remain unknown. The purposes of this study were to examine the imunopotentiating activity of various fractions of BioBran using activation of macrophages as an indicator and to analyze the chemical structure of the ingredients involved in immunoregulation.

[Methods] (1) Fractions of BioBran: (a) BioBran was dissolved in purified water, soluble fractions were divided into 50, 66, and 80% methanol precipitation fractions and an 80% methanol supernatant fraction by the alcohol separation precipitation method, and 50ppt, 66ppt, 80ppt and 80sup fractions were obtained. (b) The 80% methanol insoluble fraction of BioBran was subjected to DEAE-Sephadex A-25 column chromatography equilibrated with 20mM Na-acetate buffer (pH 5.0), and five fractions from Fr.I to Fr.V were obtained by stepwise elution with NaCl. Fr.II eluted with 0.2M NaCl was again subjected to DEAE-Sephadex A-25 column chromatography, and seven fractions from Fr.II-1 to Fr.II-7 were obtained by the elution method using a linear concentration gradient with 0 to 0.5M NaCl.

(2) Evaluation of the activation of macrophages: TGC medium (Difco) was intraperitoneally given to C57BL/6 female mice and peritoneal exudates cells (PECs) were collected after four days. A magnetic bead cell partition system (Miltenyl Biotec) was used to separate Mac-1^+ cells (peritoneal macrophages). After the cells were cultured in the presence of BioBran or each BioBran fraction for 24 hours, the culture supernatant was collected. To comparatively examine the activity of macrophages, NO^{2-} in the supernatant was measured by the Griess method, and tumor necrosis factor-alpha (TNF-α) and interleukin-1-beta (IL-1β) were determined by ELISA (Biosource).

(3) Sugar analysis of each fraction: analysis of constitutive sugar, analysis of the sugar-binding mode (methylation), analysis of fragments with enzymes, etc. were performed.

[Results and Discussion] Comparison of the macrophage activation capability of methanol precipitation fractions obtained by (1)-(a) in "Methods" revealed that the 50ppt fraction had about three times higher activity in inducing the production of NO^{2-} than unfractionated BioBran and induced a significantly large amount of inflammatory cytokines, indicating the presence of immunoregulators in 50ppt. Comparison of the macrophage activation capability of Fr.II obtained by DEAE-Sephadex A-25 ion exchange chromatography by (1)-(b) and Fr.II-4 to 6 obtained by further fractionating Fr.II using the same chromatography revealed that Fr.II-4 to -6 had four to five times higher activity in inducing the production of NO^{2-} than unfractionated BioBran. Fr.II-6 had especially higher macrophage activation. Analysis of the sugar composition of this fraction (results: Rha:Fuc:Ara:Xyl:Man:Glc:Gal = 7.6:0.6:22.2:13.7:2.7:30.2:23.0) suggests that the immunoregulator contained in BioBran is a heteropolysaccharide having a complex structure. Based on the results of analysis of the sugar-binding mode, analysis of fragments with enzymes, etc., the presumed chemical structure will be presented.