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AN IMMUNOPEPTIDE IN BEEF — PURIFICATION OF A PEPTIDE RELEASING INTERLEUKIN-1ß FROM MACROPHAGES —

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Background:

Macrophages activated with cytokines, endotoxins, or some biological response modifiers produce primary cytokines like interleukin-1 and tumor necrosis factor. These cytokines play important roles in immunological regulation and inflammatory response. Moreover, these cytokines are known to share several biological roles (1). On the other hand, in 1979, peptides derived from partial enzymatic digestion of bovine casein were shown to have opioid activity (2). Since then, bioactive peptides such as opioid peptides and immunopeptides have been detected in bovine and human milk proteins and some vegetable proteins (3-5). These findings have introduced a new criterion for defining the nutritive value of food proteins: peptides that are inactive within the protein sequence can be released during digestion, and may have hormone-like activity. Some interesting dietary and pharmaceutical applications of this discovery are indicated for the future. Bioactive components in meat and its products have also been investigated by a number of researchers (6, 7). However, bioactive peptides derived from those have not been detected yet.

Objectives:

The objectives of this study were to examine whether peptides in beef would be capable of affecting the productivity of interleukin-1 by a macrophage-like cell line, and to characterize and purify the immunopeptides.

Methods:

Cell culture. J774A.1 cells, a macrophage-like cell line purchased from Riken Cell Bank, were used for bioassay to verify macrophage activation by beef peptides. The cells (1.0×10^6 cells/ml) were cultured in serum-free RPMI-1640 medium (Gibco) supplemented with 10 μg/ml of beef peptides or lipopolysaccaride (from E.coli O55:B5, Sigma) as positive control at 37°C for 96 hours under 5% of CO_2 atmosphere. Triplicate assay was performed for each samples and control.

Determination of Interleukin-1ß (IL-1ß). IL-1ß released from macrophages was determined by Enzyme linked immunosolvent assay according to the method of Hornbeck (8).

Extraction of beef peptides. Beef round (non hormone treated) was heated in distilled water at 95°C for 10 min to inactivate endogeneous proteinase in beef, and the suspension was cooled in ice tapped water. After addition of an equal volume of 1 M acetic acid, the suspension was homogenized with a blade-type blender for 1 min. The homogenate was centrifuged at 27,000×g for 30 min, the resulting supernatant was dialyzed with Spectra Por 6 (M.W.C.O. < 1,000, Spectrum) against 0.5 M acetic acid to remove nucleotides and metal ions.

Chromatography. Column chromatography was carried out with SP-Sephadex C-25, Sephadex G-50 (Amarsham Pharmacia Biotech), and CM-Cellulofine C-200 (Seikagaku Corporation). High performance liquid chromatography (HPLC) was conducted by Shimadzu LC-10AD$_{VP}$ equipped with μBondasphere 5-C$_{18}$ (Waters), COSMOSIL 5-C$_{18}$-AR-300, and COSMOSIL 5-Ph-AR-300 (Nacalai Tesque).

Results and Discussion

Figure 1 shows IL-1ß releasing activity of beef peptides extracted with distilled water. As shown in this figure, beef peptides induced IL-1ß release from macrophages dose-dependently. The beef peptides heated at 95°C for 10 min were also induced IL-1ß production from macrophages (Figure 2). The beef peptides were fractionated with batch method using SP-Sephadex C-25. The basic fraction of beef peptides appeared to induce IL-1ß release from macrophages (Figure 3). IL-1ß releasing activities of beef peptides and its basic fraction disappeared completely after trypsin digestion of those peptides. The peptide having its molecular weight of 6,000 or lower exhibited IL-1ß releasing activity from the results of gel filtration using Sephadex G-50 and subsequent

bioassay for the fractions (Figure 4). These results suggested that IL-1ß releasing factors in beef peptides were basic peptides having molecular weight of 6,000 approximately. To purify an IL-1 releasing factor, beef peptides were extracted from 1 kg of beef round. The yield of beef peptides extracted was 2.268 g per kg of beef. After a basic fraction of beef peptides was separated with batch method using an SP-Sephadex C-25 column, cation exchange chromatography using CM-Cellulofine C-200 was carried out. The resulting active fractions (fraction number 35-39) were subjected to gel filtration using Sephadex G-50. IL-1ß releasing activities were observed the fractions containing a protein of 14,000 dalton and the other fractions containing peptides having 8,000 dalton or lower on Tris-Tricine SDS-PAGE. The peptide was further purified with HPLC equipped with COSMOSIL 5-C_{18}-AR-300 , μBondasphere 5-C_{18}, and COSMOSIL 5-Ph-AR-300 column. Finally, approximately 1 nmol of IL-1ß releasing peptide was obtained. The peptide was 7,000 dalton in the molecular weight on Tris-Tricine SDS-PAGE.

Conclusions:

We attempted to research the peptides release IL-1ß from a macrophage-like cell line in beef round, and to purify one of the immunopeptides. Major immunopeptides in beef round were basic and heat stable. An immunopeptide whose molecular weight was approximately 7,000 was purified from 1 kg of beef round.

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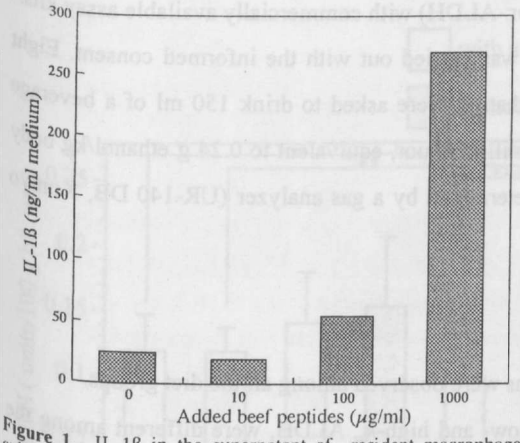

Figure 1. IL-1ß in the supernatant of resident macrophages cultured in serum-free medium containing various concentrations of a beef peptides.

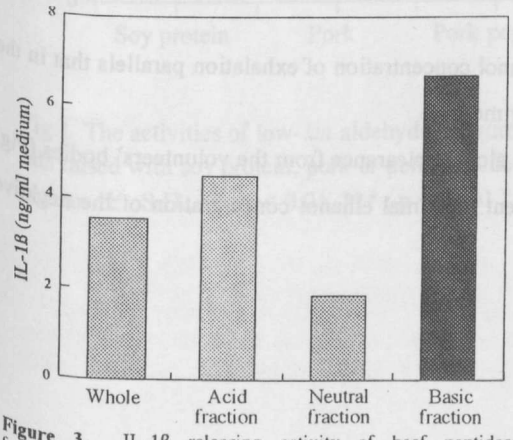

Figure 3. IL-1ß releasing activity of beef peptides fractionated with batch method using SP-Sephadex C-25.

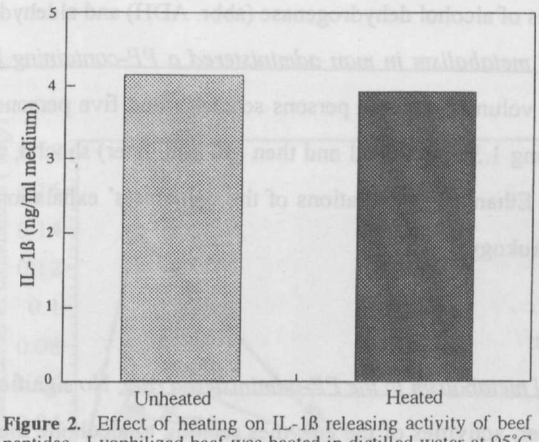

Figure 2. Effect of heating on IL-1ß releasing activity of beef peptides. Lyophilized beef was heated in distilled water at 95°C for 10 min.

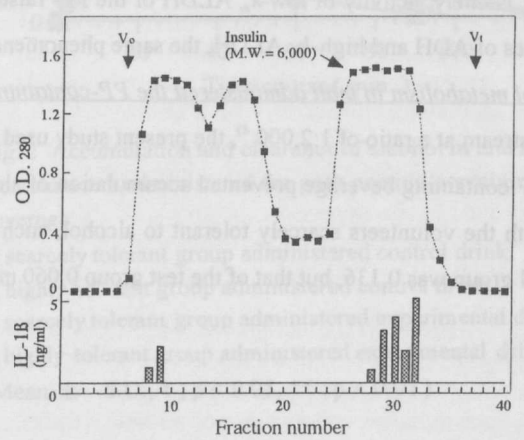

Figure 4. Gel filtration of beef extract on a Sephadex G-50 column; column size: 2.6ø×90 cm, eluent: PBS(-), frow rate: 30 ml/hr, fraction size: 15 ml/tube.