MODULATION OF LEUKOCYTE FUNCTION BY FOOD FACTORS

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1. Introduction
Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine, originally discovered by its antitumor activity, and is believed to play a role in many immunologic and inflammatory reactions. However, its overproduction might cause immunological and inflammatory diseases; for example, prolonged exposure to TNF-α might cause cachexia involving waste. In a bacterial infection, the overproduction of TNF may cause septic shock leading to acute organ failure and death. TNF-α is also a critical mediator in skin diseases such as the contact hypersensitivity reaction. The inhibition of TNF-α overproduction is therefore essential for therapy to cure an inflammatory disease including carcinogenesis.

The leaves of *Perilla frutescens* are used as a garnish with raw fish in Japan. It is believed that the aim of this use is not only as a flavor but also as an antidote to food poisoning. In a previous study, we reported that oral administration of a perilla leaf extract (PLE) to mice can inhibit the overproduction of TNF-α [1] and shows anti-inflammatory and anti-allergic activity [2]. Perilla leaf extract has also been reported to suppress anti-DNP IgE production, Th2-type cytokine production, systemic allergic reaction induced by compound 48/80, and IgA nephropathy [3-6]. However, these are parenteral effects and the active constituents were not well identified. Terpenoids, phenolics, flavonoids, cyanogenic glycosides, and anthocyanins have been reported as the chemical constituents of *Perilla frutescens*, but there has been no suggestion about the oral pharmacological effects of this plant. We investigated here the active constituents which have capability to inhibit TNF-α production, and inflammatory and allergic reactions when administered orally.

2. Results
2.1. INHIBITION OF TNF-α PRODUCTION BY PERILLA LEAF EXTRACT
Dried leaves of a green type of perilla (*Perilla frutescens* (L.) Britton var. acuta Kudo...
**forma viridis** Makino) were soaked in 5 ml of distilled water for 1 h and then homogenized for 10 min with Polytron equipment (Kinematika, Switzerland) at a power setting of 5. The homogenate was filtered through nylon mesh and centrifuged at 7,000 g for 10 min at 4°C. The resulting supernatant was passed through a membrane filter with a 0.45 μ m pore size (Millipore, Tokyo, Japan) and 4.4 ml of perilla leaf extract (PLE) was acquired. This process was repeated many times to gain the sample for isolation.

The oral effect of perilla components on systemic TNF-α production was evaluated by the method previously reported. Briefly, mice were orally administered romurtide (500 μ g/200 μ l/mouse) as a priming agent, and 3 h later were intravenously injected with OK-432 (3KE/200 μ l/mouse) as a triggering agent. Sample was orally administered with romurtide and the effect on TNF-α production was observed. Two hours after triggering, the animals were bled to obtain serum, and the serum was stored at -80°C. The TNF-α activity of the serum was evaluated by the \textit{in vitro} L929 cell cytotoxicity with the method of Ruff and Gifford, using international standard recombinant human TNF-α (National Biological Standards Board, Hertfordshire, U.K.).

In our previous study we found that oral administration of PLE can inhibit systemic TNF-α production and inflammatory and allergic ear edemas. We first investigated whether PLE can inhibit TNF-α production from LPS-stimulated peritoneal macrophage \textit{in vitro}. Glycogen-induced murine peritoneal macrophages were stimulated only by LPS and the TNF-α activity in culture supernatant was 5.5 U/ml. Pretreatment with PLE (2.5-20%) inhibited the TNF-α production in a dose dependent manner.

### 2.2. ACTIVE CONSTITUENT OF PERILLA WHICH CAN INHIBIT TNF-α PRODUCTION

We tried to isolate the active compounds, using the inhibitory activity of the TNF-α production \textit{in vitro} as an index of purification of active components. PLE was concentrated in vacuum with an evaporator and excised PLE was obtained. It was dissolved with water and applied to reverse phase chromatography with MCI gel CHP 20P (28.5 \times 2.9 cm). The column was eluted successively with 890 ml of water (W), 400 ml of 50% methanol (WM), 400 ml of methanol (M), and 400 ml of 50% acetone (WA). Each elution was evaporated and 3.30 g (W), 0.72 g (WM), 0.22 g (M), 0.12 g (WA) of excisation was obtained. In each fraction the dose response of the inhibitory activity of TNF-α production \textit{in vitro} was determined and the ED\textsubscript{50} calculated as the index for isolation. The ED\textsubscript{50} of TNF-α inhibitory activities was 12 μ g/ml (W), 100 μ g/ml (WM), 130 μ g/ml (M) and 350 μ g/ml (WA), respectively. Those fractions were also checked by TLC (silica gel plates in n-butanol: acetic acid: water 2: 1: 1), but it was proved that some constituents were present in these fractions (data not shown). We therefore further fractionated the WM and M fractions which were suggested to contain the active constituent.