Note

Macrophage Stimulating Activity of Foods

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It has long been suggested that some foods have the physiological functions of modulating immune, nerve, and endocrine systems.¹⁾ In the immune system, macrophages are important in phagocytosis, antigen-presentation, and tumor cytotoxicity. To acquire tumor cytotoxicity, macrophages should be activated by stimulants such as lipopolysaccharide. The activated macrophages secrete tumor necrosis factor, interleukin-1 (IL-1), and nitric oxide^{2,3)} as soluble effectors. Nitric oxide is an intermediate in the production of nitrite and nitrate from L-arginine.⁴⁾ In this study, we took the nitrite formation as an index of the macrophage stimulating activity to screen effective foods.

All materials used were obtained from market sources. Each of the materials was freeze-dried and pulverized. The powder was defatted with diethyl ether and the residue (10 g on a dry matter basis) was vigorously shaken with water (20 ml) at 25°C for 1 hr. The suspension was heated up to 60°C to inactivate enzymes. A water extract was obtained by centrifugation of the heat-treated suspension at $10,000 \times g$ for 20 min and then freeze-dried. The dried product (200 mg) was dispersed in 10 mM phosphate-buffered saline (PBS, pH 7.4, 1 ml) to prepare a water-soluble sample. Separately, the defatted residue (10 g on a dry matter basis) was extracted with ethanol (20 ml) at 25°C for 1 hr with mild stirring and then filtered. The filtrate was vacuum-dried. The dried product (200 mg) was dispersed in PBS (1 ml) by sonication to prepare an ethanol-soluble sample. Parts (100 μ l and 10 μ l) of the water-soluble and ethanol-soluble samples were further diluted with PBS (900 μ l and 990 μ l, respectively). Each

dispersion before and after dilution was mixed with a supplemented minimum essential medium (SMEM).⁵⁾ The sample solution was sterilized by passage through a 0.22- μ m filter, before being submitted to the following assays.

A macrophage cell line (RAW 264.7) was obtained from the American Type Culture Collection. The cells were grown on 10-cm plastic tissue culture dishes in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Bocknek Laboratories) at 37°C in a humidified incubator containing 5% CO₂ and 95% air. The macrophage cells were removed from the dishes by vigorous pipetting, and resuspended at a concentration of 1×10^6 cells/ml. Cells were plated at 200 μ l/well, allowed to adhere for 30 min, and then the medium was changed to the sample solution. After 48 hr of incubation nitrite concentrations in the cell culture supernatant were measured by a colorimetric method.⁵⁾ Data were each expressed as an average of three independent measurements with standard deviations.

IL-1 activity was measured by the IL-1 dependent growth of a T helper cell line (D10.G4.1).⁶⁾ Tumor-toxic activity was evaluated as follows. Mastcytoma P815 cells as the target of macrophages were supplied by the Japanese Cancer Research Resources Bank (JCRB). The P815 cells (1×10^5 cells/ml) were cultured with the macrophage cells (1×10^6 cells/ml) in the sample solution. After 48 hr of incubation ³H-thymidine (9 KBq/ml) was added to the culture and the cells were incubated for another 6 hr. The surviving P815 cells were harvested, and incorporated radioactivity was measured. A blank test was done by a similar procedure in the absence of macrophage cells. Tumortoxicity was expressed as the percent of inhibition of the thymidine incorporation into the target cells as follows:

$$\% \text{ inhibition} = 100 \times (1 - A_m/A_0)$$

where A_m and A_0 are the incorporated radioactivity in the presence or absence of macrophage cells, respectively.

Table I shows that the water extracts from 18 foods stimulated macrophages to produce nitrite. No stimulant was found in any ethanol extracts; water extracts *per se* did not contain nitrite. The result in Table I also indicates that foods originating from a wide range of species, particularly seeds, contain macrophage stimulants. Among the 18 foods investigated, corn, buckwheat, soybeans, chrysanthemum, garlic, tea, and bracken had higher activities than lipopolysaccharide of 10 μ g/ml. When a food contained stimulants, another one belonging to the same family had a high possibility of also containing stimulants.

The 18 water extracts (200 μ g each on a dry matter basis) containing macrophage stimulants (Table I) were dialyzed in running water to obtain their non-diffusible fractions. Each of the fractions was freeze-dried and then dissolved in PBS (1 ml). The solution was tested by the nitrite formation assay as described. Table II shows that some foods contained non-diffusible stimulants and others lost stimulants by dialysis. The foods with higher nitrite-

Table I. MACROPHAGE STIMULATING ABILITIES^a OF WATER EXTRACTS FROM FOODS

Family	Food	Part	Concentration of extract ($\mu\text{g/ml}$)	Nitrite formation ($\text{nmol}/10^6$ cells)
Gramineae	Rice	Seed	2000	43.9 ± 0.9
			200	42.3 ± 2.4
			20	30.6 ± 0.7
	Wheat	Seed	2000	13.9 ± 2.4
			200	ND ^b
	Corn	Seed	2000	80.7 ± 0.6
200			ND	
Polygonaceae	Buckwheat	Seed	2000	80.7 ± 0.6
			200	39.0 ± 2.9
			20	ND
Leguminosae	Soybeans	Seed	2000	71.0 ± 5.1
			200	10.0 ± 1.6
			20	ND
Cucurbitaceae	Kidney bean	Seed	2000	ND
	Peanut ^c	Seed	2000	ND
	Cucumber	Fruit	2000	ND
Solanaceae	Pumpkin	Fruit	2000	ND
	Tomato	Fruit	2000	ND
Solanaceae	Eggplant	Fruit	2000	ND
	Potato	Subterranean stem	2000	ND
	Red pepper	Fruit	2000	ND
	Rosaceae	Strawberry	Fruit	2000
Rosaceae	Apple	Fruit	2000	ND
	Almond ^c	Seed	2000	ND
	Nymphaeaceae	Lotus		2000
Moringeaceae	Radish	Root	2000	ND
	Cabbage	Leaf	2000	59.6 ± 1.9
			200	ND
Umbelliferae	Carrot	Root	2000	ND
	Celery	Stem	2000	33.1 ± 6.2
			200	ND
	Parsley	Leaf	2000	15.7 ± 0.4
200			ND	
Compositae	Burdock	Root	2000	ND
	Chrysanthemum	Leaf	2000	82.2 ± 3.4
			200	67.3 ± 1.9
			20	16.2 ± 1.1
	Lettuce	Leaf	2000	62.1 ± 4.4
			200	14.6 ± 0.4
Fagaceae	Chestnut	Seed	2000	ND
Moraceae	Fig	Fruit	2000	ND
Actinidiaceae	Kiwi fruit	Fruit	2000	ND
Votaceae	Grape	Fruit	2000	ND
Ebenaceae	Persimmon	Fruit	2000	ND
Liliaceae	Garlic	Bulb	2000	72.0 ± 1.3
			200	31.7 ± 3.9
			20	ND
	Onion	Bulb	2000	ND
	Leak	Leaf	2000	15.4 ± 0.7
			200	ND
	Asparagus	Sprout	2000	22.3 ± 4.5

Table I. (continued)

Family	Food	Part	Concentration of extract ($\mu\text{g/ml}$)	Nitrite formation (nmol/ 10^6 cells)
Stemonaceae	Yam	Root	2000	ND
Araceae	Taro	Root	2000	ND
	Devil's tongue	Root	2000	ND
Chenopodiaceae	Spinach	Leaf	2000	ND
Convolvulaceae	Sweet potato	Root	2000	ND
Pedaliaceae	Sesame	Seed	2000	7.9 ± 1.1
			200	ND
Piperaceae	Pepper	Seed	2000	24.7 ± 0.3
			200	21.2 ± 2.6
			20	ND
Labiatae	Beefsteak plant	Leaf	2000	ND
Zingiberaceae	Ginger	Root	2000	ND
	Turmeric	Rhizome	2000	ND
Ternstroemiaceae	Tea ^c	Leaf	2000	102.3 ± 6.5
			200	61.5 ± 0.6
			20	ND
Rubiaceae	Coffee ^c	Seed	2000	ND
Sterculiaceae	Cacao ^c	Seed	2000	ND
Rutaceae	Grapefruit	Fruit	2000	ND
Ginkgoales ^d	Ginkgonut	Seed	2000	ND
Lauraceae	Avocados	Fruit	2000	ND
Anacardiaceae	Mango	Fruit	2000	22.6 ± 0.4
			200	ND
	Cashewnut ^c	Seed	2000	ND
Caricaceae	Papaya	Fruit	2000	ND
Bromeliaceae	Pineapple	Fruit	2000	ND
Musaceae	Banana	Fruit	2000	ND
Osmundales ^d	Osmund fern	Stem	2000	ND
Filicales ^d	Bracken fern	Stem	2000	81.7 ± 3.7
			200	61.8 ± 2.2
			20	ND
Agaricales ^d	Shimeji-mushroom	Fruit body	2000	ND
	Common mushroom	Fruit body	2000	ND
Bangiales ^d	Asakusa laver	Leaf	2000	ND
Laminariales ^d	Kelp	Leaf	2000	ND
	Wakame seaweed	Leaf	2000	ND
Fucales ^d	Hijiki seaweed	Leaf	2000	ND
Chlorococcales ^d	Chlorella	Cell	2000	ND

^a Activity of lipopolysaccharide was 70 ± 3.5 at $10 \mu\text{g/ml}$.

^b Not detected.

^c Heated.

^d Order.

producing activity generally contained non-diffusible stimulants. The same samples were further measured as to IL-1 secretion and the tumor-toxic activity against P815 cells. IL-1 is one of the cytokines secreted from activated macrophages and accelerates the immune response through T cell activation. The results in Table II indicate that all the samples did not necessarily stimulate the macrophage cells for sufficient IL-1 secretion and

for distinct damage to tumor cells.

Collating all the data obtained in this work, we conclude that macrophage stimulants exist in a wide variety of foods and that they are soluble in water and stable against heat treatment. It is also concluded that several of the stimulants activate macrophage cells to a higher stage to induce secretion of IL-1 and the exhibition of tumor-toxic activity.

Table II. CHARACTERISTICS OF MACROPHAGE STIMULANTS FROM FOODS

Food	Stimulating activity of non-diffusible fraction	IL-1 excretion (Mean Δ cpm) ^a	Tumortoxicity (% inhibition)
Rice	+ ^b	2472 ± 510	99.7
Wheat	- ^c	382 ± 45	83.7
Corn	-	97 ± 15	81.8
Buckwheat	+	725 ± 153	80.6
Soybeans	+	1565 ± 277	96.0
Cabbage	+	0 ± 17	0
Celery	-	0 ± 20	91.8
Parsley	-	330 ± 183	32.5
Chrysanthemum	+	280 ± 22	3.2
Lettuce	+	305 ± 143	95.7
Garlic	-	277 ± 40	0
Leek	-	222 ± 23	0
Asparagus	-	907 ± 465	0
Sesame	-	172 ± 85	90.5
Pepper	+	192 ± 85	51.6
Tea	+	210 ± 490	0
Mango	-	140 ± 258	92.6
Bracken	+	1420 ± 193	98.6
LPS ^d		11670 ± 1037	99.9

^a Mean cpm of ³H-thymidine incorporation when the T cells were incubated with 10% culture supernatant; cpm of negative control was 1153 ± 97.

^b Active.

^c Inactive.

^d Positive control.

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