MACROPHAGE STIMULATING ACTIVITY OF MEATS AND ORGANS

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Macrophages, when stimulated with lipopolysaccharide, produce a copious amount of intermediate nitric oxide from L-arginine through an enzymatic process (Marletta,1988: Iyenger et al.,1987) , and the oxide then reacts spontaneously with molecular oxygen and water to yield nitrite and nitrate as final products (Marletta,1988: Stuehr et al.,1985). When reactive amino compounds co-exist in the system, N-nitroso compounds are also formed as final products (Miwa et al.,1989:1987). It has been proposed that nitritc oxide is one of the most important effectors for macrophage tumorcidal activity (Feldman et al., 1993, Hibbs et al., 1988,1987a,1987b). For this reason we have used nitrite formation as an index of macrophage stimulation, and reported that macrophage stimulants existed in a wide variety of plants (Miwa et al., 1990). Here we report the results of screening for macrophage - stimulating activity in meats and organs, and characterization of active compounds in swine stomach.

MATERIALS AND METHODS

Meats and organs. Meats and organs used in this experiment were prepared from cattle, swine and chicken which were slaughtered at National Institute of Animal Industry. After fat and connective tissue were removed, each of the materials was cut into small pieces. They were homogenized with an equal weight of water and centrifuged at 11,000 x g for 15 min. The supernatant was then freeze-dried . Ten water extracts containing macrophage stimulants were dialysed against distilled water to obtain their non-diffusible fractions. Each of the fractions was then freeze-dried.

Cells and medium. A macrophage cell line (RAW 264.7) was obtained from the American Type Culture Collection. The cells were grown in Dulbecco's modified Eagle medium supplemented with 10 % fetal calf serum (Bocknek Laboratories) at 37℃ in a humidified incubator containing 5 % C02 and 95 % air.

Nitrite formation assay . The freeze-dried samples were each dispersed in the supplemented MEM (Miwa et al.,1989) and stirred for 30 min. After centrifugation, the supernatant was sterilized by passage through a 0.22 μ m filter. The cultured macrophages were removed from the dishes by vigorous pipetting and resuspended at a concentration of 1 x 10^6 cells / ml. Cells were plated at 200 μl/ well, allowed to adhere for 30 min, and then the medium was changed to the sample in the supplemented MEM. After 48 h of incubation, nitrite concentration in the culture supernatant was determined by a colorimetric method (Miwa et al.1989). Data were each expressed as an average of three independent measurements .

RESULTS AND DISCUSSION

Table I shows macrophage stimulation activity of meats and organs. In beef and cattle organs, only reticulorumen and cerebrum (20.0 and 2.0 mg / ml) stimulated macrophages to produce nitirite. Water extracts per se did not contain nitrite. The nitrite production by lipopolysaccharide (10 μg/ml) was 66 nmol / 10^6 cell. In the case of pork and swine organs, six of 16 water extracts had the activity. Among the six, stomach and tongue had high activity. They stimulated macrophages at a concentration more than 2.0 mg /ml. Table 1 also shows that chicken and chicken gizzard had the macrophage stimulation activity. Chicken had the highest activity among the 38 materials investigated in this experiment and the nitrite production was 11.4 nmol / 10^6 cells at a concentration of 0.2 mg / ml. In this experiment, we found that ten water extracts from three kinds of meats and 35 organs had macrophage stimulating activity. These ten extracts containing macrophage stimulants were dialyzed against distilled water. Some of them lost the activity by dialysis and the others contained non-diffusible stimulants. The non-diffusible stimulants in swine cerebrum, swine cerebellum and chicken, chicken gizzard had higher activity than the activity before dialysis.

In this report, it is interesting that any of three stomach (cattle reticulorumen, swine stomach and chicken gizzard) had non-diffusible stimulants with high activity (Table I) . Therefore, we tried to characterize the active compounds in swine stomach. The active compounds were partially purified using ammonium sulfate precipitation and DEAE-sepharose column chromatography. The activity was resistant to pronase treatment, but was sensitive to periodic acid treatment and condroitinase treatment. According to these results, the active compound is suggested to be a kind of saccharide compounds.

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Table 1. Macrophage Stimulating Activities of Water Extracts from Meats and Organs

Part	Concentration (mg/ml)	Nitrite (nmol/10^6 cells) Cattle	Swine	Chicken
Meat	20	-	+	68.2
	2	-	-	42.6
	0.2	-	-	11.4
Stomach	20	43.9	32.1	53.1
	2	6.9	19.5	6.7
	0.2	-	3.7	-
Heart	20	-	-	-
Liver	20	-	-	-
Kidney	20	-	-	-
Spleen	20	-	-	
Adrenal grand	20	-	-	
Bile	20	-	-	
Pancreas	20	-	-	
Lung	20	-	-	
Large intestine	20	-	-	
Small intestine	20	-	-	
Uterine	20	-	14.0	
Cerebrum	20	19.1	38.9	
	2	3.7	-	
Cerebellum	20	-	18.1	
Eyeball	20	-	32.1	
	2	-	19.1	
Udder	20	-		