Appearance of Reddish Purple-Stained Cells (ARPC) Induced by Sodium Alginate in Mice

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Abstract: *To determine the optimal conditions for collecting unidentified cells, named alginate-induced reddish purple-stained cells (ARPC), sodium alginate, a heteropolyuronate derived from brown algae, was injected intraperitoneally. In the present study it was investigated if sodium alginate induced cell accumulation in the mammalian mouse peritoneal cavity. Intraperitoneal injection of sodium alginate in ddY mice resulted in great accumulation of cells that were stained reddish purple with Wright/Giemsa, together with leukocytes including neutrophils, macrophages and lymphocytes. Accumulation of the alginate-induced reddish purple-stained cells (ARPC) was found to be maximam at a dose of 10 mg/mouse. This phenomenon was not strain-specific but also observed in ICR, C57BL/6, AKR/J and BALB/c mice. Intraperitoneal injection of sodium alginate solution containing the optimal dose (10 mg/animal) induced accumulation of ARPC in mice.*

Key words: *Sodium alginate, Mice, Peritoneal cavity, Reddish purple cells (ARPC)*

It is well known that the mouse peritoneal cavity contains a set of hematopoietic cells (lymphocytes, neutrophils, eosinophils, macrophages and mast cells). These cells, which always exist in the cavity, are called resident peritoneal cells.

On the other hand, when a certain substance is injected into the peritoneal cavity, mobilization of leukocytes including neutrophils, eosinophils and macrophages into the cavity occurs immediately. These cells are named peritoneal exudate cells (PEC) and the substance is called peritoneal exudate cell-inducing substance. Many peritoneal exudate cell-inducing substances for mice, containing casein sodium [1], glycogen [2], polypeptone [3], thioglycollate broth and proteose peptone, have been developed.

The significance of injecting sodium alginate into the abdominal cavity of mice has been reported in the observation of various reactions to the immunoresponse of the living body [5].

The PEC are inflammatory leukocytes whereas the resident cells are leukocytes in a steady state. There are some differences between the expression patterns of cell surface antigens and cellular characteristics of these two peritoneal leukocyte populations. These differences are also seen between PEC induced by different agents.

Recently it was found that sodium alginate, which is a heteropolysaccharide composed of 1,4-linked mannuronate and guluronate in a block fashion [4], has the potency to elicit dynamic accumulation of leukocytes in the carp (*Cyprinus carpio* L.) peritoneal cavity when injected intraperitoneally [5]. It seemed very interesting to investigate whether or not the same phenomenon occurs in mammalian mice. The aim of this experiment is to determine the optimal conditions for collecting the unidentified cells, named alginate-induced reddish purple-stained cells (ARPC).

Materials and Methods

Reagents and media

Sodium alginate (low viscosity) was purchased from Sigma Chemical Co. (St. Louis, Mo). RPMI-1640 was obtained from Nissui Pharmaceutical Co. (Tokyo, Japan). Phosphate buffer was prepared by diluting 1 volume of Sfhrensen's phosphate buffer (pH 6.8) with 9 volumes of deionized water. Fetal bovine serum (FBS) was purchased from Nalge Nunc International (Tokyo, Japan) and used after heat inactivation at 56°C for 30 min. Wright solution and Giemsa solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Merck (Darmstadt, Germany), respectively. Eukitt and HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid)

Received: June 1, 2000
Accepted: August 31, 2000
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were obtained from O. Kindler (Germany) and Dojindo Laboratories (Kumamoto, Japan), respectively.

Animals

Female ddY, ICR, BALB/c, AKR/J and C57BL/6 mice, 7 weeks old, were purchased from Seak Yoshitomi Ltd. (Fukuoka, Japan) and acclimated for a week to laboratory conditions at 22°C in a polycarbonate cage with wood shaving. During acclimation and the experiments, mice were fed commercial pellets.

Collection of cells

Sodium alginate was dissolved in saline (0.85% NaCl) at 10 mg/ml and autoclaved. The Mice were anaesthetized with ether and injected intraperitoneally with 1 ml of the sodium alginate solution. At various times after the injection, the mice were killed under ether anaesthesia, and 5 ml ice-cold RPMI-1640 containing 25 mM HEPES (RPMI-1640-H) was intraperitoneally injected. The abdomen was massaged for 30 sec and the peritoneal fluid was collected in a polypropylene syringe with a 21 gauge needle. The PEC suspension was centrifuged in a 15 ml tube (Falcon 2096) at 1,300 rpm for 5 min at 4°C, and the pellet was washed and suspended in 1 ml RPMI-1640-H.

Counting and staining of collected cells

An aliquot (15 μl) of the collected peritoneal cell suspension was mixed with 0.3% trypan blue saline solution and subjected to cell counting in a Thoma's hemocytometer. Another aliquot containing 1–2 × 105 cells was added to 1 ml of RPMI-1640-H and deposited on a glass slide by means of a cytocentrifuge (SC-2, Tomy Seiko Co. Ltd., Tokyo, Japan). The cell preparation was air-dried, fixed with undiluted Wright solution for 1 min, stained with diluted Wright solution (Wright solution: phosphate buffer = 1: 1) for 10 min and then washed with deionized water. After being air-dried, the cells were counter-stained with Giemsa solution (1 portion/ml-phosphate buffer) for 10 min, washed with deionized water, air-dried and mounted in Eukitt-xylene. The number of leukocytes in a subpopulation was determined by multiplying the total number of PEC by the composition rate of the subpopulation.

Results and Discussion

Dose response of ARPC after sodium alginate injection

In a preliminary experiment, the injection of sodium alginate into mice elicited accumulation of peritoneal exudate leukocytes including neutrophils, eosinophils, macrophages and lymphocytes as in carp. Interestingly, unidentified cells that were stained reddish purple with Wright/Giemsa stain appeared together with these leukocytes.

A group of 5 ddY mice were injected with 0, 1, 5, 10 and 20 mg/mouse of sodium alginate, and 1 day later PEC were collected and the number of ARPC was determined. As shown in Fig. 1, the number of ARPC increased along with the increase in the injection dose in the range of 1 to 10 mg/mouse, but it decreased at the dose of 20 mg/mouse. These results indicate that 10 mg/mouse is about the optimal dosage and an excess dosage of more than 20 mg/mouse would result in lower accumulation of ARPC.

Changes in the number of ARPC after sodium alginate injection

In order to examine the fluctuation in the number of ARPC after sodium alginate injection, 50 ddY mice were injected with 10 mg/mouse of sodium alginate. Control mice (50) received saline alone (1 ml/mouse). Five mice from each group were sacrificed before injection, 1–5 days, 1, 3 and 6 month after the injection, and the number in each subpopulation in the PEC of each individual mouse was determined.

The number of ARPC reached a peak (1 × 10^7 cells/mouse) 3 days after the injection of sodium alginate,

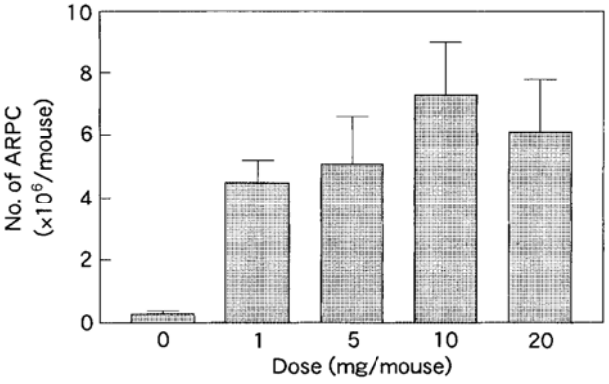

Fig. 1. Dose response of sodium alginate-induced reddish purple-stained cells (ARPC) in ddY mice to sodium alginate injection. Mice were injected intraperitoneally with sodium alginate (1–20 mg/mouse) or saline (0.85% NaCl) alone, and 24 h later the peritoneal exudate cells were collected, counted and stained with Wright/Giemsa. The number of ARPC was calculated by multiplying the number of peritoneal exudate cells by the composition rate of ARPC which was determined by morphological examination of the stained sample. Data represent the mean + S.E. for five mice.

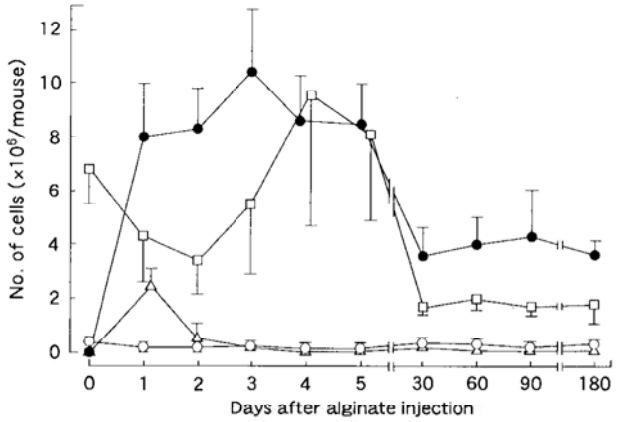

Fig. 2. Changes in the number of peritoneal exudate cells (PEC) after sodium alginate injection in ddY mice. The mice were injected intraperitoneally with sodium alginate (10 mg/mouse) and 1 day to 6 months later, PEC were collected, counted and stained with Wright/Giemsa. The number in each hematopoietic cell subpopulation was calculated by multiplying the total number of PEC by the composition rate of the subpopulation which was determined by morphological examination of the stained samples. ●, alginate-induced reddish purple-stained cells (ARPC); □, mononuclear cells; △, granulocytes; ○, connective tissue type mast cells. Data are the means and S.E. for five mice.

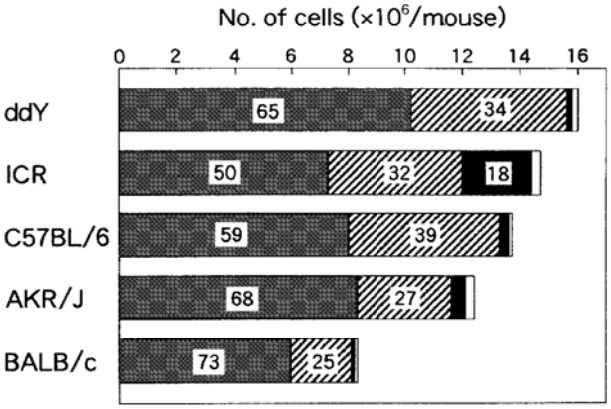

Fig. 3. Number and composition of sodium alginate-induced reddish purple-stained cells (ARPC) in various mouse strains. Mice were injected intraperitoneally with sodium alginate (10 mg/mouse), and 3 days later peritoneal exudate cells (PEC) were collected, counted and stained with Wright/Giemsa. The number in each hematopoietic cell subpopulation was calculated by multiplying the total number of PEC by the composition rate of the subpopulation determined by morphological examination of the stained sample. ■; ARPC, □; mononuclear cells, ■; granulocytes, □; connective tissue type mast cells. The number inside the graph is the mean composition percentage in the PEC (n=5).

thereafter gradually decreasing till 1 month after the injection, but it remained at the same level (about 40% of the peak) for up to 6 months (Fig. 2). On the other hand, the number of mononuclear cells (MNC) composed of macrophages and lymphocytes decreased for the first 2 days, but increased thereafter till 4 days after the injection, and decreased again to about 1/3.5 of the number before injection during the period of 1 to 6 months after the injection. Granulocytes (neutrophils and eosinophils) increased 1 day after the injection but they returned to the initial level later than 2 days after the injection. The number of connective tissue type mast cells (CTMC) was not greatly changed throughout the experiment. In control mice that received saline alone, there was no noticeable change in the number in any subpopulation throughout the experiment.

Induction of ARPC in various mouse strains

Two closed colonial strains (ddY and ICR) and three inbred strains (C57BL/6, AKR/J, BALB/c) were injected intraperitoneally with sodium alginate (10 mg/mouse), and 3 days later PEC were collected and the number and composition ratio of each cell subpopulation were determined. As shown in Fig. 3, accumulation of ARPC was elicited in all strains of mice examined and the mean number of ARPC was the highest in ddY mice and the mean composition ratio of ARPC was the highest in BALB/c mice.

There was a tendency to more PEC accumulation in closed colonial strains than in inbred strains. This is probably because closed colonial strain mice were larger than inbred strain mice.

Acknowledgments

We are very grateful to Dr. M. Matsuyama, College of Agriculture, Kyushu University, Fukuoka, Japan for his kind help.

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