Garlic  (*Allium sativum*) belongs to the Allium family of plants, which includes onions, leeks and chives, and it has been consumed as a spice and a medical herb since early times ([1–3]). Garlic contains water (~65%), carbohydrates (~30%), organosulfur compounds (~5%), small amounts of minerals (phosphorus, selenium, zinc, calcium, magnesium, sodium, iron, etc.), and vitamins (A, C, B-complex, etc.) ([4]). In constituents, the organosulfur compounds are composed of alliin, allicin, dithiins, sulfi des, ajoenes, S-allyl cysteine, and S-allyl mercaptocysteine. In addition, allicin, responsible for the characteristic pungent flavor of garlic, is converted to oil-soluble organosulfur compounds, such as diallyl disulfi de (DADS), diallyl sulfide (DAS), diallyl trisulfi de (DATS), dithiins, and ajoenes ([2, 5, 6]).

Among them, DADS is a major oil-soluble organosulfur compound, and exerts a variety of biological functions, including anti-inflammatory, immune-modulatory, and enhancing sympathetic nerve activity effects ([7–10]). Actually, an in vitro study revealed that DADS repressed the LPS-stimulated production of IL-10 in macrophages and this led to inhibition of interferon-γ-stimulated nitric oxide production from them ([11]). Thus, DADS has anti-inflammatory effects. In addition, DADS alters immune responses by promoting the expression and clustering of leucocyte function-associated antigen-1 (LF A-1) and increased LF A-1-mediated adhesion of monocytes ([12]). On the other hand, an in vivo study demonstrated that DADS administration increased the counts of total white blood cells (WBCs), including antibody producing cells, in the spleen, accompanied with increased weights of thymus and spleen ([8]), indicating that DADS promotes delivery of immune cells to such lymphoid tissues.

However, it still remains unclear how DADS affects the distribution of white blood cell subsets, which is essential to execute effective immune responses and partially regulated by adrenal glucocorticoids. Therefore, we examined the dose-dependent effects of DADS administration on the circulating number of white blood cells (WBCs) and lymphocyte subsets, and plasma corticosterone concentration in rats. Male 10-wk-old Sprague Dawley rats were divided into the DADS-free and DADS-orally administered (dose = 10, 20, and 40 mg/kg BW) groups. Blood samples were collected from the tail vein at 0, 1, 2, 4, and 6 h after the administration. DADS administration decreased dose- and time-dependently the circulating number of total WBCs, total lymphocytes, and monocytes. Within the lymphocyte subsets, the circulating number of T-lymphocytes and B-lymphocytes was significantly reduced 4 h after DADS administration in a dose-dependent manner, although that of natural killer (NK) cells was not affected. On the other hand, although DADS administration did not significantly change the circulating number of neutrophils, the circulating number of eosinophils and basophils showed a decreasing tendency after DADS administration. In contrast, plasma corticosterone concentration was increased 2 h after DADS administration in a dose-dependent manner. These results suggest that DADS administration reduces the circulating number of monocytes and lymphocytes, including especially acquired immune cells, via the action of corticosterone, and the effects are induced in a dose-dependent manner.

**Key Words**  diallyl disulfide, T-lymphocytes, B-lymphocytes, monocytes, corticosterone

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*Note*

**Diallyl Disulfide Reduced Dose-Dependently the Number of Lymphocyte Subsets and Monocytes in Rats**

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**Summary**  Diallyl disulfide (DADS) is a major sulfur compound of garlic, and exerts anti-inflammatory, immune-modulatory, and enhancing sympathetic activity effects. However, it still remains unclear how DADS affects the distribution of white blood cell subsets, which is essential to execute effective immune responses and partially regulated by adrenal glucocorticoids. Therefore, we examined the dose-dependent effects of DADS administration on the circulating number of white blood cells (WBCs) and lymphocyte subsets, and plasma corticosterone concentration in rats. Male 10-wk-old Sprague Dawley rats were divided into the DADS-free and DADS-orally administered (dose = 10, 20, and 40 mg/kg BW) groups. Blood samples were collected from the tail vein at 0, 1, 2, 4, and 6 h after the administration. DADS administration decreased dose- and time-dependently the circulating number of total WBCs, total lymphocytes, and monocytes. Within the lymphocyte subsets, the circulating number of T-lymphocytes and B-lymphocytes was significantly reduced 4 h after DADS administration in a dose-dependent manner, although that of natural killer (NK) cells was not affected. On the other hand, although DADS administration did not significantly change the circulating number of neutrophils, the circulating number of eosinophils and basophils showed a decreasing tendency after DADS administration. In contrast, plasma corticosterone concentration was increased 2 h after DADS administration in a dose-dependent manner. These results suggest that DADS administration reduces the circulating number of monocytes and lymphocytes, including especially acquired immune cells, via the action of corticosterone, and the effects are induced in a dose-dependent manner.

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the distribution of each white blood cell subset and its immune-endocrine regulatory mechanism. The distribution of each lymphocyte and granulocyte subset is essential for the immune responses, because the continuous migration of immune cells ensures the detection of antigens and neoplasms, and promotes cellular interactions that enable the immune system to execute rapid and effective responses (13–15). Furthermore, the distribution of WBCs is regulated by adrenal glucocorticoids (16). Therefore, we examined the dose-dependent effects of DADS administration on the circulating number of WBCs, such as lymphocytes (T-lymphocytes, B-lymphocytes, and NK cells), granulocytes (neutrophils, eosinophils, and basophils) and monocytes, and plasma corticosterone concentration was assayed by ELISA 2 h after the administration of DADS.

**Materials and Methods**

**Experimental procedure and animal care.** The experimental protocol used in the present study is shown in Fig. 1. Male 10-wk-old Sprague Dawley rats (CLEA Japan, Inc., Tokyo, Japan) were pre-fed for 3 d to allow adaptation to a new environment. All rats were housed in stainless steel cages at a controlled temperature (23–25°C) and relative humidity (50–60%). Lighting was automatically provided from 08:30–20:30. Animal chow (CE-2 cubic type, CLEA Japan, Inc.) and distilled water were given to the rats ad libitum (17). After the adaptation period, the rats were randomly divided into the DADS-free and DADS-orally administered (dose = 10, 20, and 40 mg/kg BW) groups (n = 8–9/group).

The present study (2011-A093) was approved by the Animal Ethics Committee, Waseda University, and conducted according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, the Physiological Society of Japan (18). The experiment was performed with the least possible pain or discomfort to rats.

**Oral administration of DADS.** DADS (99.5% purity, LKT Lab., Inc., St Paul, MN, USA) was dissolved in 2% ethanol, and then supplemented with 0.9% NaCl solution containing 10% Tween 80 as a vehicle to obtain 0.5, 1.0, and 2.0% DADS solution. Then, 0.5, 1.0, and 2.0% DADS solution were administered orally as the dosage of 10, 20, and 40 mg/kg BW to DADS-orally administered rats, respectively. The dosage of DADS was used according to the report of Munday and Munday (19). An equivalent volume of the vehicle was administered to the DADS-free rats instead of DADS solution in the same manner.

**Count analyses of WBCs.** Fifty microliters of the whole blood sample was immediately prepared with a microsyringe and diluted by 20% with cellpack, a whole blood diluent for use in a hematology analyzer (Sysmex Co., Hyogo, Japan) (15), and then the number of WBCs (total WBCs, total lymphocytes, monocytes, neutrophils, eosinophils, and basophils) was analyzed by a hematology analyzer (Model SF-3000, Sysmex Co.) based on a flow-cytometry technique with a light-emitting diode (15).

**Count analyses of lymphocyte subsets.** After 4 h from the administration of DADS, the number of T-lymphocytes, B-lymphocytes, and NK cells were determined by a direct immunofluorescent staining with a flow-cytometric analysis according to our routine methods (20). We used the IOTest Anti-Rat CD3-FITC/CD45RA-PC7/CD161a-APC (Beckman Coulter, Brea, CA, USA). Twenty five microliters of the whole blood sample was incubated with 25 μL of the primary antibody for 20 min at room temperature in the dark. Then, 1 mL of BD FACS lysing solution (Becton Dickinson, Franklin Lakes, NJ, USA) was added to the sample, and incubated for 5 min. After the centrifugation at 1,500 × g for 3 min at 4°C, the supernatant was discarded and replaced with 1 mL of phosphate-buffered saline (PBS, pH 7.4). After the filtration with Cell Strainer (a strong nylon mesh with 40 micron pores, Becton Dickinson), the cells were analyzed using FACS Calibur (Becton Dickinson).

**Assay of plasma corticosterone concentration.** Plasma corticosterone concentration was assessed using an enzyme-linked immunosorbent assay (ELISA) (YK 240 Corticosterone EIA, Yanaihara Institute Inc., Shizuoka, Japan) (20).

**Statistical analyses.** All data are presented as means ± standard error of the mean (SE). The effects of DADS administration on the circulating number of WBCs were analyzed by two-way ANOVA for repeated measures, and then estimated using Tukey-Kramer multiple comparison tests. The effects of DADS administration on the circulating number of lymphocyte subsets and plasma corticosterone concentration were analyzed by one-way ANOVA, and then estimated using Tukey-Kramer multiple comparison tests. Differences were considered significant when p was <0.05.
**Results**

**Dose-dependent effects of DADS administration on the number of WBCs**

When 20 and 40 mg/kg BW of DADS were orally administered to rats, the circulating number of total WBCs, total lymphocytes, and monocytes was gradually reduced along with the time course as compared with the values in the DADS-free rats, and the differences between the DADS-administered groups and the DADS-free group become statistically significant from 4 h after the administration (Fig. 2). In addition, dose-dependency of DADS administration effects was observed in the doses of 20 and 40 mg/kg BW but not in the dose of 10 mg/kg BW (Fig. 2). On the other hand, although the circulating number of neutrophils was not significantly changed by the administration of the different doses of DADS, the circulating number of eosinophils and basophils showed a decreasing tendency after DADS administration (Fig. 2).

**Dose-dependent effects of DADS administration on the number of lymphocyte subsets**

As shown in Fig. 3, when 20 and 40 mg/kg BW of DADS were orally administered to rats, the circulating number of T-lymphocytes and B-lymphocytes was dose-dependently reduced 4 h after the administration, although such effects were not affected by the administration of 10 mg/kg BW of DADS. On the other hand, the circulating number of NK cells was not affected by the administration of the different doses of DADS (Fig. 3).
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Dose-dependent effects of DADS administration on plasma corticosterone concentration

When 20 and 40 mg/kg BW of DADS were orally administered to rats, the plasma concentration of corticosterone was dose-dependently increased 2 h after the administration, but not affected by the administration of 10 mg/kg BW of DADS (Fig. 4). These results indicate that the circulating number of T-lymphocytes, B-lymphocytes, and monocytes, and plasma corticosterone concentration were inversely changed in response to DADS administration.

Discussion

DADS, a pungent compound of garlic, binds to and activates transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential vanilloid 1 (TRPV1) (21, 22). These ionic channels are expressed in skin and primary sensory neurons. Activation of TRPA1 and TRPV1 in sensory neuro-fibers leads to noradrenalin and primary sensory neurons. Activation of TRPA1 and one concentration increased dose-dependently plasma corticosterone concentration 2 h after the administration (Fig. 4). These results suggest that DADS administration stimulated corticosterone secretion via the activation of the HPA axis, resulting in reduction of the circulating number of T-lymphocytes, B-lymphocytes, and monocytes.

In conclusion, DADS administration reduces the circulating number of monocytes and lymphocytes, including especially acquired immune cells, via the action of corticosterone, and the effects are induced in a dose-dependent manner.

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