Inhibition of Infectious Diseases by Components from *Aloe Vera*

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The ability to eliminate *Escherichia coli* K-12 from the peritoneal cavity in the early stage of infection (48 h) was improved by the pre-administration of an aloe sample to BALB/c mice. Our results suggest that the aloe sample could inhibit infectious diseases by stimulating the host defense mechanism, especially the phagocytic and killing activities of macrophages.

**Key words:** Aloe vera; intestinal infection; innate immunity; macrophage

Gastrointestinal infection caused by such pathogenic bacteria as Salmonella which have entered the human intestinal tracts induce a critical condition that have become an annual occurrence in Japan.1,2) These infections are a major social problem, since they occur suddenly and collectively, and disease control measures have not decreased the problem in the last few decades. Although antibiotics are currently used for the medical treatment of such infections, the use of these medicines is becoming less effective in view of the increasing antibiotic resistance of bacteria. It is therefore necessary to establish a method for preventing intestinal infectious diseases as soon as possible.

*Aloe vera* has been used to treat burns and other wounds for more than 2,000 years.3,4) Several polysaccharides in the internal gel of *Aloe vera* are considered to be responsible for its wound and burn-healing, anti-inflammatory, anti-tumor and immunostimulatory properties. *Aloe* polysaccharides, especially acemannan, may enhance wound healing by activation of macrophages, which produce growth factors that influence wound repair.5,6) The major carbohydrate fraction isolated from *Aloe vera* gel, acemannan, consists of β-1,4-linked polydispersed, highly O-acetylated mannans with an average molecular weight of approximately 1,000 kDa. Acemannan may exert the following pharmacological effects: antiviral activity,7) antibacterial activity,8) the ability to activate macrophages,9) stimulation of T cells,10) reducing radiation-induced skin reactions and accelerating wound healing.11) However, the effects of the *Aloe vera* gel components on intestinal infectious diseases have not previously been reported.

We investigate in this study the effects of the soluble components contained in *Aloe vera* on infectious diseases. We examined the effect of an aloe sample on the non-specific defense system of macrophages in experiments with *Escherichia coli* K-12 (*E. coli* K-12) to elucidate the mechanism for protection against bacterial infection.

*Aloe vera* leaf pulp boiled in water was obtained from Morinaga Milk Industry Co. (Tokyo, Japan). The aloe sample was prepared by washing the leaf pulp with distilled water and then homogenizing with Polytron apparatus (Kinematica, Switzerland) at 19,000–24,000 rpm for 12 min at room temperature. To remove the insoluble components, the *Aloe vera* gel juice was centrifuged at 8,000 rpm for 15 min at room temperature, the resulting supernatant being filtered and lyophilized.

The average yield of the aloe sample from *Aloe vera* leaf pulp was about 0.59%. The lipid content of the aloe sample was determined to be 4.34% by the acid hydrolysis method. The moisture content was determined to be 8.86% with the gravimetric method by drying at 100 °C until attaining constant weight, and the ash content was gravimetrically determined by drying at 550 °C. The protein content was determined to be 2.21% by the semimicro-Kjeldahl method, and the carbohydrate content was calculated to be 64.12% as the remainder after subtracting the foregoing four components. The major component of the aloe sample was carbohydrate.

Male BALB/c mice (7 wk old) were obtained from Japan SLC, assigned to two groups of five mice/group and given distilled water with or without 5 mg/ml of the aloe sample *ad libitum* for 1 week. Water intake of a mouse was about 8 g/d. Total intake of aloe sample was calculated to be about 280 mg/mouse. These mice were maintained and used in accordance with the guidelines for the care and use of experimental animals of Tokyo University of Agriculture and Technology.

*E. coli* K-12 (2.4 × 10⁸ CFU/mouse) was intraperitoneally (i.p.) administered to the mice which had or had not been pre-treated with the aloe sample. At 0, 24 and 48 h after infection, the peritoneal cavity of each animal was washed with 5 ml of saline. The spleen was obtained from mice in each group and dipped for 1 h in PBS containing 100 μg/ml of gentamicin. After washing with PBS, the tissue was homogenized with 1 ml of 0.1% Triton X-100/PBS. The numbers of *E. coli* K-12 cells in

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the peritoneal cavity and spleen were counted after plating on Trypticase soy agar (TSA) and incubating overnight at 37°C.

The bacterial clearance in the peritoneal cavity and spleen was investigated as an indicator of the innate immune response (Fig. 1). In respect of the aloe sample-treated group, the bacterial number recovered from the peritoneal cavity of the mice was significantly less than that of the control group 48 h after infection (Fig. 1a). In respect of the spleen, the number of bacteria that had migrated from the peritoneal cavity of the aloe sample-treated mice was similar to that of the control mice up to 48 h after the challenge (Fig. 1b). These results indicate that treating with the aloe sample enhanced the bacterial clearance activity in the peritoneal cavity by activating innate immunity.

To evaluate the defensive activity of macrophages from the mice that had been treated with the aloe sample for 1 week, the activities of the binding, phagocytosis, and killing of the bacteria were assayed in vitro. To obtain macrophages from the peritoneal exudate, mice that had been pre-treated with the aloe sample were i.p. injected with 2 ml of 10% proteose peptone (Beckton, Dickinson and Company, Sparks, MD, USA) 48 h before collecting the peritoneal ascites. The cells in the peritoneal cavity fluid were collected by centrifugation (800 rpm, 5 min), suspended in an RPMI 1640 medium (Kohjin-Bio, Tokyo, Japan) containing 10% fetal calf serum (FCS; Intergen, New York, NY, USA) and put into a 24-well plate at a density of 2 × 10^5 cells/well. After incubating for 1 h at 37°C, the non-adhering cells were removed by washing with PBS.

A binding assay was then carried out. The adhering cells were infected with 1.9 × 10^5 CFU of E. coli K-12 and incubated for 1 h at 4°C to bind the macrophages and bacteria. The unbound bacteria were then removed by washing with PBS. The cells in some wells were lysed with 0.1% Triton X-100/PBS, and the lysates were diluted with PBS and plated on TSA to count the number of bacteria bound to the macrophages.

A phagocytic and killing assay was carried out after infecting by the method just described. The macrophages were incubated for 30 min at 37°C to phagocytize the bound bacteria. The cells were washed with PBS and incubated for 1 h at 4°C in an RPMI 1640 medium containing 10% FCS and 100 μg/ml of gentamicin to remove the unphagocytized bacteria. The cells were washed with PBS, lysed with 0.1% Triton X-100/PBS and plated on TSA to count the viable number of bacteria. The phagocytic activity was calculated as (CFU of phagocytized bacteria/CFU of bound bacteria)×100. The killing assay was carried out after phagocytosis of the bacteria by the method just described.

The macrophages were incubated in the RPMI 1640 medium containing 10% FCS and 100 μg/ml of gentamicin at 37°C for 30, 60 or 240 min. After each time, the cells were washed with PBS, lysed with 0.1% Triton X-100/PBS and plated on TSA to count the viable number of bacterial cells. The killing activity was calculated as (1-CFU of viable bacteria in the cells after each time/CFU of phagocytized bacteria)×100.

Figure 2a shows the activity of macrophages to bind to E. coli K-12. The macrophages obtained from the treated and non-treated mice showed similar binding activity. In respect of the phagocytic activity, 0.84% of all bound bacteria was phagocytized in the control group, whereas the macrophages from the aloe sample-treated mice phagocytized 1.21% of the bound bacteria. The phagocytosis of the aloe sample-treated group was therefore significantly enhanced (Fig. 2b). The killing activity of macrophages obtained from the aloe sample-treated mice was also significantly higher than that from the control mice after 30 and 240 min of incubation (Fig. 2c). After 60 min of incubation, there was no difference in killing activity between the treated and control mice. These findings show that an oral administration of the aloe sample would augment the activities of macrophages for phagocytosis and killing of bacteria, both being important for eliminating pathogens.

Since the aloe sample was found to have an enhancing effect on the killing activity of macrophages in vitro, we investigated its effect on the production of NO by peritoneal exudate macrophages following their stimulation with lipopolysaccharide (LPS), a bacterial cell-wall constituent. NO acts as a cytotoxic and cytostatic effector in the killing of intracellular parasites.

The peritoneal cells obtained by using the method already described were put in a 24-well plate at a density of 2.5 × 10^6 cells/well and incubated for 1 h at
The non-adhering cells were removed by washing with PBS. A 400-μl amount of Dulbecco’s modified Eagle’s medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) without phenol red containing 10% FCS and 1 μg/ml of LPS (E. coli O55:B5; Difco Laboratories, Detroit, MI, USA) was applied to the macrophages in a 24-well plate, and the culture incubated for 48 h at 37°C. Each of the culture supernatants was collected and centrifuged at 12,000 rpm for 5 min at 4°C. The concentration of nitrite, a stable product of NO, in the supernatant of the cultured macrophages was determined with a C II NO2/NO3 assay kit (Dojindo Laboratories, Kumamoto, Japan). The absorbance at 570 nm was measured with a microplate reader (EL800; Bio-Tek Instruments, Tokyo, Japan). The nitrite concentrations were determined by using sodium nitrite as the standard.

The results are shown in Fig. 3. After 48 h of incubation, the level of LPS-induced NO production by macrophages from the aloe sample-treated group was significantly higher than that from the control group. This result strongly suggests that ingesting the aloe sample would enhance the ability of activated macrophages to produce NO.

Our results indicate that the aloe sample would provide protection against infectious diseases by enhancing the functions of macrophages, suggesting that it would be useful for preventing intestinal infection when continuously ingested.

Fig. 2. Effect of the Aloe Sample on the Functions of Macrophages from the Peritoneal Exudate.
Bacterium, E. coli K-12; number of bacteria, 1.9 × 10^7 cfu/well; number of cells, 2 × 10^5 cells/well. Asterisks (*) and (**) indicate significant difference from the control (p < 0.05 and p < 0.01, respectively) by Student’s t-test.

Fig. 3. Effect of the Aloe Sample on NO Production by Macrophages from the Peritoneal Exudate.
Stimulus, LPS (1 μg/ml; E. coli O55:B5); number of cells, 2.5 × 10^6 cells/well; incubation time, 48 h. Asterisks (*) indicate significant difference from the control (p < 0.01) by Student’s t-test.

References