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Effects of Ethanol on Phagocytosis of Mouse Peritoneal Macrophages

(macrophage/phagocytosis/ethanol)

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The immunomodulatory effects of both acute and chronic alcohol use/treatment have been presumed, because chronic alcoholism increases susceptibility to bacterial infections such as pneumonia and tuberculosis. In the present study, the effects of ethanol on phagocytosis of mouse peritoneal macrophages were examined by means of fluorescent particles and flow cytometry. Phagocytosis of latex beads, zymosan (*Saccharomyces cerevisiae*, *S. cerevisiae*) and *Staphylococcus aureus* (*S. aureus*) was suppressed by ethanol in a dose-dependent manner. The results suggest that the reduced function of macrophages, at least in part, accounts for an increased susceptibility of alcoholic patients to infection.

Introduction

Excessive consumption of alcohol is associated with susceptibility to a variety of diseases [12]. Because immune surveillance provides defense in the body against cancers and pathogenic growths, the immunomodulatory effects of ethanol have been investigated. Use of alcohol *in vivo* and *in vitro* causes abnormalities in the function and structure of a broad array of cells involved in humoral and cellular immunity, including lymphocytes, Kupffer cells and macrophages [2,3,4, 7,8,9,10,13,14].

Macrophages are a component of the reticuloendothelial system which play a pivotal role in the immune system by serving as professional phagocytes in elimination of pathogens at the site of inflammation [11]. Moreover, macrophages have a tremendous capacity for immune regulation via their monokine/mediator production and accessory cell capacity. In the circulation they exist as peripheral blood monocytes and are known as macrophages upon migration from the blood into tissues. The peritoneal cavity provides an easily accessible source of macrophages.

Flow cytometry can analyze up to 10,000 macrophages within one minute, so that two important criteria of phagocytosis, percentage of phagocytic cells (PP) and phagocytic index (PI) are easily and efficiently determined [5]. By using this method, the present study examined the effects of acute ethanol on mouse peritoneal macrophage phagocytic activity for fluorescent particles, latex beads, zymosan and *S. aureus*.

Materials and Methods

The primary macrophage cultures were prepared as described previously [5], using BALB/cA Jcl mice (Nihon Clea, Tokyo) by injecting 4 ml thioglycollate medium into the peritoneum. The cells were cultured in RPMI 1640 solution containing 10% fetal bovine serum, 100 μg/ml streptomycin and 100 units/ml penicillin G in a CO_2 incubator at 37℃. The phagocytic assay was conducted within 10 days after cells were plated onto culture dishes.

Fluorescent particles (latex beads, *S. cerevisiae* and *S. aureus*) in stock solution were sonicated before use. Phagocytosis was analyzed by flow cytometry (FACStar, Becton Dickinson Immunocytometry System, Mountain View, CA, USA)

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as described previously [5]. Macrophages were incubated in the presence or the absence of ethanol with fluorescent particles at a density of 10^7 particles/dish for 30-60 min in normal external solution, which consisted of 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.5. Adhered cells were detached by trypsinization (0.25%) and subjected to flow cytometric analysis. The percentage of phagocytic cells (PP) was defined as the percentage of macrophages that ingested one or more particles. The phagocytic index (PI) was defined as the average number of particles ingested per macrophage and was calculated by dividing the total number of ingested beads by the total number of macrophages.

Fluorescent latex particles (no. 18338, 2 μm diameter) were from Polysciences, Warrington, PA, USA; Fluorescein conjugated *Saccharomyces cerevisiae* and *Staphylococcus aureus* from Molecular Probes, Inc., Eugene, OR, USA; thioglycollate, PBS(−), Hanks and RPMI 1640 medium from Nissui Pharmac., Tokyo. Other reagents were purchased from Nacalai Tesque, Kyoto.

Results and Discussion

Fig. 1a1 shows a contour plot of phagocytic cells which is based on forward scattering (FSC) versus relative fluorescence intensity (FL). The left cluster represents the cell population which does not ingest any beads and the right one represents the bead-ingested macrophages. The distribution of cell numbers versus FL intensity of Fig. 1a1 is shown in Fig. 1a2. Figs. 1b1 and 1b2 are a contour plot and a histogram in the presence of 2% ethanol. Comparing Fig. 1a1 with Fig. 1b1, the area and contour height of the non-ingested cell population were increased and those of bead-ingested cell population were decreased in the presence of ethanol. This is shown in the superimposed histogram of Fig. 1c, indicating that ethanol suppressed phagocytosis of latex beads in mouse peritoneal macrophages. Phagocytosis of beads was suppressed by ethanol at concentrations between 0.25 and 2% in a dose-dependent manner, as shown in Fig. 2.

To examine the effects of ethanol on phagocytosis in other particles, phagocytosis of zymosan and *S. aureus* were examined. As shown in Fig. 2, ethanol inhibited phagocytosis of these particles in a dose-dependent manner, the same

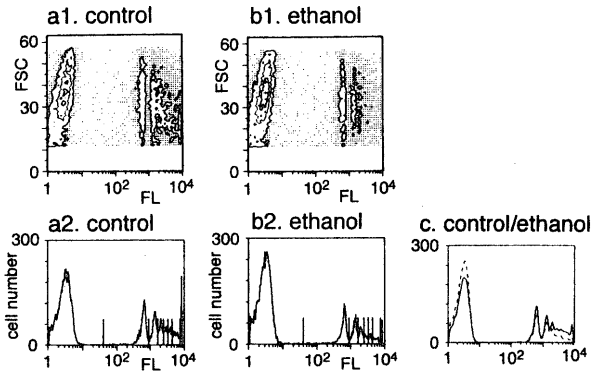

Figure 1.

Effects of ethanol on flow cytometric profiles. (a1) A control contour plot based on forward scattering (FSC) and relative fluorescence (FL). Each dot shows the area at a cell density of 1 to 4 cells/unit square. Contour lines indicate an increase in cell density of 15 cells. (a2) Histogram of cells based on FL intensities from a1. Non-ingested, 1 bead-ingested, 2 beads-ingested cell populations are indicated from the first, second and third peaks from the left, respectively. (b1) A contour plot of ethanol-treated (2%) cells. (b2) Histogram from b1. (c) Smoothed histograms of a2 and b2 are superimposed. Note that the continuous line is above the broken line in the higher FL intensity, showing that the number of bead-ingested cells is decreased by ethanol.

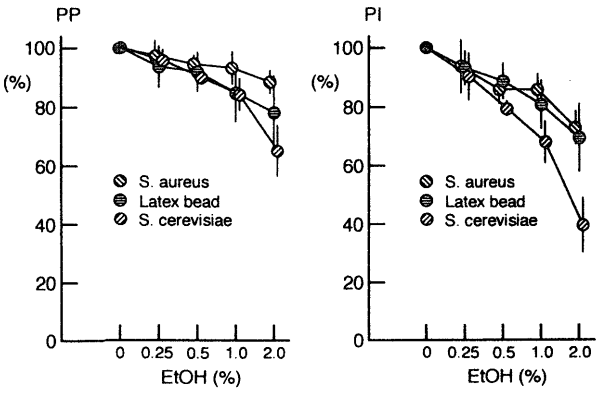

Figure 2.

Concentration-response curves showing an inhibition of phagocytosis in peritoneal macrophages by ethanol. The number of experiments for *S. aureus*, latex beads and *S. cerevisiae* are 7, 10 and 6, respectively. Data are means ± standard deviation, expressed as the percentage of control phagocytosis.

as latex beads. However, the efficacy of suppression by ethanol among *S. aureus*, latex beads and zymosan was in the order *S. aureus* < latex beads < zymosan. Though an inhibition of PI in *S. aureus* was comparable to that of latex beads, the inhibition of PP in *S. aureus* was smaller than that of latex beads. This may be due to the fact that macrophages of high phagocytic activity were not equally suppressed in the case of *S. aureus*. On the contrary, suppression of phagocytosis of latex beads was observed equally from low phagocytic activity of cells to high activity cells, as shown in Fig. 1c.

Inhibition of phagocytosis has been reported in other systems. Ethanol inhibits phagocytic activity of rabbit alveolar macrophages for latex particles [9] and *S. aureus* [8]. Ethanol also inhibits phagocytosis of unopsonized zymosan by a clonal macrophage cell line, RAW 264 [2]. Human blood monocytes cultured in growth medium containing ethanol for 1 to 7 days show impaired phagocytosis of IgG-sensitized red blood cells [14]. These reports and the present study suggest that ethanol in general inhibits phagocytosis of foreign particles and pathogens in various types of tissue macrophages.

To assess excessive consumption of alcohol and susceptibility to various diseases, the effects of chronic ethanol feeding have been reported. Peritoneal macrophages from ethanol-fed mice for 4 or 14 days demonstrated decreased phagocytic activity in *in vitro* (for opsonized zymosan) and *in vivo* (for sheep red blood cells) assays compared with macrophages from control animals [3]. Phagocytosis of sheep red blood cells *in vitro* by peritoneal macrophages is suppressed in ethanol treated mice for 7 days, compared to the cells isolated from controls [13]. Alveolar macrophages from ethanol-fed rats for 12 weeks exhibit impaired phagocytic activity [7]. Though Kupffer cells in chronically ethanol-fed rats demonstrate decreased phagocytic capacity, the phagocytic activity of hepatic endothelial cells and splenic macrophages is enhanced [10]. In phagocytic activity at the tissue level, hepatic clearance of Escherichia coli in isolated liver is depressed in ethanol-fed rats compared with control animals [4].

The type of phagocytic receptors whose function is suppressed by ethanol has been examined. Attachment mediated by Fc receptors of monocytes incubated with ethanol is suppressed [6]. The ability of peritoneal, alveolar and splenic macrophages from alcoholic rats to phagocytize through C3b and Fc receptors is impaired [1]. Because unopsonized particles in the present study were inhibited by ethanol, ethanol may also suppress certain types of phagocytosis other than C3b and Fc receptor-mediated phagocytosis.

Ethanol appears to modulate other functions of immunocytes. Ethanol enhances Platelet Activating Factor (PAF) production in nonstimulated macrophages cell line [2]. The respiratory burst in macrophages from ethanol-fed mice is enhanced [3]. Ethanol causes a decrease in the formation of erythrocyte-rosettes, a decrease in natural killer cell activity, and an increase in the number of T-helper cells in splenocytes [7]. Dietary ethanol reduces the number of T-suppresser cells and increases the number of IgM-containing B cells [13]. These data suggest that augmentation of immune function by ethanol may also be induced depending on the dose and time of exposure and specific immune system under certain circumstances, though immunosuppression is the overwhelming outcome of alcohol administration. The present study supports the notion that ethanol is a modulator of the immune system. Furthermore, on some occasions ethanol intake may be associated with a higher prevalence and greater severity of bacterial infection in humans.

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