STIMULATORY EFFECT OF CEFODIZIME ON MACROPHAGE-MEDIATED PHAGOCYTOSIS

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We evaluated the ingestion of anti-sheep erythrocyte (anti-E) IgG- and IgM-coated sheep erythrocytes by murine peritoneal macrophages exposed to cefodizime, a new semisynthetic cephalosporin, and other antibiotics. Cefodizime enhanced the ingestion of anti-E IgG-coated erythrocytes by peritoneal macrophages from CD-1 and BALB/c mice in a dose-dependent manner, but had no effect on uncoated or IgM-coated erythrocytes. Similar enhancement was observed only in the case of cefpimizole (AC-1370), among the other antibiotics examined. These results suggest that the favorable *in vivo* activity of cefodizime and cefpimizole may result from their phagocytosis-enhancing as well as antimicrobial properties.

The new semisynthetic cephalosporin, cefodizime, is characterized by a cephem ring which contains a *syn*-methoxyimino-aminothiazolyl group at the 7-position and a thiazolylthio-methyl group at the 3-position. The latter substitution is thought to provide metabolic stability and a prolonged half-life in human serum[1]. The efficacy of cefodizime in experimental murine infections is apparently superior to that predicted from *in vitro* activity[2,3]. We postulated that the enhanced *in vivo* activity of cefodizime may be due to drug-induced immunostimulation. This speculation was in part based upon the previous demonstration that a variety of agents, including lysophosphatidylcholine[4] and fibronectin[5], enhance receptor-dependent phagocytosis. Moreover, it was previously reported that cefpimizole (AC-1370), another semisynthetic cephalosporin, potentiated phagocyte function of macrophages and neutrophils[6]. Accordingly, we evaluated the ingestion of anti-sheep erythrocyte (anti-E) IgG- and IgM-coated sheep erythrocytes by murine peritoneal macrophages exposed to cefodizime and other antibiotics.

Materials and Methods

Mice

The 6~7-week old, female CD-1 and BALB/c mice were purchased from Japan Charles River Breeding Laboratories (Hino, Japan).

Antibiotics

Standard antibiotic agents were gifts from the following companies: Cefodizime, Taiho Pharmaceutical Co., Tokushima, Japan; cefotaxime, Hoechst Japan Limited, Tokyo; cefpimizole (AC-1370), Ajinomoto Co., Inc., Tokyo; cefbuperazone, Toyama Chemical Co., Ltd., Tokyo; cefminox (MT-141), Meiji Seika Kaisha, Ltd., Tokyo; cefotiam, Takeda Chemical Industries, Ltd., Osaka, Japan; cefazolin, Fujisawa Pharmaceutical Co., Ltd., Osaka; ampicillin, Meiji Seika Kaisha, Ltd., Tokyo; clindamycin, Japan Upjohn Limited., Tokyo; chloramphenicol, Sankyo Co., Ltd., Tokyo. All antibiotics were dissolved in RPMI-1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and filter sterilized immediately before use.

Anti-E IgG and IgM

Rabbit anti-E IgG at a concentration of 0.86 mg/ml and rabbit anti-E IgM at concentration of 0.27 mg/ml were obtained from Japan Immunoresearch Laboratories, Takasaki, Japan. These antibodies agglutinate E at a dilution of 1 : 10.

In Vitro Stimulation of Peritoneal Macrophages

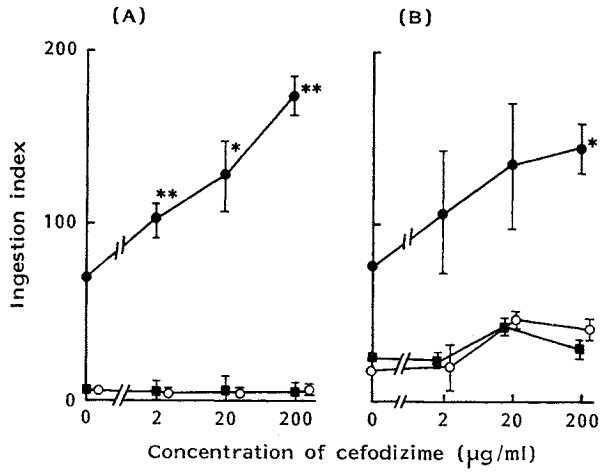
Resident peritoneal cells were harvested from untreated mice. The desired number of peritoneal cells ($2\sim3\times10^6$/ml) was suspended in RPMI-1640 with 10% heat-decomplemented (56°C, 30 minutes) γ-globulin-free fetal calf serum (GGF-FCS, GIBCO, Grand Island, NY). Aliquots of the cells were layered onto 12 mm glass cover slips (Matsunami, Osaka) which had been placed into the 16 mm wells of plastic tissue culture plates (Costar, Cambridge, MA). After 30 minutes incubation at 37°C in a humidified, 5% CO_2 incubator to effect adherence, the peritoneal cells were exposed for 60 minutes, under the same conditions, to antibiotics suspended in RPMI-1640 with 10% GGF-FCS. After treatment, cover slips were immersed in warm RPMI-1640 (37°C) and gently agitated to dislodge non-adherent cells. More than 95% of adherent cells were morphologically identified as macrophages. These peritoneal macrophages were further incubated for 5 hours in RPMI-1640 with 10% GGF-FCS prior to the ingestion assay.

Ingestion Assay

The ingestion of sheep erythrocytes (E, Japan Biotest, Tokyo) coated with sub-agglutinating concentrations of purified rabbit anti-E IgG (EA : IgG) or anti-E IgM (EA: IgM), and of uncoated E was determined as previously described[7]. Briefly, washed E were coated with sub-agglutinating concentrations of purified rabbit anti-E IgG or IgM fractions for 30 minutes at 37°C. After incubation, the coated E were washed three times with RPMI-1640. A 0.5-ml aliquot of EA : IgG, EA : IgM or E alone (2%), suspended in RPMI-1640 without GGF-FCS, was added to cover slips containing adherent cells and allowed to incubate at 37°C in a humidified, 5% CO_2 incubator for 40 minutes. Non-internalized E were lysed by immersing the cover slip in hypotonic phosphate-buffered saline for 5~10 seconds. The macrophages were fixed, air dried, and stained with Giemsa stain; ingestion was quantified microscopically. The data were expressed as ingestion index (number of ingested E per 100 macrophages). Measured values represented mean±SD of triplicate determinations based on 400 macrophages counted on each cover slip.

Fig. 1. Cefodizime-enhanced ingestion of sheep erythrocytes by peritoneal macrophages from CD-1 mice (A) and BALB/c mice (B).

Shown are uncoated E (○) and E coated with anti-E : IgG (●) or IgM (■). Bars indicate SD.



Single and double asterisks indicate $P<0.05$ and $P<0.01$, respectively, compared with control cells not exposed to antibiotics.

Statistical Analysis

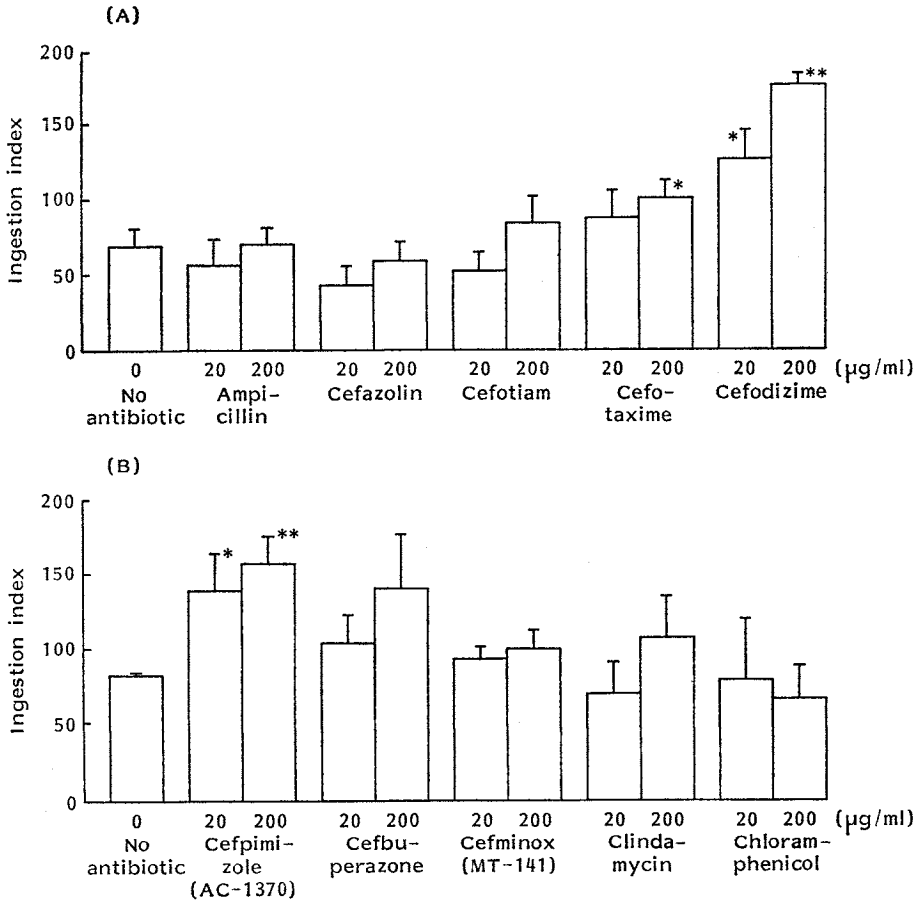
The data were analyzed by the paired t-test.

Results and Discussion

Cefodizime produced dose-dependent enhancement of EA : IgG ingestion by peritoneal macrophages from CD-1 and BALB/c mice (Fig. 1). In contrast, cefodizime caused no enhancement in the ingestion of E or EA : IgM by CD-1 macrophages, and only slight stimulation in the case of BALB/c macrophages. When various antibiotics were compared (Fig. 2), only cefodizime, cefpimizole and a higher concentration of cefotaxime produced enhanced ingestion of EA : IgG by CD-1 macrophages. The new semisynthetic cephamycins, cefbuperazone and cefminox (MT-141), did not augment macrophage-mediated phagocytosis despite particularly favorable efficacy against experimental infections in mice[8,9].

Previous reports have documented various immunomodulating activities of cephalosporin antibiotics. While at least two studies demonstrated the suppression of lymphocyte responses by cepha-

Fig. 2. Effect of various antibiotics on the ingestion of EA : IgG by peritoneal macrophages from CD-1 mice.



Data shown in panels A and B were obtained in different experiments. Bars indicate SD. Single and double asterisks indicate $P<0.05$ and $P<0.01$, respectively, compared with control cells not exposed to antibiotics.

losporins[10,11], LEYHAUSEN and his colleagues reported *in vitro* lymphocyte stimulation by cefodizime[12]. This antibiotic was also shown by LIMBERT *et al.* to augment the chemiluminescent responses of murine peritoneal macrophages and to enhance their uptake of colloidal gold[13]. More recently, it was documented that cefpimizole (AC-1370) stimulated macrophage-mediated phagocytic activity as well as neutrophil chemotaxis[6]. The binding of cefpimizole to macrophage membranes, and the enhancement of neutrophil function by culture supernatants from cefpimizole-treated macrophages[14], suggested that this treatment resulted in the production of soluble factor(s) actually responsible for stimulation of neutrophil activity.

In the present study, cefodizime produced marked, dose-dependent enhancement of macrophage-mediated phagocytosis of EA : IgG, while having no effect on the phagocytic uptake of EA : IgM or uncoated E. This differential uptake of EA : IgG and EA : IgM by murine peritoneal macrophages was reminiscent of that previously reported by BIANCO and his co-workers[15]. It is of interest that two structually distinct cephalosporins, cefodizime and cefpimizole, should exhibit similar stimulatory activity on macrophage-mediated phagocytic activity. The structural basis for this activity, and its mechanism, require further elucidation.

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