

CARRAGEENAN has been widely used as an irritant to evoke inflammation in animals or to selectively deplete macrophages *in vivo*. Although precise understanding of carrageenan activity is a prerequisite for the experimental use of this polysaccharide, the effects of this agent on host-biological systems are still poorly understood. We investigated the effect of carrageenan on serum concentrations of complement C3 and interleukin (IL)-6, a potent complement-inducing factor. Intraperitoneal administration of carrageenan (4 mg) in mice resulted in an initial fall in serum C3 (70% of control, $P < 0.05$) between 3 and 6 h, but was followed by a significant rise (180% of the control, $P < 0.05$) at 24 h. Prior to the rise in complement C3, a sharp peak of serum IL-6 was observed at 6 h after carrageenan treatment. These results indicate potential of carrageenan to enhance host complement systems, which may be associated with, at least in part, an acute induction of IL-6.

Key words: carrageenan, interleukin-6, complement C3

Acute induction of interleukin-6 and biphasic changes of serum complement C3 by carrageenan in mice

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Introduction

Carrageenan is a high-molecular-weight sulphated polygalactose obtained from marine plants.¹ This polysaccharide exerts a variety of activities in host biological systems, such as cytotoxicity to macrophages,² activation of Hageman factor³ and inhibition of complement.^{4,5} In experimental conditions, carrageenan has been widely used as a tool to evoke inflammation in animals^{6,7} or to produce a selective depletion of macrophages *in vivo*.^{8–10} Therefore, for a correct interpretation of the results of experiments using carrageenan, it is necessary to consider all the possible effects of this polysaccharide on host biological systems.

In 1965, Davies⁵ reported that carrageenan prevented the reaction of complement with sensitized red blood cells, probably by a direct inactivation of the complement. However, since then, no study has quantitatively measured the effect of carrageenan on serum complement levels. Recently, several investigators have reported that carrageenan may potentially modulate the production of inflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumour necrosis factor (TNF).^{11–13} Since these cytokines are known to play a critical role in inducing acute phase proteins including complement,¹⁴ we speculate that carrageenan may influence the host-complement system not only through direct inactivation of complement, but also production of these cytokines.

In the present study we examined the kinetics of serum concentrations of complement C3 and IL-6, a

potent complement-inducing factor, following intraperitoneal administration of carrageenan in mice.

Materials and Methods

Animals

Specific-pathogen-free male ICR mice (6 weeks old) were purchased from Charles River Japan (Kanagawa, Japan). They were housed in groups of 10 and provided with food and water *ad libitum*.

Administration of carrageenan

Iota-carrageenan (Sigma, St Louis, MO) was dissolved in pyrogen-free physiological saline, and autoclaved at 121°C for 15 min before use. ICR mice were injected intraperitoneally with 4 mg of carrageenan per mouse. The dose of carrageenan used in this study was equivalent to that used by other investigators to deplete macrophages.⁹ Mice were sacrificed in an ether-saturated chamber 0, 3, 6, 12, 24 and 48 h after the injection of carrageenan. Blood was collected by cardiocentesis and allowed to clot at 4°C for 2 h and centrifuged to obtain serum, which was stored in aliquots at –80°C before assay for complement C3 and IL-6.

Assay of serum complement C3 and IL-6

Serum concentrations of complement C3 were determined by Behring Nephelometer Analyzer (Behring Berke, Marburg, Germany) using anti-murine comple-

ment C3 antibody (Organon Teknika, West Chester, PA). The concentration of murine serum IL-6 was quantified using a sandwich-type ELISA according to the instructions provided by the manufacturer (Endogen, Boston, MA). A reference curve was obtained using recombinant mouse IL-6 and the assay had a limited sensitivity of 15 pg/ml.

Statistical analysis

Data were expressed as mean \pm SD. Differences between groups were tested for statistical significance using the Student's *t*-test. A *P* value $\leq 5\%$ was accepted as statistically significant.

Results and Discussion

As shown in Fig. 1a, carrageenan induced a biphasic response of serum concentrations of complement C3. This was characterized by a fall (approximately 70% of the control, $P < 0.05$) at 3–6 h followed by a rise (approximately 180% of the control, $P < 0.05$) in serum concentration 24 h after administration. Although it is well known that carrageenan suppresses the cell-lysing activity of complement *in vitro*,^{4,5} our data showed for the first time that carrageenan may potentially enhance the host complement system *in vivo*.

The complement system plays an important role in inflammatory and host immunological processes. In particular, complement C3 is a critical component for opsonization, cell-lysis activity and chemotaxis. To assess the function of the complement system, it may be necessary to analyse quantitatively the level of each complement component. Although this polysaccharide is known to induce an immunocompromised state by its macrophage-blocking activity,² our data suggest that carrageenan may augment complement-mediated host defence systems. Indeed, Iri-fune¹⁵ reported that carrageenan treatment (24 h

before and 48 h after infection) significantly improved the survival of mice with pneumonia using an inhalation model of *Klebsiella pneumoniae*. We also observed that intraperitoneal administration of carrageenan 24 h before induction of pleurisy enhanced the chemotactic response in the pleural cavity of mice.¹² These results and the present data suggest that carrageenan may modulate complement-mediated inflammatory and immunological responses producing an initial suppression followed by enhancement of C3.

Recent studies have indicated that administration of carrageenan *in vivo* influences the production of inflammatory cytokines (e.g. IL-1, IL-6 and TNF).^{11–13} These cytokines are important mediators not only for activation of immune cells but also for the induction of acute phase proteins, such as C-reactive protein, serum amyloid A and complements.¹⁴ As shown in Fig. 1b, a sharp peak of serum IL-6 was observed 6 h after intraperitoneal administration of carrageenan. Our data were to a large extent consistent with a previous report by Utsunomiya *et al.*¹³ in which a transient peak of serum IL-6 was observed 4 h after intrapleural inoculation of carrageenan in rats. They also showed that treatment with carrageenan resulted in a marked increase in serum concentrations of T-kininogen, an acute phase protein, at 16 h, although an initial fall in the concentration was not demonstrated. In the present study, the time-lag between peak IL-6 and peak complement C3 was 18 h, which is considerably similar to that of T-kininogen in the rat pleurisy model. It is most likely that carrageenan-induced IL-6 production may explain at least in part the rise of complement C3 in the later phase.

In conclusion, the present results indicate that carrageenan may potentially enhance the host complement system, which may be mediated by an acute induction of IL-6 production. Thus, our data suggest a novel effect for carrageenan on the host biological system.

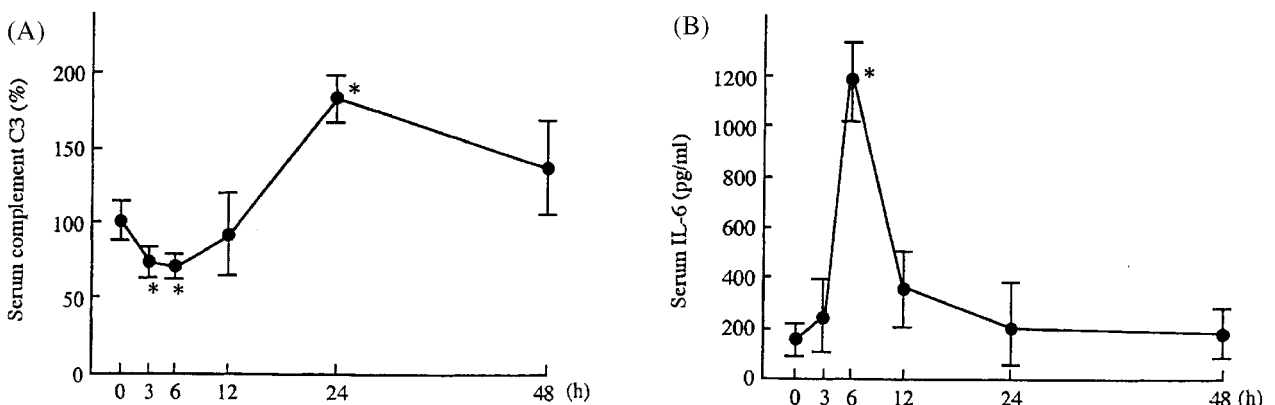


FIG. 1. Changes in serum concentrations of complement C3 (A) and IL-6 (B) in mice treated with intraperitoneal carrageenan. * $P < 0.05$ compared with the control (time = 0). Data are mean \pm SD of five mice.

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