Abstract—To elucidate the role of major chemotactic factors, cytokine-induced neutrophil chemoattractant (CINC), leukotriene B4 (LTB4) and C5a in lipopolysaccharide (LPS)-induced acute lung injury in rat, we employed three reagents: anti-CINC-1 antibody, an LTB4 receptor antagonist (ONO-4057) and an anti-complementary agent (K-76COONa). Rats were divided into five groups: (1) control group; (2) LPS group, which received intratracheal instillation of LPS (100 μg/kg); (3) Anti-CINC group, which received intratracheal coinflation of LPS with anti-CINC-1 antibody (1 mg/kg); (4) LTB4-Ra group, which received intravenous ONO-4057 (10 mg/kg) prior to intratracheal LPS; (5) Anti-C5a group, which received intravenous K-76COONa (100 mg/kg) prior to intratracheal LPS. The number of neutrophils in bronchoalveolar lavage (BAL) fluids 6 h after LPS instillation was significantly reduced in the Anti-CINC group, however, no reduction was found in either the LTB4-Ra group or Anti-C5a group. The levels of CINC-1, CINC-2α and CINC-3 in BAL fluids were significantly higher in the LPS group than in the saline-instilled control group. In vitro, the production of CINC-1 and CINC-3 from LPS-stimulated macrophages was significantly elevated compared to unstimulated macrophages 6 h later. The increase in CINC-2α production was markedly less than that of CINC-1 or CINC-3. These results indicate that CINCs, especially CINC-1 and CINC-3 play an important role in the recruitment of neutrophils to the lung in LPS-induced acute lung injury.

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is an acute inflammatory condition characterized by alveolar capillary membrane damage, neutrophilic alveolitis, pulmonary edema, and severe impairment of gas exchange (1). Although the mechanisms responsible for ARDS are not fully understood, it is known that neutrophils play an important role in experimental models of acute lung injury. Lipopolysaccharide (LPS), present in the walls of gram-negative bacte-
ria as a major component of endotoxin is a stimulus for the initiation of local acute inflammation. An experimental model of animals with many similarities to ARDS can be induced by LPS administration. Intratracheal administration of LPS causes a rapid influx of neutrophils into rat lungs (2). The toxic products released by neutrophils, such as oxygen radicals, proteases and arachidonic acid metabolites, are likely to be responsible for the development of lung injury.

A complex process that is mediated at multiple steps by cytokines, chemotactic factors, and adhesion molecules is required for the recruitment of neutrophils into alveoli. Locally produced chemotactic factors, such as interleukin (IL)-8, leukotriene B₄ (LTB₄) and complement fragment C5a, have been implicated in the recruitment of neutrophils to acute inflammatory sites (3). Various clinical and experimental evidence has strongly suggested the importance of these chemotactic factors in acute lung injury (4–7), however, their precise roles in the development of lung injury have not been defined.

Cytokine-induced neutrophil chemoattractant (CINC), a member of the C-X-C or IL-8 family in rats, is known to possess potent chemotactic activity for neutrophils (8). Several experimental studies have suggested that CINC plays a significant role in acute inflammatory response in rat lungs (9–12). Recently, four CINCs were identified in conditioned medium of granulation tissue obtained from carrageenin-induced inflammation in rats; CINC-2α and CINC-2β were novel chemoattractants differ only in the sequence of C-terminal three amino acid residues and others were CINC-1 (formerly called CINC) and CINC-3/macrophage inflammatory protein (MIP)-2 (3). CINCs have been reported to have similar chemotactic activity, suggesting their contribution to neutrophil infiltration into inflammatory sites in rats (13), however, the roles of individual CINCs in inflammatory responses are still not well understood.

In the present study, we employed three reagents, anti-CINC-1 antibody, a LTB₄ receptor antagonist (ONO-4057) and an anti-complementary agent (K-76COONa), in an attempt to elucidate the role of major chemotactic factors, CINC, LTB₄ and C5a, in the recruitment of neutrophils to the lung in a rat model of LPS-induced acute lung injury.

MATERIALS AND METHODS

Reagents. Escherichia coli LPS was purchased from Sigma Chemical Co. (St. Louis, Missouri). Mouse anti-rat CINC-1 monoclonal antibody was purchased from Immuno-Biological Lab. Co. (Gannma, Japan). An isotype-matched control antibody (mouse IgG₁) was purchased from Sigma Chemical Co. ONO-4057, a specific LTB₄ receptor antagonist (LTB₄-Ra) was a gift from Ono Pharmaceutical Co. (Osaka, Japan). K-76 sodium monocarboxylic acid (K-76COONa), an anti-complementary agent, which has been shown to inhibit complementary activity mainly at the C5 step (14), was a gift from Ohtsuka Pharmaceutical Co. (Tokushima, Japan).

Rat Models. Male Sprague-Dawley rats (Japan SLC Inc., Shizuoka, Japan) weighing between