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Role of type II phospholipase A_2 in the inflammatory process of carrageenan-induced pleurisy in rats

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Abstract

In order to investigate the role of type II phospholipase A_2 (PLA_2) in the inflammatory process, the effect of a monoclonal antibody specific to type II PLA_2 on carrageenan-induced pleurisy was studied in rats. Intravenous injection of the antibody (MB5.2), which inhibits the catalytic activity of type II PLA_2, significantly reduced both the pleural exudate volume and the intrapleural leukocyte count, while a control antibody did not have any appreciable effect. MB5.2 caused no change in the level of type II PLA_2 in the pleural fluid. These results suggest that type II PLA_2 generated at inflamed sites, at least in part, have a crucial role in the pathogenesis of acute inflammation.

Key words: Phospholipase A_2; Carrageenan-induced pleurisy; Inflammation

1. Introduction

PLA_2 is considered to play a central role in various inflammatory reactions, since it catalyzes the liberation of arachidonic acid and lysophospholipids from membrane phospholipids, and these products then are converted to proinflammatory mediators such as prostaglandins, leukotrienes, and platelet-activating factor.

The molecular nature of mammalian PLA_2 has recently been clarified. There is a 14-kDa secretory PLA_2 with two isozymes (type I and II) having different primary structures [1], and there is also an 85-kDa cytosolic form of PLA_2 [2,3]. Type II PLA_2 is often found in extracellular fluid at sites of inflammation [4–8] and the administration of type II PLA_2 results in the exacerbation of inflammation in some animal models [9–10]. In addition, inflammatory cytokines up-regulate type II PLA_2 expression by certain types of cells [11–15], while its expression is suppressed by anti-inflammatory glucocorticoids [16,17]. These observations suggest that type II PLA_2 plays a critical role in the process of inflammation. However, direct evidence supporting its involvement in inflammatory reactions has been rather limited.

In the present study, we investigated the contribution of type II PLA_2 to acute inflammation by using an anti-type II PLA_2 antibody [18] and a rat model of carrageenan-induced pleurisy.

2. Materials and methods

2.1. Preparation of monoclonal antibodies

Hybridomas producing the anti-type II PLA_2 antibody (MB5.2) [18] or a control antibody were injected into pristane-treated BALB/c mice in order to obtain monoclonal antibodies from ascites. The ascitic fluid harvested from these mice was purified by 50% ammonium sulfate precipitation followed by affinity chromatography on a protein A-Sepharose column (Pharmacia) [19], and the purified antibodies were dialyzed against 10 mM phosphate-buffered saline (pH 7.4).

2.2. Carrageenan-induced pleurisy

Male Donryu rats (Charles River, Japan Inc.) weighing 160–180 g were used. Solution (0.3 ml) of 0.5% carrageenan (Zusi Kagaku) was injected into the pleural space under ether anesthesia. After 6 h, the volume of exudate and the number of leukocytes in the pleural cavity were determined. Monoclonal antibodies were injected intravenously at various doses 30 min prior to the injection of carrageenan.

2.3. Quantification of Type II PLA_2

The type II PLA_2 content in pleural fluid was determined by a sandwich ELISA [20] using a polyclonal anti-type II PLA_2 antibody (R377) as the solid phase and a soluble biotinylated monoclonal anti-type II PLA_2 antibody (MD7.1). Immunoaffinity-purified type II PLA_2 from rat platelets was diluted to various concentrations for use as a standard for quantification.

3. Results

The monoclonal antibody MB5.2 is known to suppress the catalytic activity of type II PLA_2 [18]. When it was administered to rats with carrageenan-induced pleurisy, there was a dose-dependent reduction in both the volume of pleural exudate and the number of intrapleural leukocytes (Fig. 1). The pleural exudate volume and leukocyte count were significantly decreased by 28% and

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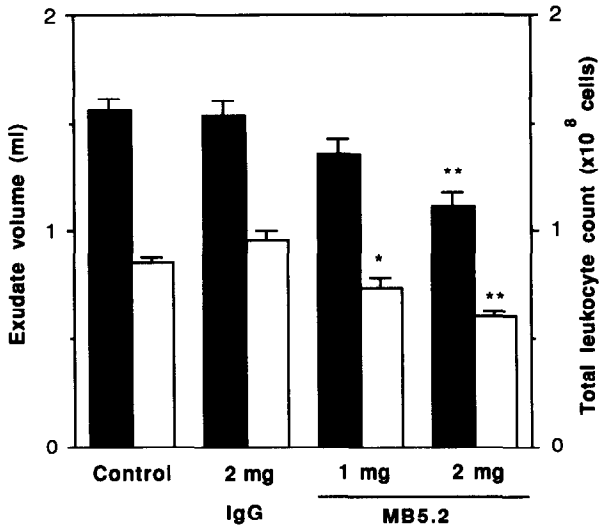

Fig. 1. Effect of the antibody MB5.2 on carrageenan-induced pleurisy in rats. The procedures were described in Section 2. Closed bars represent the pleural exudate volume and open bars represent the number of leukocytes. Values are the mean ± S.E.M. (n = 8–10). *P < 0.05 and **P < 0.01 vs. the control group.

29%, respectively, when 2 mg of MB5.2 was intravenously injected. More increased amounts of MB5.2 did not have any further effect (data not shown). The control IgG antibody did not appreciably alter the development of pleurisy.

The type II PLA_2 content in pleural fluid was determined using a sandwich ELISA (Fig. 2). The average pleural fluid level of type II PLA_2 in rats injected with carrageenan was about three-fold greater than in rats injected with saline (12 ng vs. 4 ng, respectively). No appreciable differences in the PLA_2 level were observed among rats administered either MB5.2, the control IgG antibody or vehicle only.

We have previously reported that type II PLA_2 had various pharmacological effects on several kinds of cells in vitro, it promotes eicosanoid generation by some cells [15,21] and also triggers histamine release from mast cells [22,23]. Thus, to investigate the mechanism of the inflammatory effect of type II PLA_2, we examined the influence of indomethacin (a cyclooxygenase inhibitor) and mepyramine (an anti-histamine) on the development of pleurisy (Fig. 3). Indomethacin markedly suppressed the development of pleurisy, whereas mepyramine showed no appreciable effect. Thus, type II PLA_2 might participate in the inflammatory process of carrageenan-induced pleurisy by potentiating eicosanoid synthesis rather than by triggering mast cell degranulation.

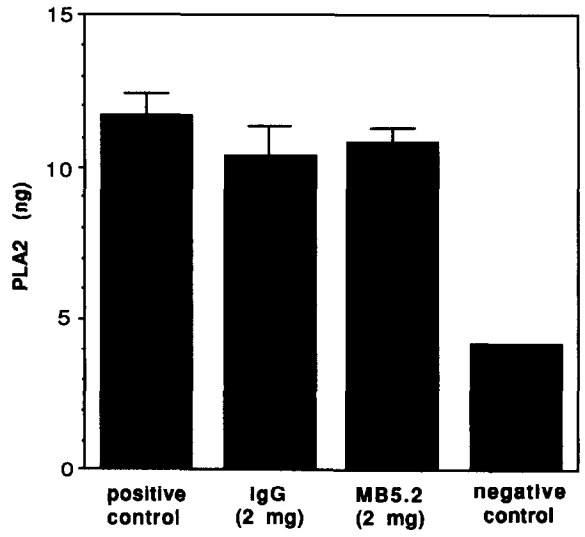

Fig. 2. Quantification of the type II PLA2 content of rat pleural fluid by sandwich ELISA. The positive control was injected with 0.3 ml of 0.5% carrageenan and the negative control was injected with 0.3 ml of saline. Values are the mean ± S.E.M. (n = 8–10).

4. Discussion

Type II PLA_2 has been detected in a wide variety of types of inflammation [4–8], and its importance in the inflammatory process has been suggested by many investigators including ourselves, although there has been little convincing evidence available. In this study, we showed that an anti-type II PLA_2 monoclonal antibody (MB5.2), which neutralizes type II PLA_2 activity [19], could significantly reduce both the volume of exudate and the number of leukocytes in rats with carrageenan-induced pleurisy. To our knowledge, this is the first direct evidence of the involvement of the catalytic activity

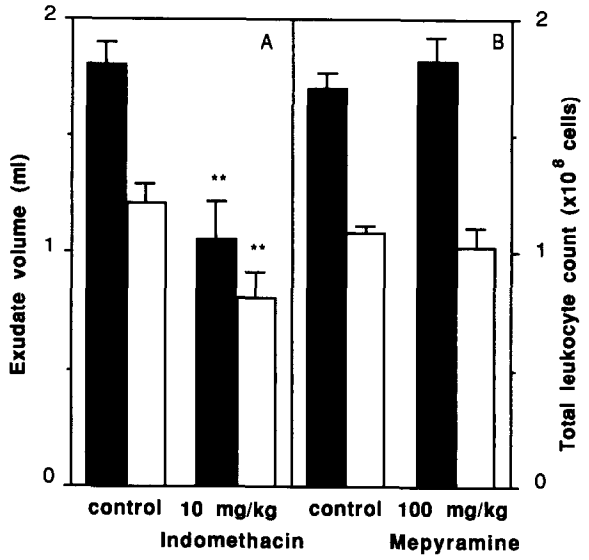

Fig. 3. Effect of indomethacin and mepyramine on carrageenan-induced pleurisy in rats. Indomethacin (10 mg/kg) or mepyramine (100 mg/kg) suspended in 0.5% methylcellulose solution was administered orally 1 h prior to the injection of carrageenan as described in Section 2. Closed bars represent the pleural exudate volume and open bars represent the number of leukocytes. Values are the mean ± S.E.M. (n = 8–10). **P < 0.01 vs. the control group.

of type II PLA_2 in the inflammatory process. Type II PLA_2 may contribute to eicosanoid generation at sites of inflammation by the hydrolysis of phospholipids in the plasma membranes of target cells and the liberation of arachidonic acid. This hypothesis is consistent with our finding that indomethacin, cyclooxygenase inhibitor, markedly suppressed carrageenan-induced pleurisy. Since the inhibitory effect of indomethacin was greater than that of the antibody MB5.2, the involvement of some other form of PLA_2, such as cytosolic PLA_2, could also be considered.

We recently demonstrated that type II PLA_2 triggers mast cell degranulation [22,23]. Since the anti-histamine drug, mepyramine, did not have any suppressive effect on pleurisy in this rat model, mast cell degranulation induced by type II PLA_2 might not be so important in this type of inflammation.

Several investigators have suggested that the presence of type II PLA_2 at sites of inflammation was the result of transduction of the protein from the plasma [24,25]. However, our present finding that the content of type II PLA_2 in pleural fluid was not affected by injection of the antibody MB5.2 despite an appreciable decrease in the pleural exudate volume and leukocyte count suggests that the source of type II PLA_2 is neither the plasma nor the leukocytes exudating into inflamed sites. Some type of cell already present in the pleural cavity might be the major source of type II PLA_2 in this pleurisy model.

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