ABSTRACT

The roles of prostaglandins (PGs) as mediators of inflammation have been extensively studied, and production of PGI₂ and PGE₂ at inflammatory sites has been reported. However, it has not yet been clarified which type of PG receptors has a major role in inflammatory exudation. To examine in vivo role of PG receptors in inflammatory exudation, we induced pleurisy in PG receptors (IP, EP₁, EP₂, EP₃, or EP₄) knockout mice by intrapleural injection of carrageenin. Pleural exudate accumulation in wild-type (WT) mice at 1 to 5 h, but not at 24 h, was significantly attenuated by the pretreatment with indomethacin, indicating that PGs are responsible for exudate formation at the early phase of pleurisy. Pleural exudation at 1 to 5 h in IP, EP₂, or EP₃ knockout mice, but not in EP₁ and EP₄ knockout, was significantly reduced compared with in WT mice. In the exudates, 6-keto-PGF₁α and PGE₂ were detected as the major PGs, each with its peak concentration at 3 h. In addition, involvement of bradykinin in the phenomenon was suggested by the fact that captopril, a kininase inhibitor, enhanced the exudate formation and increased the amount of 6-keto-PGF₁α and PGE₂, and that a bradykinin B₂-receptor antagonist inhibited the exudate formation. In contrast, leukocyte migration into pleural cavity was not influenced by indomethacin-treatment nor by these receptor deficiencies. These results demonstrate participation of EP₂ and EP₃ along with IP in pleural exudate formation but not in leukocyte migration in carrageenin-induced mouse pleurisy.
leads to edema formation or exudate accumulation. Pleurisy, an experimental model of acute inflammation, has been used as a potent tool to evaluate edema formation, enabling the assessment of both the rate and degree of exudate formation (Vinegar et al., 1973; Oh-ishi et al., 1986). In the present study, we intend to clarify the roles of PGs, in addition to PGI₂, in inflammatory exudate formation at the specified time after the carrageenin injection, pleural exudates after the carrageenin injection, 6-keto-PGF₁α levels of these PGs had already increased at 1 h, peaked at around 3 to 5 h, and then gradually decreased to about one-half of the peak level at 24 h (Fig. 1A). Indomethacin significantly suppressed the exudation rate at 5 h, but not at 16 and 24 h. The exudation rate, assessed by dye leakage into the pleural exudates for 20 min, was the highest at 1 h and decreased with time; thus, the largest plasma leakage occurred at the initiation of the pleurisy (Fig. 1B). Exudation rate at 1 h was measured as the dye exudation during the time from 40 min to 1 h after the carrageenin injection, because 40 min was the earliest time that could be measured reliably in the present experiment. Indomethacin significantly suppressed the exudation rate at 1 and 3 h (Fig. 1B), indicating the involvement of prostanoids in the early phase of the pleurisy.

Total leukocytes in the exudates increased dramatically with time, reached a plateau at 5 h, and remained fairly constant thereafter up to 24 h (Fig. 1C). Eighty to ninety percent of total leukocytes were neutrophils, whereas the resident cells in the pleural cavity consisted of mostly mononuclear cells and mast cells. Indomethacin had no significant effect on the leukocyte number in the pleural exudates throughout the time course (Fig. 1C), suggesting no involvement of prostaglandins in leukocyte migration.

Because indomethacin significantly suppressed the early phase of exudate accumulation, we focused our examination for exudate formation on the time period of 1 to 5 h after the induction of pleurisy in the following experiments.

Measurement of PGs in the Exudates. We assessed the production of PGs in the process of carrageenin-induced pleurisy in wild-type C57/BL6 mice. Among PGs measured in the exudates after the carrageenin injection, 6-keto-PGF₁α, a stable metabolite of PGL₂, and PGE₂ were the main PGs. The levels of these PGs had already increased at 1 h, peaked at 3 h, and decreased thereafter, but they remained detectable even at 24 h (Fig. 2). When the animals were pretreated with indomethacin, the levels of both PGs were almost at the lower limit of detection throughout the experimental period. The amounts of other PGs, such as PGD₂ and thromboxane B₂, were less than 0.1 ng/mouse (data not shown).
To assess the involvement of bradykinin in PG synthesis, we examined the effect of captopril, a kininase inhibitor, on the levels of PGs in the exudate. Captopril treatment significantly increased the 6-keto-PGF1α level (from 0.78 ± 0.13 to 1.43 ± 0.20 ng/mouse) and PGE2 level (from 0.17 ± 0.04 to 0.30 ± 0.07 ng/mouse) at 1 h, suggesting that the prevention of bradykinin degradation increased prostaglandin production.

Exudate Formation in PG Receptor Knockout Mice.

Next, we compared the exudate formation induced in IP-, EP1-, EP2-, EP3-, and EP4-knockout (IP-KO, EP1-KO, EP2-KO, EP3-KO, and EP4-KO) and WT mice, because indomethacin significantly attenuated the exudation and because major PGs detected in the exudates were 6-keto-PGF1α and PGE2. The exudate volume in IP-KO, EP2-KO, and EP3-KO mice at 1, 3, and 5 h was significantly less than that in WT mice (Fig. 3, A–C). The exudation rate at 1 and 3 h in IP-KO mice was significantly less than that in WT mice.
Fig. 3. Comparison of the exudate volume of carrageenin-induced pleurisy at 1 h (A), 3 h (B), and 5 h (C), and the exudation rate at 1 h (D) and 3 h (E) after carrageenin injection among the prostanoid receptor-deficient (IP-, EP1-, EP2-, EP3-, and EP4-knockout) and WT mice. The exudation rate was assessed by dye leakage into pleural exudates over 20 min at each indicated time after the carrageenin injection as described under Materials and Methods. Data represents the mean ± S.E.M. of the values obtained from indicated numbers of mice, as shown above the columns. *, P < 0.05 versus WT control; **, P < 0.01 versus WT control.

(Fig. 3, D and E), whereas those rates in EP2-KO and EP3-KO mice were slightly but not significantly less than that in WT mice. These results suggest that prostanoid receptor IP, EP2 and EP3 participate in the exudate formation.

**Pharmacological Evaluation of Bradykinin Involvement in the Exudate Formation.** We examined whether bradykinin-bradykinin B2 receptor system is involved in the mouse pleurisy; the system was reported to be involved in carrageenin-induced rat pleurisy (Harada et al., 1982; Dozen et al., 1989). The involvement of bradykinin was examined by treatment with a bradykinin B2 receptor antagonist FR173657. In WT mice, the exudate volume at 3 h was significantly suppressed by FR173657 to a similar degree as that by treatment with indomethacin, and no further suppression was found by simultaneous treatment with both inhibitors (Fig. 4). In IP-KO mice, the exudate volume at 3 h, which was significantly lower compared with that in WT mice, was significantly suppressed further by indomethacin, indicating that PG other than PGI2, possibly PGE₂, participated in the exudate formation. FR173657 significantly decreased the exudate volume to a similar degree with that by indomethacin, and no further suppression was found by simultaneous treatment with both inhibitors (Fig. 4). These results suggest that PG and bradykinin had a common pathway to stimulate the exudate formation.

We also examined the effect of captopril on the exudate volume and rate. Captopril treatment significantly increased the exudate volume in WT and EP3-KO mice at 1 h, whereas the increase in IP-KO and EP2-KO mice was slight (Table 1). For the exudation rate, significant increase was caused by captopril in WT and EP3-KO mice, but only a slight increase was seen in IP-KO and EP2-KO mice.

**Expressions of mRNAs for PG Receptors.** RT-PCR for IP, EP1, EP2, EP3, and EP4 mRNAs was performed in the tissues adjacent to pleural cavity of WT mice. Expression of mRNAs for all receptors except EP1, before and 3 h after carrageenin treatment was detected in the pleura, diaphragm, and lung, suggesting that these receptors could...
mediate signals for PGI2 and PGE2 to induce exudation (Fig. 5). Interestingly, the expression levels of these mRNAs decreased apparently in the pleura after carrageenin injection.

Discussion

We first examined the time course of carrageenin-induced pleurisy in WT mice with or without pretreatment of indomethacin. Involvement of PGs in exudate formation was suggested by the significant suppressive effect of indomethacin during the earlier phase, from the initiation up to 5 h after the carrageenin injection (Fig. 1). This feature is similar to the case in carrageenin-induced rat pleurisy, in which involvement of PGs and kinin during an earlier phase of exudation was suggested, and 6-keto-PGF1α and PGE2 were detected in the exudates (Dozen et al., 1989). However, receptor types for PGs mediating exudate formation have not yet been determined. To determine types of PG receptors involved in exudation in the present mouse model, we compared IP- or EP-knockout mice with WT mice. We demonstrated that IP, EP2, and EP3, but not EP1 or EP4, were the receptors participating in exudate formation in the carrageenin-induced mouse pleurisy model.

In consideration of the signal transduction caused by stimulation of IP and EP, the activation of either IP or EP2 results in an increased level of cAMP by coupling to Gs (Narumiya et al., 1999). However, the situation is complicated in the case of EP3 because there are several isoform receptors for EP3, all of which have different signaling. For example, among the isoforms of bovine EP3, EP3B and EP3C increase the cAMP level, but EP3A decreases it (Namba et al., 1993; Narumiya et al., 1999). At least three isoforms for mouse EP3 have been reported, and there may possibly be even more isoforms, as found in rabbit and human (Narumiya et al., 1999). Therefore, identifying the isoform responsible for the exudate formation is much more difficult and must await future investigation.

The expression pattern of PG receptor mRNAs in the tissues around the pleural cavity, such as the pleura, diaphragm, and lungs, is in line with the site of leaking vessels. This site has been shown to be the venules in the pleural tissues, using carbon particles as a marker in the carrageenin-induced rat pleurisy (Majno and Palade, 1961; Tanaka et al., 1980). Although EP4 is known to cause an increase in cAMP, the present study showed no involvement of EP4 in the exudation, suggesting that localization of EP4 differs from that of IP and of EP2. At 3 h of carrageenin injection, there was no apparent change in the expression pattern of these receptors in diaphragm and lungs. In the pleura, however, the expression levels of all these mRNA decreased. Although the reason of the decrease was not clear, it may derive from an instability of mRNAs in the pleura, where the severe inflammatory reaction took place. Precise localization of these receptors and identification of the exact leakage sites around the pleural cavity, however, remain to be clarified.

Evidence for involvement of the kinin system in the exudate formation in carrageenin-induced mouse pleurisy was obtained in the present study by examining the effects of
bradykinin B2 receptor antagonist and captopril on the exudation in mice pretreated with indomethacin. We previously reported that carrageenin, a negatively charged polysaccharide, can activate the plasma kallikrein system in human or rat plasma to produce bradykinin through activation of factor XII in the contact phase of the intrinsic blood coagulation cascade (Oh-ishi, 1982). These previous findings suggest that carrageenin injected into the pleural cavity of mice also activates the kallikrein-kinin system in the pleural fluid to produce bradykinin. In accordance with this notion, FR173657 significantly inhibited the pleural exudation, indicating that endogenous bradykinin produced in the pleural cavity enhanced the exudation by acting on the bradykinin B2 receptor. Furthermore, combined treatment with FR173657 and indomethacin did not further suppress the exudation than when treated with each agent alone, suggesting that bradykinin enhanced the exudation by stimulating prostanoid synthesis, as reported previously (Dozen et al., 1989).

From previous reports (Dozen et al., 1989; Erdos and Deddish, 2002), we suspected that treatment with captopril, which is a kininase II inhibitor that prevents the degradation of bradykinin in vivo, could enhance the response to endogenous bradykinin. As expected, the carrageenin-induced pleural exudation was significantly enhanced by captopril in WT mice in a similar manner to that reported in the rat case. In IP- or EP2-KO mice, however, there was no significant enhancement of pleural exudation by captopril, suggesting that IP and EP2 mediate further the signaling of PGI2 and thromboxane and their roles in the accumulation of exudate in rat carrageenin-induced pleurisy - a profile analysis using gas chromatography-mass spectrometry. Prostaglandins 22:881–895.


Murata T, Ushikubi F, Matsukura T, Hirata M, Yamaki K, Yamasu A, Nakano T, Utsunomiya I, and Nagashima Y (1986) Evidence for a role of the plasma kallikrein-kinin system in the earlier phase in concert with PGs is also confirmed in the mouse model of carrageenin-induced pleurisy. Furthermore, this study demonstrates that the experimental mouse pleurisy is a useful tool for evaluation of common mediators for inflammatory exudation, as was previously shown in the rat and rabbit models (Peck et al., 1978; Oh-ishi et al., 1986; Ogino et al., 1996; Saleh et al., 1997).

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References


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