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European Journal of Pharmacology 387 (2000) 111–118

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Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice

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Abstract

Intraperitoneal administration of zymosan and acetic acid induced a dose-dependent nociceptive writhing response in mice. Lavage of the peritoneal cavities with saline reduced the number of total resident peritoneal cells and caused a proportional decrease in the nociceptive responses induced by these stimuli. Furthermore, the specific reduction of the peritoneal mast cell population by intraperitoneal administration of compound 48/80 also reduced the nociceptive responses induced by zymosan and acetic acid. In contrast, enhancement of the peritoneal macrophage population by pretreatment of the cavities with thioglycollate caused an increase in the number of writhes induced by both stimuli. These data suggest that the nociceptive responses induced by zymosan and acetic acid are dependent upon the peritoneal resident macrophages and mast cells. These cells modulate the nociceptive response induced by zymosan and acetic acid via release of tumour necrosis factor α (TNF-α), interleukin 1β and interleukin 8. This suggestion is supported by the following observations: (a) pretreatment of the peritoneal cavities with antisera against these cytokines reduced the nociceptive responses induced by these stimuli; (b) peritoneal cells harvested from cavities injected with zymosan or acetic acid released both interleukin 1β and TNF-α; (c) although individual injection of TNF-α, interleukin 1β or interleukin 8 did not induce the nociceptive effect, intraperitoneal injection of a mixture of these three recombinant cytokines caused a significant nociceptive writhing response. In conclusion, our results suggest that the nociceptive activity of zymosan and acetic acid in the writhing model is due to the release of TNF-α, interleukin 1β and interleukin 8 by resident peritoneal macrophages and mast cells.

Keywords: Nociception; Interleukin 1β; Interleukin 8; TNF-α; (Tumour necrosis factor-α); Macrophage; Mast cell

1. Introduction

Over the last decade it has become clear that resident cells, by releasing cytokines, play an important role in the recognition of non-self and the development of local and systemic signs and symptoms of inflammation, such as cell migration, oedema, fever and hyperalgesia (Souza and Ferreira, 1985; Souza et al., 1988; Cunha and Ferreira, 1986; Dinarello et al., 1986; Faccioli et al., 1990; Echtenacher et al., 1996; Malaviya et al., 1996). In this context, using a model of mechanical hyperalgesia, we have been able to demonstrate that the release of cyclo-oxygenase products and sympathomimetic amines, the final mediators of inflammatory hyperalgesia (Ferreira et al., 1978; Levine et al., 1986; Nakamura and Ferreira, 1987) is preceded by the generation of cytokines by resident macrophages. Carrageenin and *Escherichia coli* endotoxin cause the release of bradykinin, which stimulates the release of tumour necrosis factor (TNF-α). TNF-α induces the release of interleukin 1β and interleukin 6, which stimulates the production of cyclo-oxygenase products, as well as interleukin 8, which stimulates the production of sympathomimetic mediators (Ferreira et al., 1988,1993; Cunha et al., 1991, 1992). In a different model, using the tail-flick method, endotoxin-induced hyperalgesia was also shown to be mediated by TNF-α and interleukin 1β (Waltkins et al., 1994, 1995). In contrast, interleukin 4 and interleukin 10 limit the inflammatory hyperalgesia by inhibiting the production of hyperalgesic cytokines and of prostaglandins. These cytokines appear to be released by resident

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0014-2999/00/$ - see front matter
PII: S0014-2999(99)00790-6

mast cells and macrophages, respectively (Poole et al., 1995; Cunha et al., 1999). The role of cytokines in the writhing model of nociception has been little investigated. In this model the nociceptive response induced by endotoxin appears to result from the release of TNF-α, interleukin 1β and interleukin 8. It was reported that administration of the supernatant of endotoxin-stimulated macrophages induces a dose-dependent nociceptive response in mice and that specific antisera against TNF-α, interleukin 1β and interleukin 8 inhibit the nociceptive activity of the supernatants (Thomazzi et al., 1997). Further, the nociceptive response induced by this supernatant is mediated by eicosanoids and sympathomimetic amines since it was inhibited by inhibitors of eicosanoid synthesis (indomethacin and paracetamol) and by the sympatholytic agents, guanethidine and atenolol (Campos et al., 1988; Thomazzi et al., 1997). Moreover, the participation of eicosanoids in the nociceptive responses induced by acetic acid and zymosan and of sympathomimetic amines in the response induced by acetic acid has also been demonstrated (Coderre et al., 1984; Doherty et al., 1985, 1987; Duarte et al., 1988; Santos et al.,1998).

In the present study, using the writhing model, we investigated the role of peritoneal resident macrophages and mast cells in the course of the nociception induced by acetic acid and zymosan, well-known nociceptive stimuli (Koster et al., 1959; Doherty et al., 1985, 1987; Duarte et al., 1988). We depleted the total peritoneal resident cells by lavage of the cavities with saline (Souza et al., 1988), increased the macrophage population by previous treatment of the cavities with thioglycollate (Cunha and Ferreira, 1986) and depleted the mast cell population by treatment with compound 48/80 (Di Rosa et al., 1971). The role of TNF-α, interleukin 1β and interleukin 8 in the process was also investigated by treating the mice with a specific antibody against these cytokines. The release of these cytokines by peritoneal cells harvested from cavities injected with zymosan and acetic acid was also determined.

2. Materials and methods

2.1. Animals

Male Swiss mice weighing 20–25 g were used for the nociceptive test. The animals were housed in a temperature controlled room (23 ± 2°C) with free access to water and food. The ethical guidelines described in the NIH Guide for Care and Use of Laboratory Animals were followed throughout the experiments.

2.2. Drugs, reagents and antisera

The following drugs were used: zymosan A (cell walls of the yeast *Saccharomyces cerevisiae*, Sigma, St. Louis, USA), glacial acetic acid (Merck, Brazil), thioglycollate (Lab Difco, São Paulo, Brazil), and compound 48/80 (Sigma, St. Louis, MO), iloprost (ZK 36374, a stable analogue of prostacyclin; Schering, AG). Murine TNF-α, murine interleukin 1β and human interleukin 8 were NIBSC (National Institute for Biological Standards and Control, UK) preparations. The following antisera were used: sheep anti-murine interleukin 1β (Poole et al., 1989), sheep anti-human interleukin 8 (Cunha et al., 1991) and sheep anti-murine TNF-α (Mahadevan et al., 1990). All reagents and drugs were diluted in phosphate buffered saline (PBS).

2.3. Nociceptive test

Nociceptive activity was tested in mice using the writhing model (Koster et al., 1959; Collier et al., 1968). To determine whether cytokines are involved in the writhing response in this model, we used acetic acid and zymosan (cell walls of the yeast *S. cerevisiae*), two stimuli known to produce inflammation as well as a writhing response in mice. Prostacyclin was used as a control because it is thought to induce nociception directly. The mice had the nociceptive stimuli injected into the peritoneal cavity and were placed in a large glass cylinder. The intensity of nociception was quantified by counting the cumulative number of writhes occurring between 0 and 30 min after stimulus injection. The writhing response consists of a contraction of the abdominal muscles together with a stretching of hind limbs. The doses of the nociceptive stimuli were: zymosan (0.25, 0.5 and 1 mg/mouse), acetic acid (0.2 ml of solutions at concentrations of 0.15, 0.3 and 0.6%, volume/volume), and iloprost (1 μg/mouse). This dose of iloprost was selected because it causes a submaximal nociceptive response. Controls were injected with saline. All stimuli were injected in a volume of 0.2 ml/mouse. The number of animals per group was 6 and the experiments were repeated 3 times.

2.4. Changes in the peritoneal resident cell population

2.4.1. Depletion of total peritoneal resident cells by peritoneal lavage

The method employed was described previously (Souza et al., 1988). Briefly, Swiss mice were anaesthetised with ether and three hypodermic needles were inserted into the abdominal cavity. Sterile saline (10 ml) was injected through the needle placed near the sternum. The abdominal cavity was then gently massaged for 1 min and the peritoneal fluid was collected via the two needles inserted into the inguinal region. This operation was repeated three times. More than 80% of the peritoneal macrophages were removed in the lavage fluid and 90% of the saline was recovered ($n = 6$). Control (sham) mice underwent insertion of the three needles and were manipulated in the same way but no fluid was injected or withdrawn. Thirty min-

utes later the animals were injected with saline (0.2 ml/mouse, $n = 10$), the stable analogue of prostacyclin, iloprost (1 μg/mouse, $n = 5$), zymosan (1 mg/mouse, $n = 10$) or acetic acid (0.2 ml of solution 0.6%/mouse, $n = 10$) and the number of writhes was counted in the interval between 0 and 30 min after injection of the stimuli. The experiments were repeated twice.

2.4.2. Increasing the peritoneal macrophage population by pretreatment with thioglycollate

Thiogycollate (3%, w/v; 3 ml) was injected i.p. and 4 days later the peritoneal macrophages were collected from a group of mice ($n = 10$), then counted and compared with the same cells obtained from a group of untreated animals (control) (Cunha and Ferreira, 1986). The other group of treated animals was used on the 4th day after being injected with saline (0.2 ml/mouse, $n = 10$), the stable analogue of prostacyclin, iloprost (1 μg/mouse, $n = 5$), zymosan (1 mg/mouse, $n = 10$) or acetic acid (0.2 ml of solution 0.3%/mouse, $n = 10$). The number of writhes was counted in the interval between 0 and 30 min after injection of the stimuli. The experiments were repeated twice.

2.4.3. Depletion of the peritoneal mast cell population by chronic pre-treatment with compound 48/80

The method used has been described previously (Di Rosa et al., 1971). The animals were pretreated with compound 48/80 for 4 days (0.6 mg/kg, i.p., twice a day for 3 days and 1.2 mg/kg on the 4th day). On the 5th day, depletion of the mast cell population was assessed in a selected group of animals ($n = 6$) by conventional light microscopy after staining the cells with toluidine blue. The counts obtained were compared with the number of cells from a group of untreated animals (control). On the 5th day other groups of treated and untreated animals were injected with saline (0.2 ml/mouse, $n = 10$), the stable analogue of prostacyclin, iloprost (1 μg/mouse, $n = 7$), zymosan (1 mg/mouse, $n = 10$) or acetic acid (0.2 ml of a 0.6% solution/mouse, $n = 10$). The number of writhes was counted in the interval between 0 and 30 min after injection of the stimuli. The experiments were repeated twice.

2.5. Effect of antisera against murine TNF-α, interleukin 1β and human interleukin 8

The mice were injected intraperitoneally with 50 μl of PBS, 50 μl of control serum or of antisera against murine TNF-α, interleukin 1β or human interleukin 8. Zymosan (1 mg/mouse) or acetic acid (0.2 ml of a 0.6%, v/v, solution) was injected i.p. 10 min later and the number of writhes was determined between 0 and 30 min. The number of animals per group was 6 and the experiments were repeated twice. In earlier experiments, we had demonstrated that these antiserum doses neutralized the mechanical hyperalgesic effects of their respective cytokines (Cunha et al., 1991; Thomazzi et al., 1997).

2.6. Production of TNF-α and interleukin 1β by peritoneal cells harvested from peritoneal cavities stimulated with zymosan and acetic acid

Saline (0.2 ml), zymosan (1 mg/0.2 ml) or acetic acid (0.2 ml of a 0.6% solution) was injected i.p. into the mice. After 15 min the peritoneal cavities were washed with saline (1 ml/cavity), and the exudates were centrifuged at 300 g for 10 min. The pelleted cells were resuspended in 200 μl of RPMI with 10% foetal calf serum and plated onto 96 well plastic tissue culture plates. The concentrations of TNF-α and interleukin 1β in the supernatants after culture for 12 h were determined by ELISA, as described elsewhere (Cunha et al., 1993). Briefly, microtiter plates were coated overnight at 4°C with antibody against TNF-α or interleukin 1β (10 μg/ml). After blocking of the plates, the samples and standard at various dilutions were added in duplicate and incubated at 4°C for 24 h. The plates were washed three times with buffer and a second biotinylated polyclonal antibody against TNF-α or interleukin 1β diluted 1/1000 was added (100 μl/well). After a further incubation at room temperature for 1 h, the plates were washed and 100 μl of avidin–horseradish peroxidase diluted 1:5000 was added. One hundred μl of colour reagent OPD (orthophenylenediamine dihydrochloride) was added 15 min later and the plates were incubated in the dark at 37°C for 15–20 min. The enzyme reaction was stopped with H_2SO_4 and absorbance at 490 nM was determined. The results are reported as means ± S.E.M. from the five wells and the experiments were repeated twice.

2.7. Nociceptive effect of TNF-α, interleukin 1β and interleukin 8

Murine TNF-α (5.0 and 100 ng/mouse), interleukin 1β (0.05 and 100 ng/mouse) or human interleukin 8 (1.0 and 100 ng/mouse) was injected i.p. into the mice and the number of writhes was determined for the period from 0 to 30 min after injection. The effect of the combination of these cytokines (100 ng of each cytokine/mouse) was also tested. The doses of the cytokines were those described in the literature (Thomazzi et al., 1997) and the number of animals per group was 6.

2.8. Statistical analysis

The results are reported as means ± S.E.M. (Standard Error of Mean) for at least five animals in each group and are representative of two independent experiments. Statisti-

cal analysis was performed with the analysis of variance (ANOVA) followed by the Bonferroni test. The differences between groups were considered significant when $P < 0.05$.

3. Results

3.1. Dose dependence of the nociceptive effects of zymosan and acetic acid

Intraperitoneal injection of increasing doses of zymosan (0.25, 0.5 and 1.0 mg/mouse) induced a dose-dependent nociceptive response in the mice (Fig. 1a, analysis of variance F: 8519, total degrees of freedom (df): 23). Although the nociceptive response induced by the zymosan dose of 0.25 mg/mouse was not different from that induced by saline (C, $P > 0.05$), the nociceptive responses induced by zymosan doses of 0.5 mg/mouse and 1.0 mg/mouse were significantly different from the control ($P < 0.01$ and $P < 0.001$, respectively). The response induced by the dose of 1.0 mg/mouse was also significantly different from that induced by the dose of 0.5 mg/mouse ($P < 0.01$). Similarly, intraperitoneal injections of increasing doses of acetic acid (0.2 ml of solutions at concentrations of 0.15, 0.3 and 0.6%) also induced a dose-dependent nociceptive response (Fig. 1b, analysis of variance F: 102, df: 23). The nociceptive response induced by acetic acid solution at 0.15% concentration was not different from that induced by saline ($P > 0.05$). However, acetic acid solutions at concentrations of 0.3% and 0.6% induced nociceptive responses significantly different from the control ($P < 0.001$). The nociceptive response induced by the 0.6% acetic acid solution was also significantly different from that induced by the 0.3% solution ($P < 0.001$).

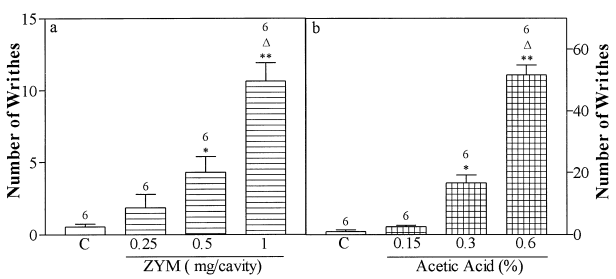

Fig. 1. Dose dependence of the nociceptive responses induced by zymosan and acetic acid. The bars show the number of writhes induced by the indicated doses of zymosan (ZYM, mg/cavity, panel a) and by 0.2 ml of acetic acid solution at the indicated concentrations (v/v, panel b). The number of writhes was determined from 0 to 30 min after injection of the stimuli. C in both panels represents the number of writhes induced by saline. The results are reported as means ± S.E.M. for the number of mice indicated at the top of each column. Analysis of variance, zymosan: F: 8519, total degrees of freedom: 23; acetic acid, F: 102, df: 23. The symbol * indicates statistically significant differences ($^{*}P < 0.01$, $^{**}P < 0.001$) between treated and control (C) groups. The symbol Δ indicates statistically significant differences ($P < 0.003$ for zymosan and $P < 0.01$ for acetic acid) compared with the dose of 0.5 mg zymosan or 0.3% acetic acid (ANOVA followed by Bonferroni's t-test).

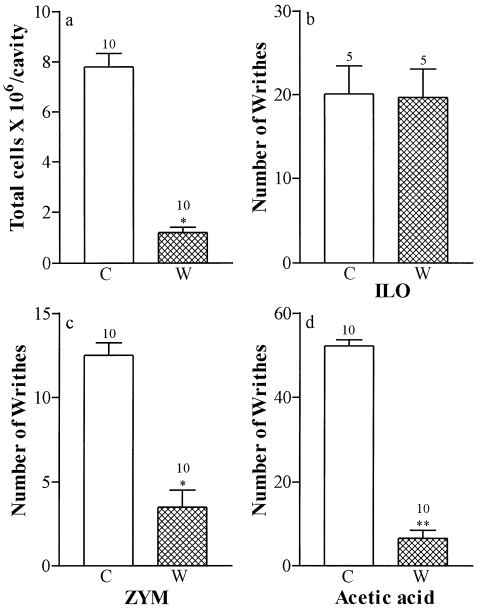

Fig. 2. Reduction in the total resident cell population of the mouse peritoneal cavity inhibits the nociceptive responses induced by intraperitoneal injection of zymosan and acetic acid but not of iloprost. Panel a: Number of resident cells in sham (C) and washed peritoneal cavities. Panels b, c and d represent the number of writhes induced by intraperitoneal injection of iloprost (ILO, 1 μg/cavity), zymosan (ZYM, 1 mg/cavity) and acetic acid (0.2 ml of a 0.6%, v/v solution/cavity) in sham (C) and washed peritoneal cavities (W), respectively. The number of writhes was determined from 0 to 30 min after injection of the stimuli and the results are reported as means ± S.E.M. for the number of mice indicated at the top of each column. Analysis of variance F: 115, df: 79. Asterisks indicate statistically significant differences ($^{*}P < 0.01$; $^{**}P < 0.001$) between the sham and washed groups (ANOVA followed by Bonferroni's t-test).

3.2. Effect of reduction of the resident peritoneal cell population on the nociceptive activities of iloprost, zymosan and acetic acid

Lavage of the peritoneal cavity of mice with saline caused a statistically significant reduction (85%, $P < 0.01$) of the total peritoneal resident cell population (Fig. 2, panel A). This procedure did not affect the nociceptive result for iloprost ($P > 0.05$, Fig. 2, panel B). In contrast, the nociceptive effects of 1 mg of zymosan (Fig. 2, panel C) and 0.2 ml of acetic acid solution at 0.6% concentration (Fig. 2, panel D) were significantly reduced, by 70% ($P < 0.01$) and 87% ($P < 0.001$), respectively.

3.3. Effect of the increase of the resident macrophage population on the nociceptive activities of iloprost, zymosan and acetic acid

Pretreatment of the peritoneal cavity of the mice with thioglycollate caused a significant increase (128%, $P <$

0.01) in the number of peritoneal resident macrophages (Fig. 3, panel A). This pretreatment did not affect the iloprost-induced nociception (1 μg/mouse, $P > 0.05$, Fig. 3, panel B). In contrast, the nociceptive effects of zymosan (1 mg/mouse, Fig. 3, panel C) and acetic acid (0.2 ml of 0.3% solution, Fig. 3, panel D) were increased by 151% ($P < 0.01$) and 123% ($P < 0.001$), respectively.

3.4. Effect of the depletion of the peritoneal mast cell population on the nociceptive activities of iloprost, zymosan and acetic acid

Chronic treatment of mice with compound 48/80 caused a significant (66%, $P < 0.001$) decrease in the peritoneal mast cell population (Fig. 4, panel A). This decrease did not affect iloprost-induced nociception (1 μg/mouse, $P > 0.05$, Fig. 4, panel B). In contrast, the nociceptive effects of zymosan (1 mg/mouse, Fig. 4, panel C) and acetic acid (0.2 ml of a 0.6% solution, Fig. 4, panel D) were significantly reduced, by 63% ($P < 0.001$) and 48% ($P < 0.001$), respectively.

3.5. Effect of anti-cytokine sera on the nociceptive activity of zymosan and acetic acid

Pretreatment of the peritoneal cavities of the mice with 50 μl of antisera against TNFα, interleukin 1β or interleukin 8 partially inhibited the nociceptive activities of zymosan (1 μg/mouse) and acetic acid (0.2 ml of a 0.6% solution) (Fig. 5, analysis of variance: zymosan F: 44, df: 29, acetic acid F: 31, df: 29). The nociceptive response induced by zymosan was inhibited by 57% ($P < 0.001$), 39% ($P < 0.001$) and 39% ($P < 0.001$) by the antisera against TNF-α, interleukin 1β and interleukin 8, respectively. Moreover, these antisera also inhibited by 69% ($P < 0.001$), 63% ($P < 0.001$) and 53% ($P < 0.01$) the nociceptive response induced by acetic acid, respectively. A control antiserum did not affect significantly ($P > 0.05$) the nociceptive activities of these stimuli.

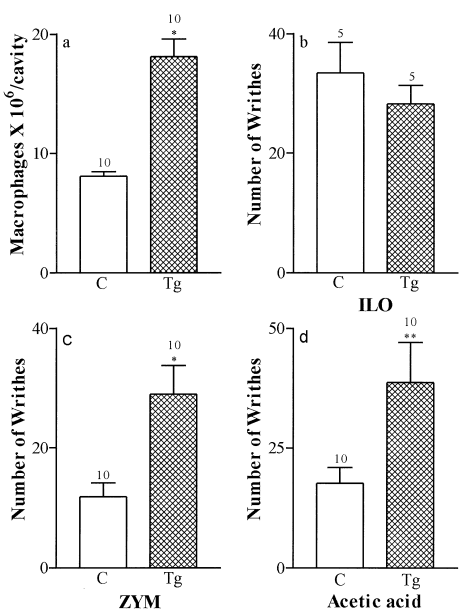

Fig. 3. Pretreatment with thioglycollate potentiates the nociceptive responses induced by zymosan and acetic acid, but not by iloprost. Panel a: Macrophage population in PBS- (C) and in thioglycollate- (Tg) pretreated groups. Panels b, c and d represent the number of writhes induced by intraperitoneal injection of iloprost (ILO, 1 μg/cavity), zymosan (ZYM, 1 mg/cavity), and acetic acid (0.2 ml of a 0.3%, v/v, solution/cavity) in control (C) and Tg-pretreated animals (W), respectively. The number of writhes was determined from 0 to 30 min after injection of the stimuli and the results are reported as means ± S.E.M. for the number of mice indicated at the top of each column. Analysis of variance F: 11, df: 79. Asterisks indicate statistically significant differences ($^*P < 0.01$, $^{**}P < 0.001$) between the control and Tg groups (ANOVA followed by Bonferroni's t-test).

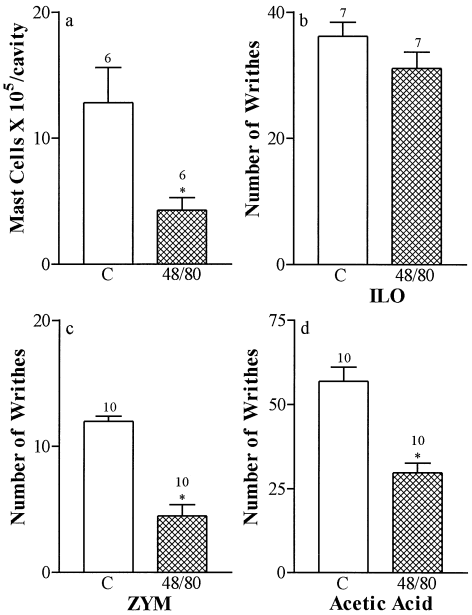

Fig. 4. Chronic pretreatment of mice with compound 48/80 the nociceptive responses induced by zymosan and acetic acid, but not by iloprost. Panel a: Number of peritoneal mast cells in PBS- (C) and compound 48/80-pretreated animals (48/80). Panels b, c and d represent the number of writhes induced by intraperitoneal injection of iloprost (ILO, 1 μg/cavity), zymosan (ZYM, 1 mg/cavity), and acetic acid (0.2 ml of a 0.6%, v/v, solution/cavity) in PBS- (C) and 48/80 compound-pretreated cavities (48/80), respectively. The number of writhes was determined from 0 to 30 min after injection of the stimuli and the results are reported as means ± S.E.M. for the number of animals indicated at the top of each column. Analysis of variance F: 208, df: 79. Asterisks indicate statistically significant differences ($^*P < 0.001$) between the control and 48/80 groups (ANOVA followed by Bonferroni's t-test).

3.6. Production of TNF-α and interleukin 1β by peritoneal cells stimulated with zymosan and acetic acid

Peritoneal cells harvested from the cavities of mice 15 min after administration of zymosan (1 mg/0.2 ml) or acetic acid (0.2 ml of a 0.6% solution) and incubated *in vitro* for 12 h released significant amounts of TNF-α ($P < 0.001$) and interleukin 1β ($P < 0.001$) into the supernatant compared to the release of cells harvested from cavities injected with saline (Fig. 6, analysis of variance

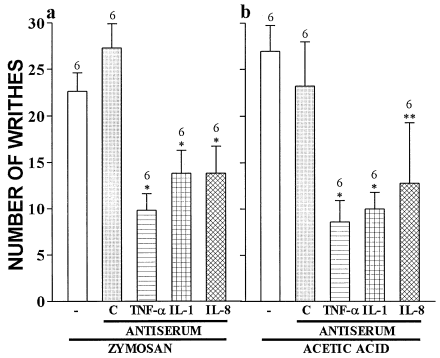

Fig. 5. Effect of antisera against murine TNF-α, interleukin 1β and human interleukin 8 on the nociception induced by zymosan and acetic acid. The animals were pretreated with an intraperitoneal injection of PBS (−), 50 μl of control serum (C) or of antisera against TNF-α, interleukin 1β (IL-1) or interleukin 8 (IL-8) and injected i.p. 10 min later with zymosan (1 mg/cavity, panel a) or acetic acid (0.2 ml of a 0.6% v/v solution/cavity, panel b). The number of writhes was determined from 0 to 30 min after injection of the stimuli and the results are reported as means ± S.E.M. for the number of animals indicated at the top of each column. Analysis of variance for zymosan F: 44, df: 29; acetic acid, 31, df: 29. Asterisks indicate statistically significant differences ($^{*}P < 0.001$, $^{**}P < 0.01$) between antiserum and saline treatments (ANOVA followed by Bonferroni's t-test).

F: 67, df: 28). The amounts of TNF-α and interleukin 1β released by the cells obtained from the peritoneal cavities injected with zymosan were not different from those released by the cells obtained from the peritoneal cavities injected with acetic acid ($P > 0.05$)

3.7. Nociceptive effects of interleukin 1β, interleukin 8 and TNF-α.

Intraperitoneal injection of interleukin 1β (0.05 and 100 ng/mouse), interleukin 8 (1.0 and 100 ng/mouse), or TNF-α (5.0 and 100 ng/mouse) did not induce significant nociceptive responses in mice ($P > 0.05$). In contrast, the combination of the three cytokines (100 ng of each/mouse) induced a significant nociceptive response ($P < 0.001$) compared with the nociceptive response induced by saline (Table 1, analysis of variance F: 43, df: 47).

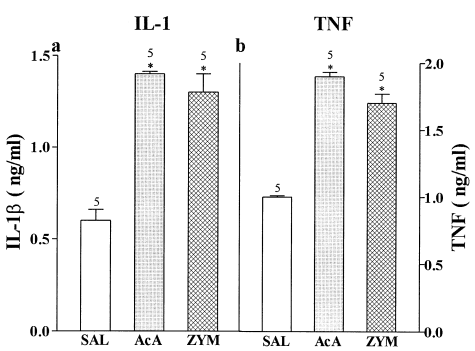

Fig. 6. Production of interleukin 1β and TNF-α by cells harvested from peritoneal cavities injected with acetic acid or zymosan. The animals were injected with saline (SAL, 0.2 ml/cavitiy), acetic acid (AcA, 0.2 ml of a 0.6%, v/v, solution/cavity) or zymosan (ZYM, 1 mg/cavity) and 15 min later the peritoneal cells were harvested and incubated in vitro for 12 h. The concentrations of interleukin 1β (IL-1, panel a) and TNF-α (panel b) were then determined by ELISA. The results are reported as means ± S.E.M. for the five wells. Analysis of variance: F: 67, df: 28. Asterisks indicate statistically significant differences ($P < 0.001$) compared to saline (ANOVA followed by Bonferroni's t-test).

Table 1
Nociceptive effect of recombinant TNF-α, interleukin 1β (IL-1β) or interleukin 8 (IL-8)
The number of writhes was determined from 0 to 30 min after cytokine injection and the results are reported as means ± S.E.M. for the indicated number of animals. Analysis of variance F: 43, df: 47.

Cytokine	Total number of writhes
Control	
PBS	1.0 ± 0.2 ($n = 6$)
Interleukin 1β	
0.05 ng/cavity	1.9 ± 0.7 ($n = 6$)
100 ng/cavity	1.4 ± 0.6 ($n = 6$)
Interleukin 8	
1.0 ng/cavity	2.1 ± 0.7 ($n = 6$)
100 ng/cavity	1.4 ± 0.5 ($n = 6$)
TNF-α	
5.0 ng/cavity	1.0 ± 0.3 ($n = 6$)
100 ng/cavity	2.8 ± 0.8 ($n = 6$)
IL-1β + IL-8 + TNF-α	
100 ng each	10.5 ± 2.6[a] ($n = 6$)

[a] Indicates statistically significant differences ($P < 0.001$) between cytokine- and PBS-injected animals. (ANOVA, followed by Bonferroni's t-test).

4. Discussion

We now report that intraperitoneal administration of zymosan or acetic acid induces a dose-dependent writhing response in mice. The doses of zymosan and acetic acid were similar to those described by other authors, who demonstrated that these stimuli cause nociception by a mechanism involving eicosanoids and/or sympathomimetic amines (Doherty et al., 1985, 1987; Duarte et al., 1988; Santos et al., 1988) . We and other investigators have shown previously that eicosanoid and sympathomimetic amines also mediate the mechanical hyperalgesia induced by inflammatory stimuli such as carrageenin and lipopolysaccharide (Ferreira et al., 1978, 1993; Moncada et al., 1975; Nakamura and Ferreira, 1987; Tracey et al., 1995). Moreover, the release of prostaglandins or sympathomimetic amines is secondary to the release of pro-inflammatory cytokines by the tissue resident cells (Ferreira et al., 1988, 1993; Cunha et al., 1991, 1992).

To investigate whether the resident cells are involved in the release of the nociceptive mediators in the writhing model, we analyzed the nociceptive activities of zymosan

and acetic acid in animals depleted of peritoneal resident cells. Lavage of the peritoneal cavities with saline reduced the population of resident cells by 86%. In these animals the nociceptive responses induced by zymosan and acetic acid were reduced by 73% and 87%, respectively. However, lavage of the cavities did not affect the nociceptive response to iloprost, a stable analogue of prostacyclin, which is a final nociceptive mediator (Ferreira et al., 1978). This fact excludes the possibility that the reduction in nociception observed when zymosan and acetic acid are injected into the washed cavities is a consequence of the decrease in the nociceptive responsiveness of the cavities due to the washing procedure. In agreement with this conclusion, washing of the peritoneal cavities with saline also did not reduce the responses to chemotactic factors such as MNCF (macrophage-derived neutrophil chemotactic factor), FMLP (*N*-formyl-methionyl-leucyl-phenylalanine) and C5a (fifth component of activated complement), which cause neutrophil migration by mechanisms not dependent on resident cells (Souza et al., 1988; Ribeiro et al., 1997). Thus, these results suggest that zymosan and acetic acid induce nociception through a mechanism dependent on resident cells.

Lavage of the peritoneal cavities with saline removes most of the free cells present in the cavities, including macrophages and mast cells (Souza et al., 1988). To determine which of these cell types are stimulated by zymosan or acetic acid, we injected these agents into mouse peritoneal cavities in which the number and activity of the macrophages had been increased by previous treatment with thioglycollate (Cunha and Ferreira, 1986), or in which the mast cell population was depleted by chronic pretreatment with compound 48/80 (Di Rosa et al., 1971). Neither treatment affected the nociceptive activity of iloprost, suggesting that these procedures also did not interfere with the nociceptive responsiveness of the peritoneal cavity. Furthermore, in previous experiments we had shown that neither thioglycollate nor compound 48/80 treatment interfered with the responsiveness of the cavities to the neutrophil chemotactic factors, C5a or FMLP (Ribeiro et al., 1997). The number of writhes induced by zymosan and by acetic acid was increased by pretreatment of the peritoneal cavities with thyoglycollate. In contrast, reduction of the mast cell population by pretreatment of the mice with compound 48/80 significantly reduced the nociceptive responses to zymosan and acetic acid. These data clearly suggest that the writhing response induced by zymosan and acetic acid in mice depends upon the activation of both peritoneal macrophages and mast cells.

We next investigated the possibility that zymosan and acetic acid stimulate the release of TNF-α, interleukin 1β and interleukin 8 by the peritoneal cells and that these cytokines mediate their nociceptive activities. Mouse peritoneal cavities were treated with antisera against TNF-α, interleukin 1β and interleukin 8 and zymosan or acetic acid injected intraperitoneally. We chose these antibodies because TNFα, interleukin 1β and interleukin 8 mediate the release of eicosanoids and symphathomimetic amines in a model of mechanical hyperalgesia (Cunha et al., 1992). Furthermore, these cytokines mediate the nociceptive activity of lipopolysaccharide-stimulated macrophage supernatants in mice (Thomazzi et al., 1997). All three antisera partially inhibited the nociceptive responses to zymosan and acetic acid. This partial inhibition was not due to the use of inadequate doses of the antisera, since the same doses were able to abolish the mechanical hyperalgesic effects of their respective cytokine (Cunha et al., 1992). These results suggest that the nociceptive activities of zymosan and acetic acid are mediated by TNF-α, interleukin 1β and interleukin 8 which are released by resident peritoneal macrophages and mast cells. Confirming this suggestion, it was observed that peritoneal cells harvested from the cavities 15 min after zymosan or acetic acid administration and incubated in vitro for 12 h released significant amounts of TNFα and interleukin 1β into the incubation medium.

Furthermore, further supporting the idea that TNF-α, interleukin 1β and interleukin 8 are concomitantly involved in the writhing response, we showed that no cytokine alone caused nociception, but that the three injected together into the peritoneal cavities of mice caused a significant nociceptive response. Thus, the nociceptive effect results from a synergistic effect rather than from an additive effect of these three cytokines.

In conclusion, our results suggest that the nociceptive activity of zymosan and acetic acid in the writhing model may be due to the release of TNF-α, interleukin 1β and interleukin 8 by resident peritoneal macrophages and mast cells.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Ieda R. dos Santos, Giuliana Bertozzi Francisco and Sérgio Roberto Rosa. This work was supported by grants from FAPESP, Pronex and CNPq (Brazil).

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