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INFLAMMATORY RESPONSE INDUCED BY INTRAPLEURAL INJECTION OF ANTISERUM TO IgE IN RAT

AN EVALUATION OF THE ROLE OF HISTAMINE*

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Abstract—Intrapleural injection of antiserum to rat IgE (anti-IgE) into rats resulted in release of histamine from mast cells and rapid effusion of fluid and plasma proteins into the pleural cavity. By 4 hr this was followed by infiltration of neutrophils. These responses were dependent on the amount of anti-IgE injected, and maximal responses were greater than those obtained with compound 48/80. The effusion of fluid and protein, but not the infiltration of cells, was partially suppressed by prior treatment with the H_1 histamine receptor antagonist mepyramine (5 mg/kg, s.c.) or the H_2 antagonist metiamide (100 mg/kg, s.c.) and was almost totally suppressed (85–88%) when both drugs were administered simultaneously. Neither methysergide (1 and 4 mg/kg, s.c.) nor indomethacin (5 and 10 mg/kg, i.v.) had an effect on the responses to anti-IgE. Although it seemed likely that histamine was a primary mediator of increased vascular permeability, the intrapleural injection of histamine agonists or histamine in large amounts (50 μg) provoked a much less intense response than did anti-IgE. The effects of injected histamine may not, therefore, mimic those induced by histamine released from mast cells *in situ*. The intrapleural injection of histamine releasers such as anti-IgE may serve as a useful model to test the therapeutic efficacy of antihistamine drugs. The present results also confirm previous reports that localized neutrophil infiltration occurs after mast cell degranulation.

Histamine is released from mast cells in response to some inflammatory stimuli, but its importance as an inflammatory mediator in relation to other products released during mast cell degranulation has not been well defined except in a few instances. The injection of compound 48/80 or dextrans into rat pleural cavity or the scalding of rat paws, for example, was shown to induce rapid histamine release and development of edema [1, 2]. The time course and extent of histamine release were highly correlated with the initial edema response to scalding [1] or to the injection of dextrans of various molecular weights [3]. The initial edema reaction was much less intense in rats pretreated with compound 48/80 or a combination of H_1 and H_2 histamine receptor antagonists [4, 5]. Delayed responses to heat or ultraviolet injury, however, could not be attributed to histamine release [1, 6]. Some workers have concluded that any effect of histamine release in response to inflammatory stimuli must occur during the early phases of the inflammatory reaction [7, 8]. Moreover in rats, serotonin and other tissue mediators are much more potent inflammatory agents than is histamine when these substances are injected into the rat paw or the pleural cavity [9–11].

To analyze the role of mast cell derived mediators, and in particular histamine, we thought that it might be instructive to use specific mast cell degranulating agents that are now available commercially. We report here that mast cell degranulation induced by intrapleural injection of antisera to IgE resulted in extensive plasma protein leakage and neutrophil infiltration. Evidence is presented that the leakage of plasma proteins was mediated primarily by histamine but that this response was not mimicked by injection of histamine. The responses are compared with those induced by a chemical mast cell degranulating agent, compound 48/80, and a nonhistamine releasing inflammatory agent, carrageenan [4, 9, 10].

MATERIALS AND METHODS

Materials. Sheep and goat antisera to rat IgE were purchased, respectively, from Miles Laboratories Inc., Elkhart, IN, and from Pel Freez Biologicals, Rogers, AR; histamine (di HCl) was purchased from Mann Research Laboratories, Inc., New York, NY; carrageenan (Viscarin carrageenan, RENJ-6755) and compound 48/80 were obtained, respectively, from Marine Colloids, Inc., Springfield, NJ, and Burroughs Wellcome Inc., Tuckahoe, NY. Metiamide and impromidine (tri HCl) and 2-pyridyl-β-ethylamine were gifts from Dr. Robin Ganellin, SKF Laboratories, Welwyn Garden City, England. Methysergide maleate was supplied by Sandoz Pharmaceuticals, East Hanover, NJ. Other materials were purchased from the following companies: indomethacin from the Sigma Chemical Co.; pyrilamine (mepyramine) maleate from K & K Laboratories,

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Inc., Hollywood, CA; dimethylsulfoxide from Pierce, Rockford, IL; Dulbecco's phosphate-buffered saline (without Ca^{2+} and Mg^{2+}) from GIBCO, Long Island, NY; and bovine serum albumin (fraction V) from the Armour Pharmaceutical Co., Chicago, IL. Sheep serum (96 mg protein/ml) was prepared from freshly drawn blood by the Division of Research Services (Poolesville Farm), NIH.

Male Sprague–Dawley rats (240–270 g body wt) were obtained from the Division of Research Services and Taconic Farms, Inc., Germantown, NY. The rats were housed at 24° and were maintained on Purina laboratory chow and distilled water *ad lib*.

Preparation and injection of drugs and inflammatory agents. Indomethacin (25 or 50 mg) or methysergide maleate (10 or 40 mg) was dissolved in 200 μl dimethyl sulfoxide and then diluted with 10 ml normal saline. Pyrilamine maleate (5 mg/ml), metiamide (100 mg/ml), histamine (0.5 mg/ml), impromidine (0.5 mg/ml), 2-pyridyl-β-ethylamine (5 mg/ml) and compound 48/80 (0.5 mg/ml) were dissolved in normal saline. The drug solutions were neutralized with NaOH or HCl and diluted with saline where necessary; the amounts indicated refer to the free base. Lyophilized antiserum to IgE (Miles Laboratories, Code No. 64-352) was reconstituted in 2 ml sterile water as recommended by the supplier. The reconstituted serum (78 mg protein/ml) was diluted with normal saline, and 0.1 ml of the diluted anti-IgE serum was then injected into the pleural cavity. Unless specified otherwise, the anti-IgE provided by Miles was used throughout this study.

Indomethacin was injected i.v. in a volume of 0.5 ml. Other drugs were injected s.c. in a volume of 0.25 ml. Vehicle (saline or 2% dimethylsulfoxide in saline) was injected into control rats. Thirty minutes after administration of drug, rats were lightly anesthetized with ether, and the solution of carrageenan (500 μg/0.1 ml), compound 48/80 (10–100 μg/0.1 ml) or diluted anti-IgE (0.1 ml) was injected into the pleural cavity. To study the response of rats to histamine, impromidine and 2-pyridyl-β-ethylamine, 0.1 ml of each of these agents was injected intrapleurally.

Collection and processing of pleural exudate. Thirty minutes or four hours after the intrapleural injection of inflammatory agents, rats were killed in an ether-saturated chamber. The chest was opened, the exudate was removed by aspiration, and the cavity was washed with 1 ml of 5 mM EDTA in Dulbecco's phosphate-buffered saline (without Ca^{2+} and Mg^{2+}). The volume reported was corrected by subtracting 1 ml from the observed total volume.

A portion of the exudate (plus washings) was centrifuged at 600 *g* for 10 min at 4°, and the supernatant fluid was assayed for protein by the method of Lowry *et al.* [12] using bovine serum albumin (fraction V) as a standard. Another portion was removed to analyze total cellular and extracellular histamine as previously described [2, 4] by a single step enzymatic assay [13]. Extracellular histamine accounted for a minor portion (<2% in vehicle-treated rats and generally <10% in IgE-treated rats) of the total histamine in the exudate. Data reported here are expressed as total histamine recovered from the cell pellets.

The remaining exudate was processed for total and differential cell counts as previously described [2]. For total cell number, cells were suspended in a mixture of Isoton and LAS reagent (Fisher Scientific Co., Fairlawn, NJ) and counted in a Particle Data Counter (Particle Data Inc., Elmhurst, IL). For differential cell counts, samples of the cell suspension (about 150,000 cells) were centrifuged onto glass slides in a cytocentrifuge (Shandon Elliott, Shandon Scientific Co., London, England). The slides were air-dried and stained with 0.3% (w/v) polycrome methylene blue in a Hema-Tek slide stainer (Ames Co., Division of Miles Laboratories Inc., Elkhart, IN). For further identification of eosinophils, additional slides were stained with fast green stain (0.2%, w/v) (Fisher Scientific Co.) and counterstained with neutral red (1%, w/v) (Fisher Scientific).

All variables measured, i.e. cell numbers, protein, fluid and histamine, are total amount per cavity and are given as mean ± S.E. of the mean value.

RESULTS

Characteristics of inflammatory response to anti-IgE. Within 30 min after the intrapleural injection of various doses of sheep antiserum to rat IgE (Miles Laboratories Inc.), fluid and protein had entered the pleural cavity space and by 4 hr white cells had infiltrated as well (Fig. 1). Cell infiltrates were largely neutrophils; no significant increases in eosinophils or other cell types were observed (Table 1). These responses depended on the amount of antisera injected over the range 2.5 to 100 μl (0.2 to 7.8 mg protein) antisera. Other studies (Table 2) indicated similar responses to goat antisera to rat IgE from another manufacturer. Normal sheep serum induced neither histamine release (data not shown) nor inflammatory response. It was evident, however, that injected serum proteins were cleared from the cavity.

The histamine content of pleural cells collected from saline-injected rats ranged between 6.5 and 8.1 μg (legend, Fig. 1). Significant ($P < 0.05$) histamine release from the pleural cells was apparent by 30 min with the injection of as little as 5 μl (0.4 mg protein) of the antisera. Maximal release was observed with 40–100 μl (3.1 to 7.8 mg protein) of antisera (Fig. 1).

Histamine release appeared to be complete by 30 min, as intracellular histamine levels showed little further decrease between 30 min and 4 hr. The inflammatory response was sustained, however, for at least 4 hr. At this time the concentration of protein in the exudate (58–81 mg/ml) in response to the highest dose of anti-IgE was similar to that of plasma (70 ± 5 mg/ml) (Fig. 1).

Effects of histamine agonists and antagonists. There was a barely detectable infiltration of fluid or protein when histamine was injected directly into the pleural cavity, and even a dose as high as 50 μg had small effect (Fig. 2). The responses to the H_2 and H_1 histamine receptor agonists impromidine and pyridylethylamine were also small. The smallest responses were observed with impromidine, the greatest with pyridylethylamine, and the mixture of

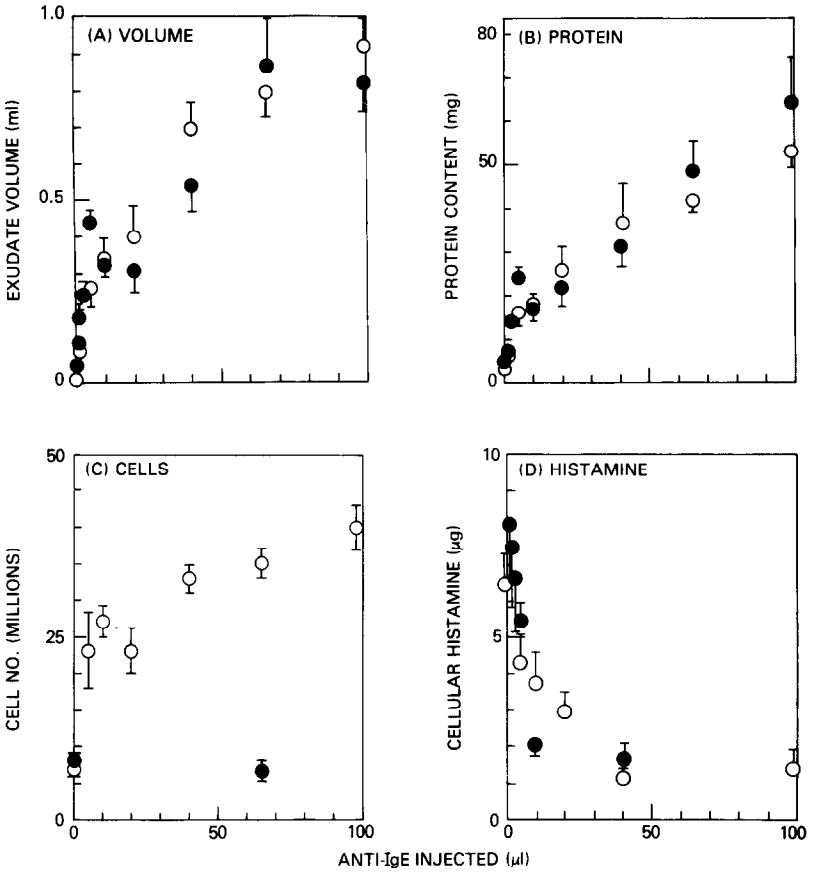

Fig. 1. Inflammatory response evoked by intrapleural injection of different doses of antisera to rat IgE as determined by volume (A), protein content (B), number of cells (C), and histamine content (D) of exudates collected 30 min (●) and 4 hr (○) after the injection of saline or the indicated amount of antisera (diluted to 100 μl). The histamine content of washings (see Materials and Methods) collected from the pleural cavity of saline-injected rats ranged between 6.5 ± 0.7 and 8.1 ± 1.5 μg. Other values and bars are means ± S.E.M. of the histamine content of exudates from anti-IgE-injected rats (N = 5–8).

the H_1 and H_2 agonist was intermediate between these two extremes (Fig. 2). The doses of agonists used produced the maximum response attainable, and no further attempt was made to determine order of potency.

Despite the weak inflammatory reaction to the histamine and its agonists, antihistamine drugs were very effective in suppressing the inflammatory response to anti-IgE (Fig. 3). Mepyramine (5 mg/kg) or metiamide (100 mg/kg), given subcutaneously

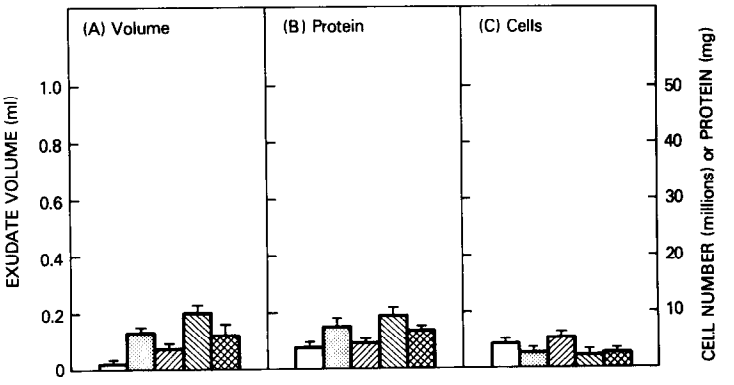

Fig. 2. Inflammatory response to histamine and histamine agonists injected into the pleural cavity. The volume (A), protein content (B) and number of white cells (C) of exudates collected 30 min after the injection are shown. Key: saline (□), histamine, 50 μg (▦), impromidine, 50 μg (▨), pyridylethylamine, 500 μg (▧), and impromidine, 50 μg, plus pyridylethylamine, 500 μg (▩). All drugs were injected in a volume of 0.1 ml. Mean and S.E.M. of values obtained from five to six rats are shown.

Table 1. Cell populations in exudates collected 30 min and 4 hr after intrapleural injection of antisera to rat IgE (anti-IgE)*

Anti-IgE injected (μl)	N	Cell No. (millions)			
		Monocytes	Eosinophils	Mast cells	Neutrophils
30 min					
0	6	5.8 ± 0.4	0.6 ± 0.1	0.6 ± 0.1	0
1.25	6	5.2 ± 0.3	0.8 ± 0.1	†	0.2 ± 0.2
40	6	4.3 ± 0.3	0.7 ± 0.1	†	1.1 ± 0.2
4 hr					
0	10	5.3 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0
5	6	9.2 ± 0.9	0.9 ± 0.3	†	12.5 ± 1.3
40	7	4.0 ± 0.2	0.6 ± 0.1	†	29.0 ± 1.5
66	7	4.1 ± 0.3	0.8 ± 0.2	†	29.6 ± 0.7
66‡	8	4.4 ± 0.9	0.5 ± 0.2	†	29.7 ± 1.0

* Values are mean ± S.E.M. of the number (N) of animals indicated. Antisera was diluted in normal saline (volume shown is the actual amount of antisera injected: 100 μl contained 7.8 mg protein) and injected in a volume of 0.1 ml intrapleurally. The cell number was calculated from differential cell counts and total cell count.

† Almost all recognizable mast cells were degranulated to various extents.

‡ Rats were pretreated with indomethacin (10 mg/kg, i.v.)

30 min before the injection of antiserum, partially suppressed fluid and protein infiltration and when given in combination they almost totally suppressed (85–88%) infiltration of both fluid and protein. Cell infiltration, however, was not suppressed by either drug although a slight reduction in cell count was observed when the drugs were given in combination (Fig. 3). As reported by others [11, 14], the antihistamines may partially inhibit mast cell degranulation: the histamine contents of pleural cells from the saline- and the anti-IgE-injected rats were 7.7 ± 0.5 and 3.4 ± 0.7 μg, respectively, compared to 4.6 ± 0.9 μg in rats pretreated with a combination of the two antihistamine drugs. In another experiment (ten animals/group), the values were 2.5 ± 0.5 and 3.8 ± 0.4 μg histamine, respectively, for rats treated with anti-IgE alone and those treated with antihistamines before injection of anti-IgE. These values represent a 29 and 44% reduction in histamine release.

Comparisons with other inflammatory agents and antagonists. As with anti-IgE, compound 48/80 injected intrapleurally induced fluid and protein infiltration (Table 3). There was, however, no well defined relationship between dose and response over the range of doses tested, and the maximal effects observed were less than those obtained in response to anti-IgE (compare Table 3 with Fig. 1). In agreement with previous data [2], no cell infiltration was observed with high doses of compound 48/80, but

Table 2. Inflammatory response 30 min following the intrapleural injection of antisera to rat IgE and other agents: Control experiments*

Treatment (mg protein or drug)	N	Exudate composition		
		Volume (ml)	Protein (mg)	Cell No. (millions)
Experiment 1				
Saline	6	0.02 ± 0.01	4 ± 1	2 ± 1
Sheep serum (0.5)	5	0.07 ± 0.02	4 ± 1	2 ± 1
Anti-IgE† (0.4)	6	0.46 ± 0.03	26 ± 2	1 ± 1
Anti-IgE‡ (0.9)	6	0.11 ± 0.04	7 ± 1	2 ± 1
Anti-IgE‡ (8.9)	6	0.46 ± 0.07	24 ± 2	2 ± 1
Experiment 2				
Saline	5	0.04 ± 0.03	2 ± 1	7 ± 1
Sheep serum (9.6)	5	0.02 ± 0.03	4 ± 1	ND§
Anti-IgE† (7.8)	5	0.96 ± 0.13	70 ± 12	ND
Compound 48/80 (0.05)	5	0.57 ± 0.09	37 ± 4	8 ± 2

* Values are mean ± S.E.M. All samples were diluted in isotonic salone to give a final volume of 0.1 ml.

† Sheep antisera to rat IgE, Miles Laboratories Inc. (78 mg protein/ml).

‡ Goat antisera to rat IgE, Pel Freez Biologicals (89 mg protein/ml).

§ Not determined.

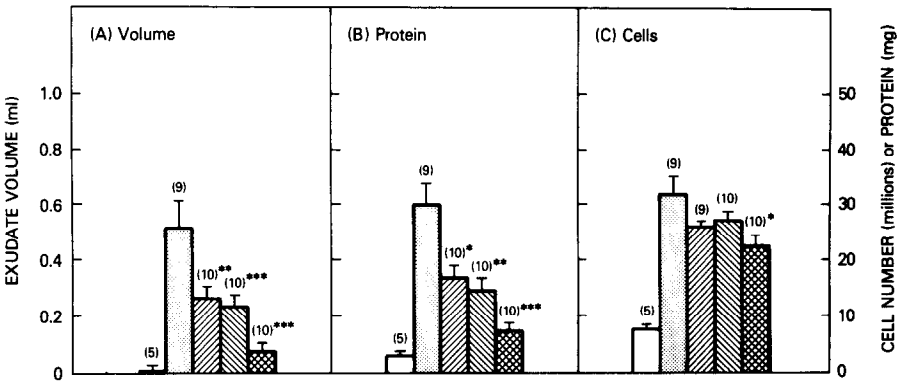

Fig. 3. Effects of H_1 (mepyramine, 5 mg/kg, s.c.) and H_2 (metiamide 100 mg/kg, s.c.) histamine antagonists, given individually or in combination, on the inflammatory response to anti-IgE. The antihistamine drugs or saline were administered 30 min before the intrapleural injection of the antisera (66 μl). Exudates were collected 4 hr thereafter. Bars are mean and brackets S.E.M. of values obtained for the number of animals indicated in parentheses. Key: saline-injected rats (□), rats injected with anti-IgE (▩) or rats injected with anti-IgE but previously injected with metiamide (▨), mepyramine (▧) or both metiamide and mepyramine (■). Asterisks indicate significant differences from rats treated with antisera alone: (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$.

modest cell infiltration was observed in the present studies with doses lower than the 50 μg dose used previously. Neutrophils accounted for 11–62% of the cells present in these exudates compared with 0–4% in saline-injected rats.

Indomethacin did not reduce the inflammatory reaction to anti-IgE (Table 1 and Fig. 4) or compound 48/80 (Table 3) at doses (5 and 10 mg/kg, i.v.) that reduced appreciably the inflammatory responses to carrageenan (Table 3).* The anti-serotonin drug methysergide (1–4 mg/kg) was also without effect when tested against anti-IgE and com-

* The lipoxygenase inhibitor nordihydroguaiaretic acid (50–100 mg/kg) and the lipoxygenase/cyclooxygenase inhibitor BW 755c (20–50 mg/kg) also had minimal effects on fluid, protein and cell infiltration induced by intrapleural injection of anti-IgE but they did suppress the responses to carrageenan (three experiments).

Table 3. Inflammatory response 4 hr following the intrapleural injection of compound 48/80 or carrageenan: Effect of indomethacin*

Treatment (dose, μg)	Inhibitor	N	Exudate composition Volume (ml)	Protein (mg)	Cell No. (millions)
Compound 48/80					
0		6	0.02 ± 0.01	4 ± 0.5	5 ± 1
10		7	0.33 ± 0.09†	16 ± 2†	15 ± 3†
25		9	0.31 ± 0.04†	17 ± 1†	11 ± 1†
50		9	0.34 ± 0.04†	18 ± 2†	8 ± 1
100		5	0.40 ± 0.07†	29 ± 3†	5 ± 1
Compound 48/80					
50	Vehicle	6	0.81 ± 0.12	39 ± 6	ND‡
50	Indomethacin	6	0.74 ± 0.28	41 ± 5	ND
Carrageenan					
500	Vehicle	9	0.83 ± 0.04	53 ± 2	113 ± 6
500	Indomethacin	6	0.35 ± 0.05†	22 ± 3†	65 ± 6†

* Values are mean ± S.E.M. for the number (N) of animals indicated. Compound 48/80 or carrageenan was injected intrapleurally in a volume of 0.1 ml. As noted in the second column some animals were injected with vehicle or indomethacin (5 mg/kg), i.v., 30 min prior to intrapleural injection of inflammatory agents.

† Significant difference from saline- or vehicle-treated rats, $P < 0.01$.

‡ Not determined.

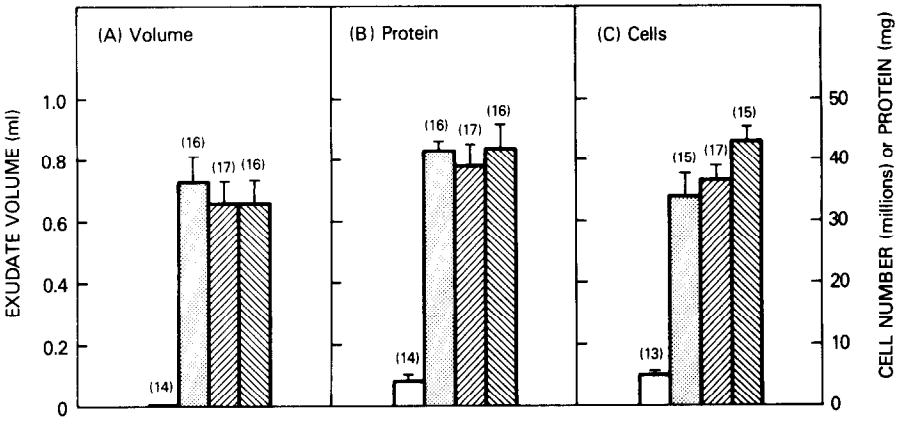

Fig. 4. Effect of indomethacin on the inflammatory response to anti-IgE. The drug was administered in doses of 5 or 10 mg/kg, i.v., 30 min before the intrapleural injection of the antisera (66 μl); the exudates were collected 4 hr thereafter. Volume (A), protein content (B) and number of white cells (C) in the exudates are shown. Bars and brackets depict mean ± S.E.M. of values for the number of animals indicated in parentheses. Key: saline (□), antisera (▨) or anti-IgE given to rats previously injected with indomethacin, 5 mg/kg (▨) or 10 mg/kg (▨).

pound 48/80. In a typical experiment, fluid and protein content of the exudate at 30 min was 0.53 ± 0.06 ml and 34 ± 4 mg for rats given anti-IgE and 0.55 ± 0.04 ml and 33 ± 2 mg for rats given methysergide (1 mg/kg, s.c.) before the intrapleural injection of anti-IgE (66 μl or 5.2 mg protein).

DISCUSSION

Early studies by Dale and others raised the possibility that histamine was an important inflammatory mediator in tissues. These reports showed that histamine produced a wheal and flare reaction [15] and was present in and released from tissues [16, 17] in sufficient quantities to initiate such reactions. Dale, however, cautiously noted that there was "no justification for assuming that histamine is the only cell constituent which injury liberates" [18]. Indeed, mast cell degranulation is now known to be associated with release or generation of a variety of active factors which include serotonin (in rat), histamine, prostaglandins, leukotrienes, chemotactic factors (for eosinophils and neutrophils) and proteolytic enzymes [see 19–23]. Although increases in plasma histamine levels have been shown to correlate with pathophysiological reactions in certain types of urticarial and anaphylactic phenomena ([24–28] and see [29]), the specific role of histamine in these reactions is still unclear. In experimental models of inflammation, as opposed to hypersensitivity reactions, histamine is present in exudates produced in response to various stimuli [1, 5, 7, 8]. In some cases, the time–course and extent of histamine release are highly correlated with the degree of initial edema reaction, but these findings alone do not preclude the involvement of other possible mediators [9].

The present studies were undertaken to evaluate the usefulness of the intrapleural route of administration of anti-IgE as a model to evaluate the effects of mast cell degranulation *in vivo*. Antisera to IgE has been widely used to induce mast cell degranulation *in vitro* [e.g. 30, 31], and *in vivo* [e.g. 23, 32], and the pleural cavity provides a convenient site for collection of inflammatory mediators and infiltrates. As has been observed upon injection of anti-IgE into rat skin [23, 32], injection of the antibody into the pleural cavity resulted in infiltration of plasma proteins and neutrophils into the cavity. Effusion of proteins and fluid but not neutrophil infiltration were suppressed by H_1 and H_2 histamine receptor antagonists which may act, in part, as antidegranulating agents. Indomethacin and methysergide were without effect. The same pattern was observed following the intrapleural injection of compound 48/80; the leakage of plasma protein was prevented by antihistamines [4] but not by indomethacin (Table 3) or methysergide. These results suggested that cyclooxygenase-derived products of arachidonic acid and serotonin were not responsible for protein leakage, but it should be noted that serotonin was reported to be a potent inflammatory agent when injected into the rat paw [10, 11] but not when it was injected into the rat pleural cavity [10].

In contrast to anti-IgE and compound 48/80, carrageenan does not cause histamine release [4, 9], and the inflammatory response is suppressed by indomethacin (Table 3; and Refs. 2 and 33) but not by antihistaminic drugs [4]. These results indicate that the activities of drugs currently available can be used to distinguish inflammatory reactions in which histamine is the principal mediator from those in which it is not.

Interestingly, histamine is reported to be a weak inflammatory agent in rat [9–11] and, as reported here, the intrapleural injection of histamine agonists or histamine evoked small responses. The implication from these results is that injection of exogenous histamine may not mimic the effects of histamine released *in situ* and that a particular mediator should not be dismissed merely because it has weak or transient actions upon injection. As noted elsewhere, histamine is rapidly eliminated or inactivated in the rat pleural cavity [2, 4]. Injected histamine might also be less effective than that released locally in

high concentration from mast cells in the adventitia of small blood vessels. Synergistic action between histamine and, for example, prostaglandin E_1 [34, 35], which by itself has little permeability increasing activity [36], may be an additional factor. The persistence of the inflammatory response 4 hr after injection of anti-IgE when histamine release was complete certainly suggested that secondary mediators might be involved.

The neutrophil infiltration caused by antisera to IgE (and in low doses, compound 48/80) was largely resistant to the actions of the antihistamines and indomethacin. It would therefore appear unlikely that histamine or cyclo-oxygenase products were important mediators of this response. A high molecular weight neutrophil chemotactic factor has been detected in human leukemia basophils, human lung fragments [25], human serum during reactions associated with mast cell degranulation [21, 25, 37, 38] and rat mast cell granules [23, 32]. Soluble chemotactic factors have been isolated from the rat mast cell granule matrix and a highly purified factor (an oligopeptide of mol. wt 1400) has been shown to be capable of eliciting neutrophil infiltration in submicrogram quantities when injected into rat skin [23, 32]. Cell infiltration induced by the granule factors was not blocked by prior treatment with a combination of H_1 and H_2 histamine receptor antagonists, whereas partial attenuation of cell infiltration that was induced by anti-IgE was observed upon treatment with these drugs [39]. In the latter study, skin blueing reactions to anti-IgE were only partially suppressed with the antihistamine drugs. Thus, the effects noted with injection of anti-IgE into the rat pleural cavity are in general agreement with those observed in the studies in rat skin [23, 32, 39] even though subtle differences may exist with the two routes of administration. However, studies in the rat pleural cavity offer distinct advantages because of the ease with which exudates can be collected and analyzed.

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