EFFECTS OF ANTI-INFLAMMATORY DRUGS ON TRIPLE VACCINE-INDUCED PLEURISY IN RATS

Hisashi SATOH, Kyoichi SHIMOMURA, Sueo MUKUMOTO, Kaname OHARA and Jo MORI
Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., Yodogawa-ku, Osaka 532, Japan
Accepted June 5, 1982

Abstract—Effects of various anti-inflammatory drugs on triple vaccine-induced pleurisy, a model of delayed hypersensitivity, were examined and compared with those on carrageenin-induced pleurisy in rats. Steroidal drugs depressed markedly the volume of exudate and the number of leucocytes in both types of pleurisy. Gold compounds also depressed both types of pleurisy. Non-steroidal anti-inflammatory drugs were apt to show depressive effects on carrageenin-induced pleurisy, especially on increased exudate volume. BW755C produced a depressive effect on carrageenin-induced pleurisy, but on triple vaccine-induced pleurisy, BW755C produced only a slight depressive effect. Cyproheptadine produced a slight depressive effect on carrageenin-induced pleurisy, but not on triple vaccine-induced pleurisy. Promethazine had a slight depressive effect on both types of pleurisy. D-penicillamine and levamisole did not show any depressive effects on triple vaccine-induced pleurisy. The results show that reported mediators in carrageenin-induced pleurisy (prostaglandin, serotonin, leukotriene B, etc.) are not relevant to triple vaccine-induced pleurisy. Specific lymphokines and/or degraded products of complement may participate in the latter. This triple vaccine-induced pleurisy seems to be a good model for screening non-steroidal anti-inflammatory drugs which have steroidal-like activity.

Intrapleural injection of various agents, including carrageenin (1, 2), dextran (3), and silver nitrate (4), have been reported to induce inflammation in the pleural cavity of rats. Exudate rich in leucocytes accumulates according to the course of inflammation. The intensity of inflammation is related to the volume of exudate and the number of leucocytes in the exudate. Recently, Dieppe et al. (5) have reported that the intrapleural injection of Bordetella pertussis in sensitized rats produces an inflammatory reaction of delayed hypersensitivity in the pleural cavity. Mononuclear cells were more numerous than polymorphonuclear cells in the leucocyte population of the exudate in vaccine-induced pleurisy, whereas, most of the leucocytes were polymorphonuclear cells in the inflammation induced by intrapleural injection of carrageenin (2).

Effects of anti-inflammatory drugs on carrageenin-induced pleurisy have been studied (1, 2). On the other hand, the effects of drugs on pleurisy of delayed hypersensitivity have been studied with only a few drugs (5, 6). Therefore, we studied the effects of some drugs on vaccine-induced pleurisy in comparison with those on carrageenin-induced pleurisy in rats.
MATERIALS AND METHODS

Animals: Five to 10 male Sprague-Dawley rats, weighing 250–350 g, were used in one group.

Vaccine-induced inflammation of delayed hypersensitivity in the pleural cavity (vaccine pleurisy): The triple vaccine (pertussis organisms, diphtheria, and tetanus toxoid) was purchased from the Research Institute for Microbial Disease, Osaka University, Japan. Animals were sensitized by subcutaneous injection of 0.2 ml of an emulsion, made from a mixture (V/V: 50/50) of triple vaccine and Freund’s complete adjuvant (Difco), into the dorsal surface of the right front and hind paws. In the preliminary experiments, Freund’s incomplete adjuvant was also used in some groups. At various days after sensitization, rats were challenged intrapleurally with 0.1 ml of triple vaccine under light ether anaesthesia. Intrapleural injection was carried out according to the method of Tarayre et al. (7). At various intervals after challenge, the rats were killed by chloroform anaesthesia; and the pleural exudate was collected, and its volume was measured. The pleural cavity was then washed with 1.0 ml of Medium 199 to collect the remaining cells. The total leucocytes were counted with a Coulter counter. A differential count of the mononuclear and polymorphonuclear cells was performed on the Giemsa stained smear preparation.

Carrageenin-induced inflammation in the pleural cavity (Carrageenin pleurisy): Rats were killed by chloroform anaesthesia 4 hr after intrapleural injection with lambda carrageenin (0.1 ml of 1% solution, Nakarai Chemicals, Kyoto). The volume of the pleural exudate was measured, and the number of leucocytes was counted according to the method described above.

To examine the effects of the drugs on vaccine pleurisy, all drugs were given twice to sensitized rats 1 hr before and 24 hr after challenge with the triple vaccine. In the case of carrageenin pleurisy, the drugs were given to animals 1 hr before intrapleural injection of carrageenin.

Drugs: Dexamethasone (Uclaf, Paris); indomethacin (August Brandes, Hamburg); ibuprofen (Brufen®, Kaken, Tokyo); promethazine (Pyretia®, Shionogi, Osaka); aspirin (Hoei-Yakko, Osaka); cyproheptadine hydrochloride (Nihon Merck-Banyu, Tokyo); auranofin (SK & F, Philadelphia); gold sodium thiomalate (Myochrysine®, Merck, Sharpe & Dohme, West Point); diclofenac sodium, hydrocortisone acetate, triamcinolone, BW755C, D-penicillamine, and levamisole (synthesized in our research laboratories, Osaka).

All drugs except gold sodium thiomalate were dissolved in or suspended with 0.5% methylcellulose aqueous solution. Gold sodium thiomalate was dissolved in saline. All drugs were given orally except gold sodium thiomalate which was given intramuscularly. The doses of drugs except the gold compounds were expressed in terms of the salts. The doses of gold compounds were expressed in terms of gold.

Statistical analysis: P values were calculated according to the Student’s t-test. ED50 was calculated according to Litchfield-Wilcoxon’s method with a computer program.

RESULTS

1. Optimal conditions for inducing vaccine pleurisy: Time intervals between sensitization and challenge, and the pleural responses 48 hr after challenge were studied. The amount of an antigen used for sensitization and challenge was 10⁹ pertussis organisms. As shown in Table 1, the reaction peaked on the 12th day. In the next experiment, the time intervals between challenge and sampling were varied, while the amount of antigen was 10⁹ organisms and the sensitization period
was 12 days. As shown in Table 2, the volume of exudate and the number of leucocytes showed a peak at 48 hr after challenge. Effects of different amounts of antigen on the development of pleurisy is shown in Fig. 1. The volume of exudate and the number of leucocytes increased with the increase of antigen from $10^7$ to $10^9$ organisms. Freund's complete adjuvant gave a stronger response than Freund's incomplete adjuvant. From these results, we induced vaccine pleurisy, using the antigen in an amount of $10^9$ organisms (mixed with Freund's complete adjuvant), sensitizing rats 12 days before challenge, and sampling 48 hr after challenge. We could collect $2.3 \pm 0.4$ ml ($n=10$) of exudate containing about $10^8$ leucocytes. Mononuclear cells always occupied more than a half of the leucocyte population. On the other hand, in the rats not sensitized previously, we could collect $0.2 \pm 0.0$ ml ($n=5$) of exudate containing about $3 \times 10^7$ leucocytes, in which polymorphonuclear cells occupied more than a half of the leucocyte population.

2. Effect of drugs on vaccine pleurisy:

Results are shown in Table 3. Steroidal drugs, dexamethasone, triamcinolone, and hydrocortisone strongly and dose dependently reduced the volume of exudate and the number of leucocytes. Dexamethasone being the strongest. When auranofin, a new oral gold compound for treatment of rheumatoid arthritis, was given orally, it was as effective as gold sodium thiomolate given intramuscularly. Both gold compounds showed effects comparable to that of hydrocortisone. Non-steroidal anti-inflammatory drugs, aspirin

| Day after sensitization | Volume of exudate (ml) | Number of leucocytes | Number of polymorphonuclear cells ($\times 10^6$ cells/sample) | Number of mononuclear cells |
|------------------------|------------------------|-----------------------|-------------------------------------------------------------|-----------------------------|
| 0                      | 0.3±0.1                | 34.1±6.6              | 18.4±2.7                                                   | 15.7±4.2                    |
| 3                      | 0.2±0.1                | 40.5±5.5              | 24.5±3.3                                                   | 16.0±2.5                    |
| 6                      | 0.7±0.2                | 57.4±7.8*             | 27.2±2.3*                                                  | 30.2±6.9                    |
| 9                      | 0.8±0.2*               | 62.2±6.9*             | 26.8±3.4*                                                  | 36.4±7.4*                   |
| 12                     | 2.3±0.4*               | 109.8±8.7*            | 44.9±4.7*                                                  | 64.9±6.4*                   |
| 21                     | 2.2±0.7*               | 98.0±17.7*            | 37.8±5.0*                                                  | 60.2±13.0*                  |
| 40                     | 1.4±0.2*               | 95.9±9.0*             | 42.9±3.8*                                                  | 53.0±6.0*                   |

The amount of antigen used for sensitization and challenge was $10^9$ pertussis organisms. Results are presented as a mean±S.E. Control animals were challenged just after sensitization.

*: Significant difference from control at $P<0.05$.

| Time after challenge (hr) | Volume of exudate (ml) | Number of leucocytes | Number of polymorphonuclear cells ($\times 10^6$ cells/sample) | Number of mononuclear cells |
|---------------------------|------------------------|-----------------------|-------------------------------------------------------------|-----------------------------|
| 6                         | 0.7±0.2                | 46.2±6.4              | 37.4±5.8                                                   | 8.7±1.4                     |
| 24                        | 2.6±0.5                | 64.4±3.6              | 31.8±2.5                                                   | 22.6±2.1                    |
| 48                        | 2.4±0.5                | 122.7±13.6            | 50.1±5.1                                                   | 72.6±8.5                    |
| 64                        | 1.1±0.2                | 68.7±12.0             | 22.4±5.1                                                   | 46.2±7.9                    |

The amount of antigen used for sensitization and challenge was $10^9$ pertussis organisms. Results are presented as a mean±S.E.
Table 3. Effects of drugs on triple vaccine-induced pleurisy

| Drug             | Dose (mg/kg, p.o.) | Inhibition (%) | ED50 (mg/kg) | Inhibition (%) | ED50 (mg/kg) |
|------------------|--------------------|----------------|--------------|----------------|--------------|
|                  |                    | Volume         |              | Leucocyte      |              |
| Dexamethasone    | 0.01               | 0              |              | 16.5           |              |
|                  | 0.1                | 87.5           | ca. 0.04     | 86.0           | 0.03         |
|                  | 1                  | 100            |              | 100            | (0.001–0.06) |
| Triamcinolone    | 1                  | 21.7           |              | 50.4           |              |
|                  | 10                 | 71.4           | 4.7          | 50.0           | 2.3          |
|                  | 32                 | 76.2           | (0.7–29.2)   | 63.5           | (0.02–270.9) |
|                  | 100                | 85.7           |              | 74.9           |              |
| Hydrocortisone   | 1                  | 0              |              | 27.8           |              |
|                  | 10                 | 50.0           | 18.3         | 43.5           | 25.5         |
|                  | 32                 | 50.0           | (4.2–79.2)   | 38.6           | (1.1–558.1)  |
|                  | 100                | 87.5           |              | 73.0           |              |
| Gold sodium thiomalate | 3.2 (i.m.)       | 0              |              | 0              |              |
|                  | 10                 | 47.4           | 12.8         | 34.3           | 18.2         |
|                  | 32                 | 84.2           | (6.3–26.2)   | 62.3           | (10.1–32.7)  |
| Auranofin        | 3.2                | 0              |              | 0              |              |
|                  | 10                 | 38.1           | 16.5         | 34.3           | 24.2         |
|                  | 32                 | 71.4           | (6.9–39.0)   | 62.3           | (4.3–136.2)  |
| Diclofenac sodium| 3.2                | 0              |              | 29.7           |              |
|                  | 10                 | 4.3            | 31.7         | 49.9           | 13.7         |
|                  | 32                 | 55.6           | (14.8–68.2)  | 45.6           | (2.5–75.7)   |
|                  | 100                | 88.9           |              | 90.3           |              |
| Indomethacin     | 3.2                | 0              | >10          | 0              | 3.2<ED50<10  |
|                  | 10                 | 30.0           |              | 60.3           |              |
| Drug          | Dose (mg) | ED50 (mg) | ED50<320 | ED50<ED50<100 | ED50<100<320 |
|--------------|-----------|-----------|----------|----------------|---------------|
| Aspirin      | 100       | 0         | 100<ED50<320 | 0               | 100<ED50<320 |
|              | 320       | 81.5      |          |                | 76.5           |
| Ibuprofen    | 100       | 0         | >320     | 0.7            | ca. 280        |
|              | 320       | 0         |          |                | 57.9           |
| BW755C       | 10        | 8.8       |          | 16.2           |                |
|              | 32        | 17.1      |          | 20.9           |                |
|              | 100       | 23.5      |          | 23.5           |                |
|              |           |           |          |                |                |
| Cyproheptadine| 10       | 0         | >100     | 17.2           | ca. 60         |
|              | 32        | 28.6      |          | 47.3           |                |
|              | 100       | 42.9      |          | 48.3           |                |
| Promethazine | 10        | 0         | 10<ED50<100 | 0              | 10<ED50<100    |
|              | 100       | 90.0      |          | 71.7           |                |
| D-Penicillamine | 32    | 0         |          | 0              |                |
|              | 100       | 0         |          | 0              |                |
| Levamisole   | 10        | 0         |          | 0              |                |
|              | 32        | 16.7      |          | 13.4           |                |

The amount of antigen used for sensitization and challenge was 10⁹ pertussis organisms. All drugs were given twice to sensitized rats 1 hr before and 24 hr after challenge with triple vaccine. The volume of exudate and the number of leucocytes in the pleural cavity were measured 48 hr after challenge. All drugs except gold sodium thiomalate were given orally. Gold sodium thiomalate was given intramuscularly. Figures in parenthesis are the 95% confidence limits.
Fig. 1. Effects of Freund's adjuvant and triple vaccine on exudate volume and leucocyte count in the pleural cavity of rats. The amount of antigen used for sensitization was from $10^7$ to $10^9$ pertussis organisms. The pleural exudate and the number of leucocytes were measured 12 days after sensitization and 48 hr after challenge with triple vaccine.

and ibuprofen, had weak effect or no effect. On the other hand, the effects of indomethacin and diclofenac sodium on the number of leucocytes were equal to those of the steroidal drugs. BW 755C produced only a slight depressive effect. Promethazine had a slight effect, but cyproheptadine had no effect. D-penicillamine and levamisole had no effect. On the basis of the ED50, the rank in order of activity was: steroidal drugs $>$ gold compounds $\geq$ indomethacin = diclofenac sodium $>$ promethazine $\geq$ BW755C, aspirin, ibuprofen, cyproheptadine, D-penicillamine, and levamisole.

3. Effects of drugs on carrageenin pleurisy: Carrageenin in the dose used produced an accumulation of $1.0\pm0.1$ ml ($n=5$) of exudate containing $8-9\times10^7$ leucocytes at 4 hr after injection. In contrast to vaccine pleurisy, the main component leucocytes in the exudate were polymorphonuclear cells (neutrophils, over 90%).
The effects of the drugs are shown in Table 4. Steroidal drugs, dexamethasone, triamcinolone, and hydrocortisone strongly reduced the volume of exudate and the number of leucocytes. Gold compounds, non-steroidal anti-inflammatory drugs, BW755C, and cyproheptadine also decreased both indexes of carrageenin pleurisy. Promethazine had a slight effect. In all cases, the volume of exudate was depressed more strongly than the number of leucocytes.

DISCUSSION

Although Dieppe et al. (5) used Bordetella pertussis vaccine to induce vaccine pleurisy, we used triple vaccine in the present experiments because the vaccine containing only pertussis is not commercially available here. Therefore, we ran some preliminary experiments to obtain the best conditions for inducing vaccine pleurisy by triple vaccine. The results showed that we can get the maximum response when we use the following experimental conditions: an amount of triple vaccine which contains 10⁹ pertussis organisms as antigen, 12 days for sensitization, and 48 hr for inflammation development. Although each component of the triple vaccine can independently cause the skin reaction or the cell mediated reaction in rats (8, 9) and humans (10), and some differences between Bordetella pertussis and triple vaccine could be expected, the present results agree well with those obtained by Dieppe et al. (5) with Bordetella pertussis vaccine.

In carrageenin pleurisy, steroidal drugs inhibited both exudate and leucocyte accumulation to the same extent, non-steroidal anti-inflammatory drugs inhibited the volume of exudate more effectively than accumulation of leucocytes, and gold compounds showed responses similar to those of steroidal drugs. The results agree well with those of Vinegar et al. (2) who showed that steroidal drugs prevent neutrophils from participating in the acute inflammatory response and thereby decrease the quantity of enzymes available for synthesis of the vasoactive prostaglandin intermediate; whereas non-steroidal anti-inflammatory drugs do not affect the mobilization of neutrophils, but prevent the activity of the vasoactive prostaglandin synthesizing enzyme. Steroidal drugs have been reported to inhibit neutrophil chemotaxis (11–13) and non-steroidal anti-inflammatory drugs have been reported to inhibit prostaglandin synthesis (14–16). BW755C, which has been reported to inhibit lipoxygenase activity besides cyclooxygenase activity (17), depressed both indexes of carrageenin pleurisy. Therefore, 5-hydroxy-eicosatetraenoic acid (5-HETE), leukotriene B, etc., which have been reported to be potent chemotactic factors synthesized from arachidonic acid by lipoxygenase (18–20), may also be involved in carrageenin pleurisy. Cyproheptadine was more effective than promethazine, an antihistamine agent; therefore, serotonin may also play some role in carrageenin pleurisy.

As in carrageenin pleurisy, the accumulation of leucocytes at the site of inflammation may also be one of the initial important processes in vaccine pleurisy. Steroidal drugs and gold compounds may suppress this process because they exert depressive effects on the volume of exudate and leucocyte accumulation in a almost similar manner to that in carrageenin pleurisy. However, prostaglandin intermediates may not be important chemical mediators in vaccine pleurisy because aspirin and ibuprofen, inhibitors of prostaglandin synthesis, had a weak effect or no effect; and the effect of indomethacin was weaker than that on carrageenin pleurisy in which prostaglandins were reported to be mediators of inflammation (21). BW755C had only a slight depressive effect. Therefore, 5-HETE, leukotriene B, etc. are not so important in vaccine pleurisy. Cyproheptadine had no effect, and promethazine had only a
Table 4. Effects of drugs on carrageenin-induced pleurisy

| Drug          | Dose (mg/kg, p.o.) | Volume | Inhibition (%) | ED50 (mg/kg) | Inhibition (%) | ED50 (mg/kg) |
|---------------|--------------------|--------|----------------|--------------|----------------|--------------|
|               |                    |        |                |              | Leucocyte      |              |
| Dexamethasone | 0.01               | 0      | 29.5           |              | 58.9           | 0.04         |
|               | 0.1                | 85.7   | ca. 0.05       | 91.9         | (0.004–0.5)    |
|               | 1                  | 100    |                |              |                |              |
| Triamcinolone | 0.1                | 0      | 4.8            |              | 24.1           | 2.9          |
|               | 1                  | 44.4   | ca. 1.0        | 49.6         | (0.8–10.4)     |
|               | 3.2                | 100    |                | 78.9         |                |              |
|               | 10                 | 100    |                |              |                |              |
| Hydrocortisone| 1                  | 14.3   | 22.5           |              | 41.9           | 15.0         |
|               | 10                 | 57.1   | 35.1           |              | (0.9–39.5)     |
|               | 100                | 100    | (0.9–39.5)     | 73.2         | (1.4–162.1)    |
|               |                    |        |                |              |                |              |
| Gold sodium thiomolate | 3.2 (i.m.) | 22.6   | 5.4            |              |                |              |
|               | 10                 | 44.4   | 22.7           |              | ca. 48.4       |
|               | 32                 | 66.7   | (3.5–53.8)     | 43.3         |                |              |
| Auranofin     | 1                  | 0      | 0              |              |                |              |
|               | 3.2                | 55.6   | ca. 3.2        | 21.9         | 21.8           |
|               | 10                 | 100    |                | 43.7         | (4.8–99.0)     |
|               | 32                 | 100    |                | 50.3         |                |              |
| Diclofenac sodium | 0.1          | 33.3   | 28.0           |              | 23.7           |
|               | 1                  | 33.3   |                |              |                |              |
|               | 10                 | 66.7   | 28.7           |              | ca. 54.2       |
|               | 32                 | 71.4   | (0.2–11.6)     | 43.6         |                |
|               | 100                | 100    |                | 66.5         |                |
| Drug          | Dose (mg/kg) | Volume (ml) | Cell Count (x10^6) |
|--------------|-------------|-------------|--------------------|
| Indomethacin | 0.1         | 22.2        | 15.3               |
|              | 1           | 55.6        | 36.1               |
|              | 3.2         | 77.8        | 42.8               |
|              | 10          | 88.9        | 41.7               |
| BW755C       | 3.2         | 0           | 0                  |
|              | 10          | 14.3        | 65.8               |
|              | 32          | 57.1        | (9.7–63.3)         |
|              | 100         | 100         | (24.1–179.5)       |
| Ibuprofen    | 3.2         | 0           | 0                  |
|              | 32          | 42.9        | 17.4               |
|              | 320         | 85.7        | 48.4               |
| Aspirin      | 3.2         | 0           | 0                  |
|              | 32          | 60.0        | 38.3               |
|              | 100         | 60.0        | (8.8–177.5)        |
|              | 320         | 90.0        | 47.5               |
| Cyproheptadine| 1           | 14.3        | 7.8                |
|              | 10          | 50.0        | 32.0               |
|              | 32          | 75.0        | (1.4–60.3)         |
|              | 100         | 87.5        | 34.9               |
|              |             |             | (5.3–229.6)        |
| Promethazine | 10          | 28.6        | 30.2               |
|              | 32          | 42.9        | 30.7               |
|              | 100         | 57.1        | >100               |
|              |             |             | (6.3–518.6)        |

Drugs were given to rats 1 hr before intrapleural injection of lambda carrageenan (0.1 ml of 1% solution). The volume of exudate and the number of leucocytes in the pleural cavity were measured 4 hr after challenge. All drugs except gold sodium thiomolate were given orally. Gold sodium thiomolate was given intramuscularly. Figures in parenthesis are the 95% confidence limits.
weak effect. Therefore, serotonin is not involved, and histamine is not so important in vaccine pleurisy. Dieppe et al. (5) have reported that D-penicillamine and levamisole were effective on *Bordetella pertussis* vaccine-induced pleurisy when these compounds were given in a 'long through-dosing' regime in which animals were predosed for three weeks, prior to sensitization, and dosing was continued throughout the reaction. In the present experiments, the two compounds were given two times only around the time of challenge. It appears that the two compounds depress the pleurisy by modification of the sensitization process; and for that modification, repeated dosings during the sensitization period may be required. These results suggest that different chemical mediators were involved in carrageenin and vaccine pleurisies. In the former model, prostaglandin, serotonin, 5-HETE, leukotriene B, etc. may be involved; whereas in the latter, the mediators are not clear. Undefined mediators (lymphokines ?) and/or degraded products of complement may be involved in vaccine pleurisy.

It is interesting that indomethacin and diclofenac sodium were effective on the vaccine pleurisy. However, more extensive experiments are required to clarify the mode of action of these two drugs, steroidal drugs, and gold compounds.

Pleurisy is a useful model for studying anti-inflammatory drugs because we can simultaneously and quantitatively measure the number of inflammatory cells mobilized and edema volume; these and the measurement of lysosomal enzymes (22, 23), chemotactic factors (24), levels of cyclic nucleotides (25), prostaglandins, and other mediators (21) enable us to study the effects of drugs and their modes of action. So far, carrageenin has been mainly used to induce pleurisy (1, 2, 21). Ackerman et al. (26) have reported that the number of mononuclear cells mobilized into the pleural cavity peaked at 48 hr after intrapleural injection of carrageenin, and mononuclear cells occupied more than a half of the cell populations; and they regarded this stage of inflammation as a model of chronic inflammation because chronic inflammation, rheumatoid arthritis, etc. caused by an immunological response is characterized by large increases of mononuclear cells at the inflammatory sites. In their report, dexamethasone depressed the number of mononuclear cells significantly but indomethacin did not, though the latter is used in chronic inflammation clinically. The main difference between the carrageenin pleurisy and the present vaccine pleurisy as chronic inflammatory models can be summarized as follows: vaccine pleurisy is caused by immunological responses as in rheumatoid arthritis, but carrageenin pleurisy is not; and due to the difference of the cause of inflammation, mediators may be different between the two models. Thus, it is interesting to compare the vaccine pleurisy with the carrageenin pleurisy in detail in order to know the action mechanisms of drugs and mediators in inflammation.

**REFERENCES**

1) Di Rosa, M., Giround, J.P. and Willoughby, D.A.: Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenin and turpentine. J. Pathol. Bact. 104, 15–29 (1971)

2) Vinegar, R., Truax, J.F. and Selph, J.L.: Quantitative studies of the pathway to acute carrageenan inflammation. Fedn Proc. 35, 2447–2456 (1976)

3) Hurley, J.V., Ryan, G.B. and Friedman, A.: The mononuclear response to intrapleural injection in the rat. J. Pathol. Bact. 91, 575–587 (1966)

4) Fontagne, J., Stochla, C. and Lechat, P.: Plurésie expérimentale provoquée chez le rat par le nitrate d'argent: Rôle des médiateurs chimiques et action de quelques substances anti-inflammatoires sur modèle expérimental. J. Pharmacol. Paris 5, 13–23 (1974)

5) Dieppe, P.A., Willoughby, D.A., Huskisson, E.C. and Arrigoni-Martelli, E.: Pertussis vaccine
pleurisy: A model of delayed hypersensitivity. Agents and Actions 6, 618–621 (1976)

6) Tarayre, J.P. and Lauressergues, H.: Action of phenylbutazone, cyclophosphamide and prednisonolone on pleurisy due to Bordetella pertussis hypersensitivity in the rat. Archs int. Pharmacodyn. Thér. 235, 165–169 (1978)

7) Tarayre, J.P., Dellion, A. and Lauressergues, H.: The influence of various methods of sensitization on delayed hypersensitivity to Bordetella pertussis in the Sprague Dawley rat. Archs int. Pharmacodyn. Thér. 228, 162–170 (1977)

8) Rowley, D.A., Chutkow, J. and Attig, C.: Severe active cutaneous hypersensitivity in the rat produced by hemophilus pertussis vaccine. J. exp. Med. 110, 751–769 (1959)

9) Dekaris, D., Veselic, B. and Tomazic, V.: In vitro studies of delayed hypersensitivity: Inhibition of macrophage spreading in rats sensitive to tuberculin and diphtheria toxoid. Immunology 20, 363–372 (1971)

10) Galant, S.P., Flod, N., Shimizu, I., Granger, G.A. and Groncy, C.E.: Relationship between cutaneous delayed hypersensitivity and cell-mediated immunity in vitro responses assessed by diphtheria and tetanus toxoids. J. Allergy Clin. Immunol. 60, 247–253 (1977)

11) Rivkin, I., Foschi, G.V. and Rosen, C.H.: Inhibition of in vitro neutrophil chemotaxis and spontaneous motility by anti-inflammatory agents (39518). Proc. Soc. exp. Biol. Med. 153, 236–240 (1976)

12) Perper, R.J., Sanda, M., Chiena, G. and Gronski, A.L.: Leucocyte chemotaxis in vivo II. Analysis of the selective inhibition of neutrophil or mononuclear cell accumulation. J. Lab. clin. Med. 84, 394–406 (1974)

13) Borel, J.F.: Effect of some drugs on the chemotaxis of rabbit neutrophils in vitro. Experimenta 29, 676–678 (1973)

14) Vane, J.R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biology 231, 232–235 (1971)

15) Smith, J.B. and Willis, A.L.: Aspirin selectively inhibits prostaglandin production in human platelets. Nature New Biology 231, 235–237 (1971)

16) Ferreira, S.H., Moncada, S. and Vane, J.R.: Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature New Biology 231, 237–239 (1971)

17) Higgs, G.A., Flower, R.J. and Vane, J.R.: A new approach to anti-inflammatory drugs. Biochem. Pharmacol. 28, 1959–1961 (1979)

18) Siegel, M.I., McConnell, R.T., Bonser, R.W. and Cuatrecasas, P.: The production of 5-HETE and leukotriene B in rat neutrophils from carrageenan pleural exudates. Prostaglandins 21, 123–132 (1981)

19) Kuehl, F.A., Jr. and Egan, R.W.: Prostaglandins, arachidonic acid, and inflammation. Science 210, 978–984 (1980)

20) Ford-Hutchinson, A.W., Bray, M.A., Doig, M.V., Shiple, M.E. and Smith, M.J.H.: Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leucocytes. Nature 286, 264–265 (1980)

21) Katori, M., Ikeda, K., Hamada, Y., Uchida, Y., Tanaka, K. and Oh-ishi, S.: A possible role of prostaglandins and bradykinin as a trigger of exudation in carrageenan-induced rat pleurisy. Agents and Actions 8, 109–112 (1978)

22) Ennis, R.S., Granda, J.L. and Posner, A.S.: Effect of gold salts and other drugs on the release and activity of lysosomal hydrolases. Arthritis and Rheumatism 11, 756–764 (1968)

23) Ammendola, G., Di Rosa, M. and Sorrentino, L.: Leucocyte migration and lysosomal enzymes release in rat carrageen Pleurisy. Agents and Actions 5, 250–255 (1975)

24) Parente, L., Koh, M.S., Willoughby, D.A. and Kitchen, A.: Studies on cell motility in inflammation. II. The in vivo effect of anti-inflammatory and anti-rheumatic drugs on chemotaxis in vitro. Agents and Actions 9, 196–200 (1979)

25) Bartelli, A. and Schinetti, M.L.: Activity of tolmetin on levels of cyclic nucleotides in experimental pleurisy. Arzneim.-Forsch. 29, 779–781 (1979)

26) Ackerman, N., Tomolonis, A., Miram, L., Kheiht, J., Martinez, S. and Carter, A.: Three day pleural inflammation: A new model to detect drug effects on macrophage accumulation. J. Pharmacol. exp. Thor. 215, 588–596 (1980)