Pertussis Vaccine Pleurisy: A Model of Delayed Hypersensitivity

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Abstract

Male Wistar rats have been sensitized to *Bordetella pertussis* using a mixture of Freund's incomplete adjuvant and pertussis organisms. Intrapleural challenge 12 days later with pertussis produced a marked delayed inflammatory response, maximal at 48 hours and dominated by influx of mononuclear cells. Dosing with D-penicillamine (25 mg/kg) and levamisole (5 mg/kg) at the time of challenge produced a significant enhancement of the reaction. A long period of dosing with either drug, or treatment with indomethacin (3 mg/kg), suppressed the response. The relevance of this to the testing and mode of action of antirheumatic drugs is discussed.

Introduction

Many experimental models, such as adjuvant arthritis [1] and the rabbit fibrin arthritis [2], have been developed in an attempt to aid the understanding of the aetiology, pathogenesis and treatment of rheumatoid arthritis. However, no model has been found that perfectly simulates the disease, and although responsive to anti-inflammatory drugs few such animal models have been sensitive to the effective, but slow-acting antirheumatic drugs, such as D-penicillamine [3] and levamisole [4].

There is much evidence to implicate delayed hypersensitivity as being at least partly responsible for the chronic inflammatory changes in rheumatoid arthritis [5]. In view of this, simple models of immunologically-induced inflammation are being developed in this laboratory, instead of complex attempts to reproduce rheumatoid disease in experimental animals [6].

This paper reports preliminary data of a model of delayed hypersensitivity, developed in the rat pleural space, using *Bordetella pertussis* organisms as antigen. The main interest in this model is the dramatic change observed following treatment with the antirheumatic drugs D-penicillamine and levamisole.

Methods

Inbred, male Wistar rats (200-250 g wt.) were used for all experiments.

Equal volumes of Freund's incomplete adjuvant (Difco) and pertussis vaccine (Lister Institute) were mixed. 0.2 ml of the mixture, containing $4 \times 10^{10}$ organisms, was injected into the dorsal surface of one hind-paw and one fore-paw, to sensitize the animals.

At varying time intervals after sensitization, the animals were challenged intrapleurally. Pertussis vaccine was dialysed against a saline column to remove preservative and diluted in saline to achieve a concentration of about $1 \times 10^{11}$ organisms/ml; the concentration was checked by turbidimetry (absorption of light at 450µ wavelength). 0.1 ml of this suspension was injected intrapleurally. Animals were sacrificed at 6, 12, 24, 48 and 72 hours thereafter, and exudate and cells in the pleural space collected in medium 199, using siliconized pipettes. The total volume of exudate, total cell count and differential cell count were measured. Control animals were injected with 0.1 ml saline intrapleurally and cells harvested as for test animals; control values have been subtracted from the total cell count in test animals.

The effects of indomethacin (3 mg/kg), D-penicillamine (25 mg/kg) and levamisole (5 mg/kg) have been assessed. Each drug was administered orally, on a once daily basis. Three different drug regimes were used, as shown in Figure 1. First, a 'long through-dosing' regime, in which animals were pre-dosed for three weeks, prior to sensitization, and dosing was continued throughout the reaction. Secondly, a 'short pre-dose' regime, drugs being administered 48 hours, 24 hours and 1 hour before sensitization, and dosing was continued throughout the reaction. Secondly, a 'short pre-dose' regime, drugs being administered 48 hours, 24 hours and 1 hour prior to challenge, and at 24 hours after challenge.

The results were analysed by Student's t-test.

Results

Intrapleural injection of the challenge dose of pertussis produced a small inflammatory reaction in non-sensitized animals, as shown in Figure 2. This response was maximal at 6 hours and dominated by polymorphonuclear cells.
Drug-dosing regimes. All drugs were given orally, on a once daily basis.

Sensitisation Challenge

<table>
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<th>Days</th>
<th>-20</th>
<th>-16</th>
<th>-12</th>
<th>-8</th>
<th>-4</th>
<th>0</th>
<th>+4</th>
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</table>

Figure 1

Reaction to challenge in non-sensitized animals. (Total cell volumes are expressed as cells obtained minus the number harvested from control animals.)

PERTUSSIS VACCINE PLEURISY IN NON - SENSITISED ANIMALS

The reaction was identical in animals sensitized with either pertussis vaccine or adjuvant alone.

Sensitization with pertussis in incomplete Freund's adjuvant caused a marked swelling and some ulceration of the injected feet. There were no lesions apparent elsewhere and no evidence of secondary inflammatory lesions as in adjuvant disease. The animals remained healthy and maintained their weight throughout the reaction.

The reaction to challenge in sensitized animals was much greater than that in the non-sensitized group, and is shown in Figure 3. The volume of exudate is ten times that of the non-specific reaction, is maximal at 48 hours, and dominated by mononuclear cells.

The results shown in Figure 3 are based on a 12-day interval between sensitization and challenge. The effect of varying the time interval is shown in Figure 4. The reaction was greatest with a 12-day interval.

The influence of drugs was assessed, using the optimal 12-day interval between sensitization and challenge, and at 48 hours after challenge. The results are shown in the Table. The 'long through-dosing regime' caused reduction of the response with levamisole, D-penicillamine and indomethacin. Dosing prior to sensitization with D-penicillamine enhanced the response, but with the other two agents there was no difference. Dosing at the time of challenge caused a marked enhancement of the response by levamisole and D-penicillamine, and reduction by indomethacin.

PERTUSSIS VACCINE PLEURISY IN SENSITISED ANIMALS

Figure 3

Effect of varying the time interval between sensitization and challenge, on the response seen 24 hours after challenge.