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Pharmacological investigation of acute cellular accumulation in immunological air pouch inflammation

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Abstract

Immunologically-mediated cellular accumulation was measured 24 hours after antigenic challenge using a rat subcutaneous air pouch model. This response was inhibited by treatment with prednisolone, colchicine, anti-thymocyte serum and systemic antigen. In contrast, administration of a range of other pharmacological and clinically active agents had little effect. The profile of inhibitory activity suggested that this response was mainly due to delayed type hypersensitivity with little anaphylactic or Arthus-type component.

Introduction

Immunologically-mediated accumulation of inflammatory cells has been demonstrated in animal models at several inflammatory sites, including the guinea pig, rat and mouse pleural cavity [1–7], rat skin [8–10], and the rat subcutaneous air pouch [11–14]. However, pharmacological investigations of this type of response have been relatively few and often limited to small numbers of agents. Furthermore, differences in methodologies, dosing schedules and end-points have made comparison of these data somewhat difficult. The present studies were intended to investigate the actions of an extensive range of pharmacological and clinically active agents on the effector arm of acute cellular accumulation in the same model, namely immunological inflammation in the rat subcutaneous air pouch [15].

Materials and methods

Animals

Male CFHB rats (Interfauna) weighing 130–150 g were used throughout these experiments in groups of 5–6 and were housed on wood shavings in plastic cages with free access to food and water.

Induction of immunological air pouch inflammation

The sensitisation and induction of immunological air pouch inflammation were carried out as described previously [15]. Methylated bovine serum albumen (MBSA, Sigma) was dissolved in saline (10 mg/ml) and emulsified with an equal volume of Freund's complete adjuvant (FCA, Difco). Rats were sensitised by subcutaneous injection of 0.4 ml emulsion in the base of tail (2 mg MBSA per rat). Eight days later, 20 ml of air was injected s.c. to form an oval air pouch on the dorsal surface which was reflated with 10 ml of air 2–4 days later. Fourteen days after sensitisation, rats were challenged into the six day old air pouch with 2 mg of heat aggregated (1 hour at 70 °C) MBSA suspended in 1 ml of saline.

Measurement of cellular accumulation

Twenty-four hours after challenge the rats were sacrificed and the pouch contents were harvested

Table 1
Effect of various agents on 24 hour cellular accumulation in the immunologically inflamed rat subcutaneous air pouch.

Treatment	Dose (mg/kg)	Route	Total cells ($\times 10^6$) (mean ± SEM)	n	Percentage change
Control			39.1 ± 5.0	8	
Disodium cromoglycate (DSCG)[a]	10	i.v.	38.6 ± 3.7	6	− 1
Control			62.2 ± 11.6	8	
Cyproheptadine hydrochloride	2 × 10	p.o.	74.1 ± 12.1	7	+19
Control			59.6 ± 4.8	8	
Cimetidine	2 × 100	p.o.	49.6 ± 7.6	6	−17
Control			51.2 ± 10.0	7	
Bromelain	10	i.v.	46.1 ± 6.4	6	−10
Control			46.0 ± 10.2	7	
Cobra venom factor (CVF)[b]	–	i.p.	40.7 ± 4.5	6	−12
Control			65.7 ± 9.0	10	
Aprotinin[c]	–	i.v.	54.5 ± 8.6	7	−17
Control			39.6 ± 3.6	8	
Indomethacin	2 × 5	p.o.	29.3 ± 3.3	6	−26
BW755C	2 × 50	p.o.	32.3 ± 6.2	6	−18
Prednisolone	2 × 5	p.o.	18.9 ± 2.5	6	−52**
Control			51.2 ± 10.0	7	
Colchicine	1	i.v.	19.0 ± 4.1	6	−63*
Control			44.0 ± 7.8	7	
Anti-thymocyte serum (ATS)[d]	–	i.v.	15.3 ± 1.1	7	−65**
Control			48.2 ± 3.5	8	
MBSA	10	i.v.	26.7 ± 1.7	5	−45**
Control			47.8 ± 7.7	9	
Azathioprine	2 × 100	p.o.	37.7 ± 4.8	6	−21
Cyclophosphamide monohydrate	2 × 50	p.o.	37.7 ± 4.9	6	−21
Control			39.6 ± 3.6	8	
Cyclosporine	2 × 50	p.o.	30.4 ± 7.6	6	−23
Control			47.8 ± 7.7	9	
Hydroxychloroquine sulphate	2 × 100	p.o.	52.5 ± 16.8	6	+10
Control			62.2 ± 11.6	8	
Levamisole hydrochloride	2 × 50	p.o.	78.1 ± 12.8	7	+26
Control			59.6 ± 4.8	8	
Penicillamine	2 × 100	p.o.	88.2 ± 16.5	6	+48

Agents were administered 30 minutes before challenge or at −30 minutes and +6 hours.
[a] Immediately before challenge; [b] 100 units/kg as two doses 6 hours apart on the day before challenge; [c] 2 × 500000 units/kg; [d] 10 ml/kg as two doses 6 hours apart on the day before challenge; n number of rats per group.
* $p < 0.05$; ** $p < 0.005$ (Student's t-test).

as described previously [15]. Total leukocytes were counted using an automatic cell counter and expressed as cells per rat. In some experiments, differential counts were performed microscopically using Turk's stain.

Drug treatments

All agents were obtained from Sigma except cobra venom factor (CVF, Cordis), aprotinin (Bayer), BW775C (Roussel), prednisolone (Roussel), azathioprine (Wellcome), cyclophosphamide monohydrate (Koch Light), cyclosporine (Sandoz), hydroxychloroquine sulphate (Winthrop), levamisole hydrochloride (Aldrich), penicillamine (Dista). Rabbit anti-rat thymocyte serum (ATS) was prepared as described elsewhere [16]. Drugs were administered as indicated either p.o. dissolved or suspended in vehicle (5% acacia, 0.01% Tween 80 in tap water), or i.p. and i.v. dissolved in saline at a dose volume of 10 ml/kg. The doses are expressed as the salt.

Statistical analysis

The mean ±SEM was calcuated for groups of 5–10 rats and comparisons were made using Students *t*-test. Results are expressed as percentage change compared to the appropriate control group.

Results

The results shown in Table 1 are a combination of data obtained from ten separate experiments in which the control 24 hour cell response was $39-66 \times 10^6$ cells which were predominantly neutrophils (>90%). Negligible exudate formation was observed as reported previously [15].

The anti-allergic agent disodium cromoglycate (DSCG), and cyproheptadine and cimetidine (anti-histamine/serotonin agents) had little effect on cellular accumulation. Similarly, kininogen depletion with bromelain, decomplementation with cobra venom factor (CVF), and the protease inhibitor aprotinin were also without effect. The cyclooxygenase inhibitor indomethacin and the dual inhibitor of cyclooxygenase and lipoxygenase BW755C had little effect on the cell response whereas the corticosteroid prednisolone gave profound inhibition. Intravenous administration of colchicine, anti-rat thymocyte serum (ATS), and specific antigen also gave marked inhibition. The clinically active immunosuppressants azathioprine, cyclophosphamide and cyclosporine, and the "disease modifying" anti-arthritic agents hydroxychloroquine, levamisole and penicillamine had no significant effect, although some potentiation was observed for penicillamine.

Discussion

The present study was an attempt to elucidate some of the mechanisms of acute cellular accumulation in a model of immunological inflammation in the rat subcutaneous air pouch. The mediation of immune-based cell accumulation is complex and may represent a mixed response involving elements of mast cell-dependent anaphylaxis/late-phase responses, antibody-dependent Arthus-type mechanisms, and lymphocyte-dependent delayed type hypersensitivity (DTH). Indeed, characteristics resembling all these types of reaction have been reported in different versions of rat air pouch inflammation]12–14]. Our previous publication described the time course characteristics of the present model in detail and revealed a delayed onset inflammatory response of long duration which lead us to initially suspect a DTH-dominated aetiology [15].

Mast cell products have been reported to influence DTH [17] and in particular cell accumulation in a model of allergic air pouch inflammation [14]. The present data failed to indicate a role for mast cell products in 24 hour cellular accumulation since the anti-allergic agent DSCG and histamine/serotonin receptor antagonism with cyproheptadine and cimetidine had little effect. However, actions on earlier vascular and cell events as reported elsewhere [14] cannot be discounted in the present study. Similarly, kininogen depletion with bromelain [18] had no effect suggesting no important role for kinins in this response. Complement depletion with cobra venom factor and protease inhibition with aprotinin have been reported to inhibit the neutrophil-dependent reversed passive Arthus (RPA) reaction [10, 19] but these measures were found to be without effect in our model suggesting an absence of this component. Also, indomethacin and BW755C had little effect indicating a lack of eicosanoid involvement. These agents have previously been shown to inhibit 24 hour irritant-induced leukocyte accumulation in the rat given either as a single dose in the carrageenin pleurisy model [20], the carrageenin air pouch model [21] and in the saline-sponge model [22], or as two doses in the carrageenin-sponge model [23].

The glucocorticoid prednisolone profoundly inhibited the cell response in agreement with the literature [11], but the lack of effect of indomethacin and BW755C suggested that it probably exerts its inhibitory activity in this situation via phospholipase A_2 – independent mechanisms as suggested elsewhere [24]. The inhibitory action of colchicine on most forms of acute cellular accumulation was confirmed and supports previous reports using other models [25]. The marked inhibitory activity of ATS provided direct evidence of lymphocytic mediation and supported previous observations [16]. One common criticism of the use of antisera of this type is the possibility of non-specific anti-inflammatory activity via complement depletion [26]. However, the lack of effect of cobra venom factor and the reported short duration of anti-serum-mediated complement depletion [26] made

this unlikely. Administration of specific antigen to systemically pre-empt immune effector mechanisms also gave profound inhibition of the cell response. This was not associated with signs of systemic toxicity as would have been expected in rats sensitised for anaphylactic or Arthus reactions and may be similar to the desensitisation response reported in guinea pigs following systemic challenge of DTH-sensitised animals [27].

The clinically active immunosuppressants, azathioprine, cyclophosphamide, and cyclosporine have been poorly studied in models of immunological cellular accumulation using dosing schedules comparable to that used in the present study. Azathioprine has been reported to have no effect on the rat pleural RPA cell response [5] and also had no effect in this study. Cyclosporine had little effect and has similarly been reported to be inactive in a mouse pleural DTH model despite inhibition of exudate formation [7].

The clinically active "disease modifying" anti-arthritis agents have been the subject of a small number of relevant studies. Hydroxychloroquine had little effect on cellular accumulation in agreement with previous reports for chloroquine using the rat and guinea pig pleural cavity [2, 5]. Levamisole had little effect in the present study and has also been reported to have no effect in rat RPA antigen-induced pleurisy [4, 5] although in two studies potentiation of the cell response was observed [3, 8]. Penicillamine also had no effect in the rat RPA reaction and gave potentiation elsewhere [3, 8] and in the present report. However, striking "bell-shaped" dose response relationships have been reported for levamisole and penicillamine making comparison with the present data tentative [8].

In general, drug studies on acute immunological cellular accumulation have often provided inconsistent data for numerous possible reasons. These include the obvious methodological differences in species, drug dose levels and dosing schedules, plus other considerations such as the influence of non-specific and mixed immune reactions [3, 8] antigen/antibody ratios [6] and method of assessment of the cell response. This latter point may be of particular importance since at pleural and air pouch sites only extravascular cells are harvested, whereas at dermal sites using radiolabelled cell [8] or marker enzyme methods [9, 10] cells at several stages of the extravasation process may be counted. One additional consideration involves the use of animals sensitised using FCA since we and others [15, 28, 29] have observed an apparent FCA-dependent increased inflammatory potential and thus it is possible that some modification of expected drug actions may also occur.

In conclusion, the profile of drug actions reported in the present study suggests that DTH forms the major mechanism of cellular accumulation in this model and supports our previous observations [15]. However other putative mediators of cellular accumulation such as platelet activating factor and interleukin-1 were not investigated in the present study due to the lack of availability of suitable manipulative agents. Indeed, these mediators have been detected in models of this type and their possible role can thus not be discounted [30, 31].

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