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A role for endogenous histamine in interleukin-8-induced neutrophil infiltration into mouse air-pouch: investigation of the modulatory action of systemic and local dexamethasone

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1 When injected into a 6-day-old mouse air-pouch, human recombinant interleukin-8 (IL-8; 0.03–3 μg) induced, in a dose-dependent fashion, an accumulation of neutrophils which could be reliably assessed 4 h after the injection. No protein extravasation was measured above the values obtained with the vehicle alone (carboxymethylcellulose, CMC, 0.5% w/v in phosphate-buffered solution, PBS).

2 The IL-8 effect (routinely evaluated at 1 μg dose) was inhibited neither by local administration of actinomycin D (1 μg) nor by systemic treatment with indomethacin (1 mg kg^{-1}, i.v.), BWA4C (5 mg kg^{-1}, p.o.), methysergide (6 mg kg^{-1}, i.p.) and RP67580 (2 mg kg^{-1}, i.p.).

3 Treatment of mice with the H_1 antagonist, mepyramine (1–10 mg kg^{-1}, i.p.) resulted in a dose-dependent inhibition of the cell accumulation elicited by the chemokine, with a maximal reduction of approximately 50–60%. The mepyramine effect was not due to a non specific reduction of neutrophil function, since treatment with this drug (6 mg kg^{-1}, i.p.) did not modify the cell infiltration measured in response to a challenge with interleukin-1β (20 ng) or with the vehicle CMC to any extent. Moreover, treatment of mice with mepyramine did not modify cell counts in a peripheral blood film with respect to controls. Two other H_1 antagonists, chemically unrelated to mepyramine, diphenhydramine (9 mg kg^{-1}, i.p.) and triprolidine (0.5 mg kg^{-1}, i.p.), inhibited IL-8-induced migration to a similar extent (≈50–60%), whereas the H_2 antagonist, ranitidine (5 mg kg^{-1}, i.p.) was without effect.

4 The concept that endogenous histamine could be involved in the IL-8 effect was strengthened in two ways: (i) addition of histamine (0.2–2 μg) to a small dose of IL-8 (0.3 μg) potentiated the cell elicitation induced by the chemokine without having any effect on its own; (ii) IL-8-induced neutrophil accumulation was greatly impaired in animals depleted of mast cell amines by sub-chronic (5 day) treatment with compound 48/80 according to an established protocol.

5 The glucocorticoid dexamethasone (Dex; 1–50 μg per mouse, i.v., corresponding approximately to 0.03–1.5 mg kg^{-1}, given i.v. 2 h prior to challenge with IL-8) potently inhibited neutrophil infiltration with an approximate ED_{50} of 5 μg per mouse (≈0.3 mg kg^{-1}, i.v.). Passive immunisation of mice with a polyclonal sheep serum raised against the steroid-inducible anti-inflammatory protein lipocortin 1 (LC1) abolished the inhibitory action of Dex whereas a control serum was without effect.

6 Local administration of Dex at a dose which was ineffective when given systemically (1 μg) also reduced neutrophil migration induced by IL-8, either alone or in combination with histamine. This local inhibition (≈50%), also seen with hydrocortisone (30 μg), was prevented by the concomitant administration of the steroid antagonist RU38486 (10 μg) indicating the involvement of glucocorticoid receptor in the response.

7 These findings characterize further the mechanisms underlying PMN recruitment induced by IL-8 *in vivo*, and point to a role for histamine. The anti-inflammatory action of the glucocorticoids, as in some other models, appears to be LC1-dependent when these drugs are given systemically and LC1-independent when the steroids are given locally.

Keywords: Inflammation; interleukin-8; histamine; dexamethasone; lipocortin 1

Introduction

Interleukin-8 (IL-8) is the prototype of a new class of chemotactic cytokines recently named chemokines (Miller & Krangel, 1992). Since its discovery (Yoshimura *et al.*, 1987) it has always been clear that the polymorphonuclear leucocyte (PMN) was the main target cell for this cytokine. Indeed, IL-8 induces the classical pattern of phenomena typical of PMN activation, i.e. enzyme release, superoxide and leukotriene generation, β_2-integrin activation and *in vitro* chemotaxis (Carveth *et al.*, 1989; Schroder, 1989; Baggiolini *et al.*, 1989). The administration of IL-8 into specific tissue sites *in vivo* causes a potent and selective PMN accumulation which is distinguishable from the migration induced by other pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor (TNF) as it does not require continuing DNA-dependent RNA synthesis (Rampart & Williams, 1988; Colditz *et al.*, 1989; Foster *et al.*, 1989; Rampart *et al.*, 1989). In this respect, IL-8 is considered to be a direct chemoattractant as are C5a and leukotriene B_4. Moreover, it is very likely that the endogenous release of IL-8 is involved in the chemotactic action of IL-1 (Huber *et al.*, 1991) although this interrelationship has yet to be confirmed *in vivo*.

We have recently characterized a murine air-pouch model for the evaluation of PMN migration using IL-1 as a stimulus (Perretti & Flower, 1993). In this study it was possible to determine that IL-1 acted through a type I receptor-mediated mechanism and in a manner dependent upon *de novo* protein synthesis. Moreover, a role for endogenous PAF, but not for arachidonic acid metabolites, was proposed on the basis of the differential effects of selective drugs. IL-1-induced PMN migration was sensitive to the

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anti-inflammatory glucocorticoid hormone, dexamethasone (Dex). The inhibition which followed systemic treatment with Dex was mediated by the steroid-inducible anti-inflammatory protein lipocortin 1 (LC1), whereas the inhibition observed after local treatment with Dex was LC1-independent (Perretti & Flower, 1993).

In the present study we have used the murine air-pouch model to investigate the immunopharmacological modulation of IL-8-induced PMN migration. We describe the kinetics of the PMN accumulation into the air-pouch caused by this cytokine and the effect of various anti-inflammatory drugs which were evaluated to investigate potential endogenous mediators of the action of IL-8.

Methods

Mouse air-pouch model

Male Swiss Albino mice (22–25 g; Tuck, Essex) were used for all the experiments. Air-pouches were formed by s.c. injection of 2.5 ml of air on day 0 and day 3 (Perretti & Flower, 1993). Six days after the initial injection of air, mice (28–32 g) received a local injection of human recombinant IL-8 (72aa monocyte-derived form, generous gift of Dr I. Lindley, Sandoz Forschunginstitut, Wien, Austria) in 0.5 ml of carboxymethylcellulose (CMC; BDH, Dorset) 0.5% (w/v) in sterile phosphate-buffered solution (PBS). Control mice received CMC alone. This protocol was chosen on the basis of preliminary experiments, confirming the findings observed with IL-1 (Perretti & Flower, 1993), that is a poor migration in the absence of CMC. Using PBS + BSA 0.01% (w/v) as vehicle only $0.07 \pm 0.03 \times 10^6$ PMN and $0.21 \pm 0.11 \times 10^6$ PMN per mouse were recovered in the absence and presence of 1 μg IL-8, respectively (mean ± s.e.mean, $n = 4$ in both cases, not significant).

At various times after IL-8 administration mice were killed by CO_2 exposure and the pouches washed thoroughly with 2 ml of PBS containing 1 mM ethylenediaminetetracetic acid (EDTA) and 50 u ml^{-1} heparin. Lavage fluids were centrifuged at 220 *g* for 10 min at 4°C and the pellet was resuspended in 2 ml of PBS-EDTA-heparin. Leucocytes were counted after staining (1:10) in Turk's solution (crystal violet 0.01% w/v in acetic acid 3%) in an improved Neubauer hemocytometer. The number of PMN recovered from each pouch was then calculated.

In further experiments the migration caused by local administration of 20 ng human recombinant IL-1β (generous gift of Dr L. Parente, IRIS, Siena, Italy) was assessed, this dose of the cytokine being chosen on the basis of a previous study (Perretti & Flower, 1993). Pouches were washed at the 4-h time point, and PMN infiltration measured as described above.

Protein concentration in cell-free lavage fluids was measured according to the methodology described by Bradford (1976).

Drug treatment

The effect of putative inhibitors and/or antagonists was assessed either by local administration or by systemic treatment.

The role of *de novo* RNA synthesis was ascertained by co-administration into the air-pouch of actinomycin D (1 μg) together with IL-8 (1 μg). The potential synergism between IL-8 and histamine was evaluated in co-administration experiments in which a mixture of the cytokine, at a dose of 0.3 μg, and histamine (0.2 to 4 μg) in 0.5 ml CMC was injected in the air-pouches at time 0. The leucocyte accumulation was measured 4 h later.

The potential role of inflammatory mediators was investigated by systemic administration of selective drugs. Indomethacin (Sigma, Poole, Dorset) was given i.v. into the tail vein. Mepyramine (maleate, May and Baker Ltd., Essex), diphenhydramine (hydrochloride, Research Biomedical International, Natick, MA, U.S.A.), triprolidine (hydrochloride, Research Biomedical International, Natick, MA, U.S.A.), methysergide (maleate, Sandoz, U.K.), RP-67580 (*3aR,7aR*)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl]perhydroisoindol-4-one; generous gift of Dr C. Garret, Rhone-Poulenc Rorer, Vitry-sur-Seine, France) and ranitidine (Sigma, Poole, Dorset) were administered i.p. whereas BWA4C (N-(3-phenoxycinnamyl)-acetohydroxamic acid; The Wellcome Research Laboratories, Beckenham, Kent) was given by oral gavage; in all cases drug treatment was performed 15–20 min before IL-8 challenge. PMN migration was always evaluated 4 h following cytokine administration.

The inhibitory action of the anti-inflammatory glucocorticoid, Dex (sodium phosphate salt, David Ball Laboratories, Warwick) was carefully investigated. Mice were treated with Dex (1–50 μg per mouse corresponding to 0.03–1.5 mg kg^{-1}) i.v. 2 h before the local administration of IL-8. The role of endogenous LC1 in this action of the steroid was evaluated by passive immunisation of the mice with a specific anti-LC1 (αLC1) sheep antiserum (50 μl s.c., 24 h before Dex) using the time and dose protocol found to prevent Dex inhibition of IL-1-induced migration (Perretti & Flower, 1993). Control animals received an identical volume of normal sheep serum (Sigma). To evaluate its effectiveness following local treatment, Dex (at the dose of 1 μg shown to be effective against IL-1-induced cell migration, see Perretti & Flower, 1993) was given directly into the pouch either with IL-8 (1 μg) or with the mixture of IL-8 plus histamine (0.3 μg and 2 μg, respectively) as described above. The glucocorticoid nature of the Dex effect observed following local treatment was confirmed in two ways: first, the action of another glucocorticoid, hydrocortisone (30 μg; sodium succinate salt, Upjohn Ltd, U.K.) was assessed; second, the effect of the specific compound with anti-glucocorticoid properties, RU38486 (10 μg; Roussel-Uclaf, Paris, France) upon Dex-induced inhibition was evaluated.

To deplete mast-cell amines an established protocol was followed (Di Rosa *et al.*, 1971). Mice received six doses of compound 48/80 (Sigma) at 0.6 mg kg^{-1} i.p. at 12 h intervals followed by three doses of the same agent at 1.2 mg kg^{-1} i.p., after which the experiment was started by local administration of CMC alone or in combination with 1 μg IL-8. Control mice received repeated doses of PBS (5 ml kg^{-1}, i.p.) prior to challenge with the cytokine. Migration was assessed 4 h later.

In some experiments blood samples were obtained by cardiac puncture from a number of mice 4.5 h after treatment with mepyramine, or 2 h after treatment i.v. with Dex and the total number of leucocytes measured with a Coulter Counter (Coulter Electronics, Luton, Bedfordshire). The percentage of PMN and mononuclear cells was then assessed by staining in Turk's and the total number of each cell type then calculated.

Data and statistics

Data, PMN (10^6) migrated per mouse, are reported either as total migration or as net migration by subtracting the effect of CMC alone: this is stated for each table and figure. Statistical differences between treatments were assessed by analysis of variance followed by the Bonferroni test. Values of probability less than 0.05 were taken as significant.

Results

Characterization of IL-8-induced PMN migration into the mouse air-pouch

The dose of 1 μg of human recombinant IL-8 caused a time-dependent PMN infiltration into the pouch, with a max-

imal rate of influx between 2 and 4 h (3.1×10^6 PMN per h). The cell accumulation reached maximum by the 8-h time point and was greatly reduced by 24 h (Figure 1a). At the 4-h time point, the cumulative data for CMC and CMC + IL-8 1 μg were as follows (mean ± s.e.mean): $1.56 \pm 0.18 \times 10^6$ PMN ($n = 47$) and $5.84 \pm 0.28 \times 10^6$ PMN per mouse ($n = 70$), respectively ($P < 0.01$). The IL-8 effect appeared to be specific for PMN because no mononuclear cell infiltration was observed at any time point. PMN infiltration was not accompanied by any increase in plasma protein extravasation above that caused by CMC alone (2.18 ± 0.14 and 1.88 ± 0.12 mg protein in CMC and CMC plus 1 μg IL-8 groups, respectively, $n = 5$). The 4-h time point was selected and used in all subsequent experiments.

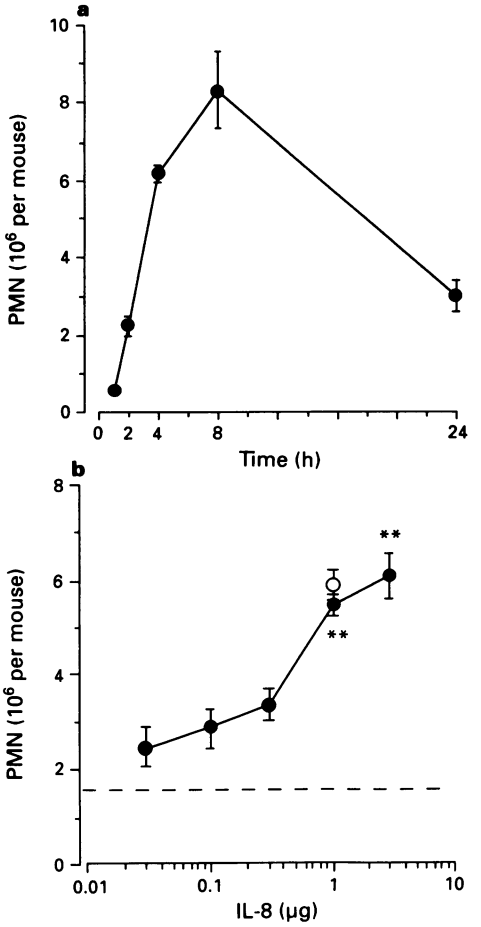

Figure 1 Characterization of interleukin-8 (IL-8)-induced PMN infiltration into the mouse air-pouch. (a) Time-course: 1 μg of IL-8 in 0.5 ml CMC was injected at time 0 directly in the air-pouch. At different times following treatment with the cytokine mice were sacrificed and PMN infiltration measured. Values are mean ± s.e. mean of 5–6 mice per time point. (b) Dose-response: different doses of IL-8 were injected in 0.5 ml CMC at time 0 and PMN migration evaluated 4 h later (●). Values are mean ± s.e.mean of 6–8 mice per group. In one case, actinomycin D 1 μg was injected concomitantly with IL-8 1 μg and migration evaluated 4 h later (○). The dotted line indicates the migration measured with CMC alone ($1.6 \pm 0.7 \times 10^6$ PMN, $n = 5$). $^{**}P < 0.01$ vs CMC.

The IL-8 effect was dose-dependent. Figure 1b shows that the dose of 0.3 μg per pouch caused a consistent effect, with an apparent peak observed at 3 μg. The dose of 1 μg, which caused a consistent infiltration and corresponded to ≈80% of maximal migration (Figure 1b), was selected and used in experiments to evaluate the potential role of inflammatory mediators. IL-8-induced PMN infiltration was not dependent upon DNA-dependent RNA synthesis: co-administration of actinomycin D (1 μg) was without effect on the response elicited by 1 μg IL-8 (Figure 1b).

Drug effect

The PMN accumulation which follows IL-8 administration into the pouch does not appear to depend upon arachidonic

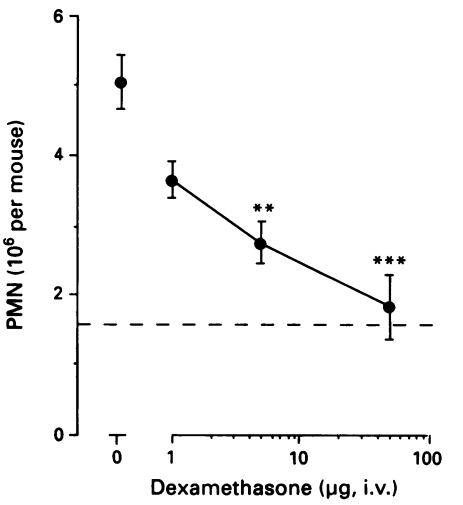

Figure 2 Systemic treatment with dexamethasone (Dex) inhibits interleukin-8 (IL-8)-induced PMN migration. Mice received an intravenous treatment with Dex 2 h prior to challenge with 1 μg of IL-8 (in 0.5 ml CMC directly into the pouch). PMN infiltration was measured 4 h later. Values are mean ± s.e.mean of 5–7 mice per dose. The dotted line indicates CMC-induced migration ($1.69 \pm 0.28 \times 10^6$ PMN, $n = 11$). $^{**}P < 0.01$ and $^{***}P < 0.001$ vs controls (dose 0 group).

Table 1 Drug effects on interleukin-8 (IL-8)-induced PMN migration

Treatment	*PMN* (10^6 per mouse)	*% inhibition*
CMC	1.90 ± 0.19 (4)	–
IL-8 1 μg	5.70 ± 0.64 (8)	0
+ Methysergide 6 mg kg^{-1}, i.p.	4.60 ± 0.83 (7)	19
CMC	1.90 ± 0.19 (4)	–
IL-8 1 μg	4.40 ± 1.18 (4)	0
+ Indomethacin 1 mg kg^{-1}, i.v.	3.40 ± 0.37 (4)	23
CMC	1.40 ± 0.72 (3)	–
IL-8 1 μg	4.75 ± 0.54 (4)	0
+ BWA4C 5 mg kg^{-1}, p.o.	3.50 ± 0.50 (6)	26
CMC	1.40 ± 0.50 (3)	–
IL-8 1 μg	5.65 ± 0.49 (4)	0
+ RP67580 2 mg kg^{-1}, i.p.	7.45 ± 1.50 (4)	– 31

Either vehicle or selective drugs were administered 10–20 min before local challenge with IL-8 (0.5 ml in carboxymethylcellulose (CMC) 0.5%). Control mice received CMC alone. In all cases migration was measured at the 4 h time-point. Results (mean ± s.e.mean, n) of each single experiment are reported. No treatment was statistically different from its respective IL-8 group.

acid metabolism, since both the cyclo-oxygenase inhibitor, indomethacin, and the 5'-lipoxygenase inhibitor, BWA4C, exerted no significant inhibition on this migration, when tested at doses which have been demonstrated to inhibit the appropriate enzyme (Table 1). The 5-hydroxytryptamine (5-HT) antagonist, methysergide, and the tachykinin antagonist to the NK_1 receptor, RP-67580, were also without effect (Table 1).

Systemic Dex was a powerful inhibitor of IL-8-induced migration with an ED_{50} of approximately 5 μg per mouse and a maximal inhibition of 91% if assessed as net migration (Figure 2). Passive immunisation of mice, according to a pre-determined protocol (Perretti & Flower, 1993), with a specific αLC1 sheep serum completely prevented the action of Dex (Figure 3). By contrast, a control sheep serum was without effect. In the absence of Dex treatment, the αLCl antiserum did not modify IL-8-induced migration (Figure 3). Dex 5 μg dose was also tested on the number of circulating PMN finding no effect with respect to control mice at the 2 h time point (mean ± s.e.mean): $0.69 \pm 0.20 \times 10^6$ PMN ml^{-1}, $n = 4$, and $0.80 \pm 0.17 \times 10^6$ PMN ml^{-1}, $n = 8$, in PBS- and Dex-treated mice, respectively.

Dex exerted a profound inhibition of IL-1-induced cell migration also after local injection into the pouch at time 0, an effect which is LC1-independent (Perretti & Flower, 1993). At the same dose used in the previous study (1 μg), local Dex significantly inhibited IL-8-induced PMN migration (Table 2). This inhibition was not due to a systemic absorption and action, inasmuch as 1 μg Dex was ineffective when given i.v. at time 0 (mean ± s.e.mean): $4.08 \pm 0.79 \times 10^6$ PMN ($n = 5$) and $3.48 \pm 0.43 \times 10^6$ PMN per mouse ($n = 5$), not significant, in PBS- and Dex-pretreated mice, respectively, in response to IL-8 1 μg at 4-h time point. Moreover, the effect of local Dex was mimicked by hydrocortisone, although a higher dose was necessary to inhibit PMN migration to a similar extent (Table 2). Dex-induced inhibition was prevented by co-injection of the antagonist RU38486, which alone had no action on the IL-8 response (Table 2).

The role of endogenous histamine on IL-8-induced PMN migration

A dose-dependent inhibition of IL-8-induced PMN migration was consistently observed with the selective H_1 antagonist, mepyramine (Figure 4) with a maximal inhibition of 50–60%. The action of mepyramine was not the result of a non-specific depression of PMN function because at a dose (6 mg kg^{-1}, i.p.) which greatly affected PMN infiltration caused by IL-8, this drug did not modify the cell influx measured in response to IL-1β (Table 3). Similarly, mepyramine treatment did not alter the 4 h-migration observed with CMC alone (mean ± s.e.mean): $1.56 \pm 0.48 \times 10^6$ PMN ($n = 5$) and $2.33 \pm 0.42 \times 10^6$ PMN per mouse ($n = 6$) in PBS- and mepyramine-treated mice, respectively. To exclude the possibility that mepyramine lowered peripheral PMN, the number of circulating mononuclear leucocytes and

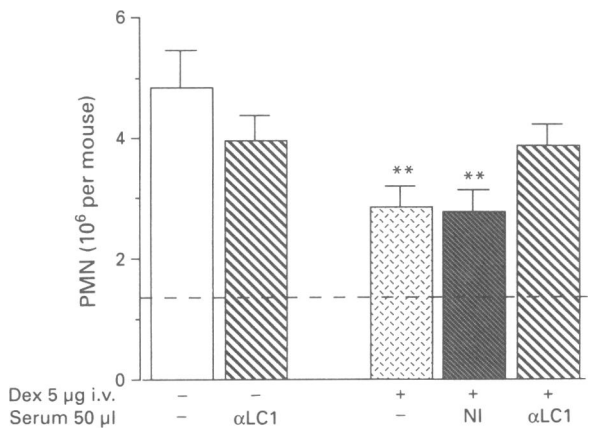

Figure 3 Anti-lipocortin 1 (αLC1) polyclonal serum prevents the inhibition exerted by systemic dexamethasone (Dex) upon interleukin-8 (IL-8)-induced PMN infiltration into the mouse air-pouch. Mice received either PBS (0.2 ml s.c.), non-immune (NI) or αLC1 sheep serum (50 μl s.c. in both cases) 24 h prior to the i.v. administration of Dex (5 μg per mouse). IL-8 (1 μg in 0.5 ml CMC) was injected 2 h after the steroid, and the PMN migration assessed at 4 h-time point. Values are mean ± s.e.mean for 9–12 mice per group. The dotted line indicates the migration measured with CMC alone ($1.36 \pm 0.13 \times 10^6$ PMN, $n = 6$). $^{**}P < 0.01$ vs PBS/PBS group.

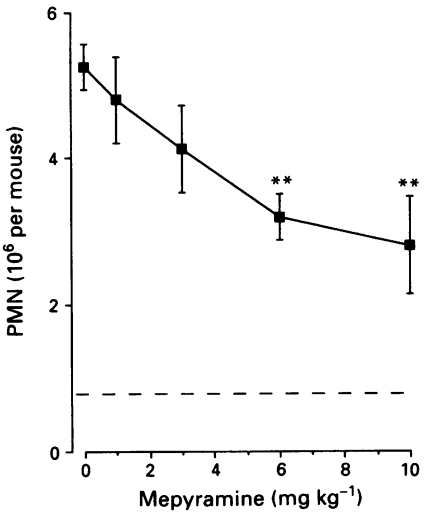

Figure 4 Mepyramine inhibits interleukin-8 (IL-8)-induced PMN influx into the mouse air-pouch. Mice received either PBS (10 ml kg^{-1}, i.p.) or mepyramine (i.p.) 10–20 min before local administration of IL-8 (1 μg in 0.5 ml CMC). PMN migration was measured 4 h later. Values are mean ± s.e.mean for 5–12 mice per dose. The dotted line indicates CMC-induced migration ($0.69 \pm 0.12 \times 10^6$ PMN, $n = 11$). $^{**}P < 0.01$ vs PBS-treated group (dose 0 group).

Table 2 Local effect of dexamethasone (Dex) and hydrocortisone on interleukin-8 (IL-8)-induced PMN migration into mouse air-pouch

Pretreatment	*PMN* (10^6 per mouse)	*% inhibition*
PBS	5.00 ± 0.42 (18)	0
Dex	3.00 ± 0.36** (20)	40
RU38486	4.67 ± 0.66 (6)	7
Dex + RU38486	4.12 ± 0.66 (6)	18
Hydrocortisone	2.43 ± 0.62** (6)	51

All treatments were done by co-administration with IL-8 (1 μg/0.5 ml carboxymethylcellulose (CMC)). Doses used: Dex 1 μg; RU38486 10 μg; hydrocortisone 30 μg. PMN influx was measured at 4 h-time point. Values are mean ± s.e.mean (n) and are reported as total migration since the effect of CMC ($1.17 \pm 0.38 \times 10^6$ PMN, $n = 7$) has not been subtracted. % inhibition was calculated vs IL-8-induced migration in control group (PBS-pretreated group).
$^{**}P < 0.01$ vs PBS-treated group.

PMN after i.p. treatment with either PBS (10 ml kg^{-1}) or mepyramine (6 mg kg^{-1}) was counted. The values found were: $4.29 \pm 0.6 \times 10^6$ ml^{-1} mononuclear cells and $0.74 \pm 0.06 \times 10^6$ ml^{-1} PMN in control animals ($n = 4$), and $4.33 \pm 0.36 \times 10^6$ ml^{-1} mononuclear leucocytes and $0.80 \pm 0.19 \times 10^6$ ml^{-1} PMN in mepyramine-treated mice ($n = 4$, not significant). The mepyramine effect was mimicked by two other H_1 antagonists, diphenhydramine and triprolidine, whereas the H_2 antagonist, ranitidine, was inactive (Figure 5).

Histamine co-injection with IL-8 into the air-pouch potentiated PMN infiltration. A significant and consistent migration was measured with IL-8 0.3 μg in the presence of 2 μg (10 nmol) histamine, a dose which did not potentiate the mild infiltration caused by CMC alone (Table 4). The histamine effect followed a bell-shaped curve, with an optimal dose of 2 μg (Figure 6). To further clarify the mechanism underlying the effectiveness of the local treatment with Dex, the effect of the steroid upon the IL-8/histamine mixture was evaluated. At a dose of 1 μg, Dex greatly affected (60% inhibition) the PMN accumulation measured under these conditions (Figure 7).

IL-8-induced PMN migration was impaired in mice depleted of mast cell amines by sub-chronic treatment with compound 48/80 (Figure 8). Cell accumulation in response to IL-8 was reduced by ≈50% in depleted animals, with no effect on the aspecific stimulus CMC (Figure 8).

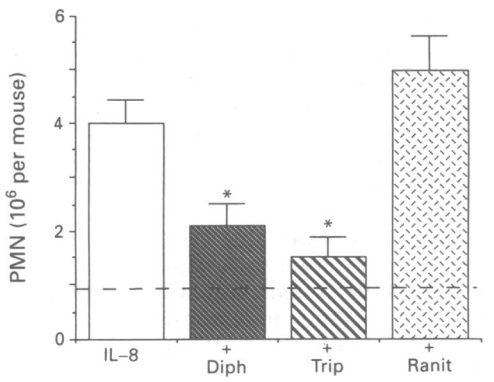

Figure 5 Effect of diphenhydramine (Diph), triprolidine (Trip) and ranitidine (Ranit) on interleukin-8 (IL-8)-induced PMN influx into the mouse air-pouch. Mice received either PBS (10 ml kg^{-1}, i.p.), Diph (9 mg kg^{-1}, i.p.), Trip (0.5 mg kg^{-1}, i.p.) or Ranit (5 mg kg^{-1}, i.p.) 10–20 min before local administration of IL-8 (1 μg in 0.5 ml CMC). PMN migration was measured 4 h later. Values are mean ± s.e.mean for six mice. The dotted line indicates CMC-induced migration ($0.89 \pm 0.15 \times 10^6$ PMN, $n = 6$). *$P < 0.05$ vs PBS-treated group.

Discussion

In this study we have observed a selective and consistent PMN infiltration following IL-8 administration into a 6-day-old subcutaneous air-pouch in the mouse. The kinetics of

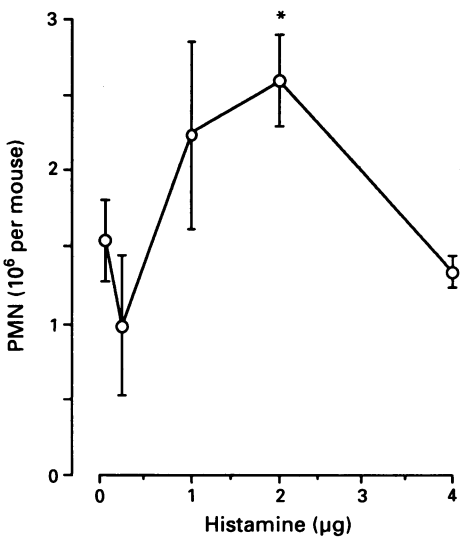

Figure 6 Dose-dependent histamine potentiation of interleukin-8 (IL-8)-induced PMN migration. Local treatment with IL-8 0.3 μg alone or with different doses of histamine was made at time 0, and PMN migration evaluated 4 h later. Values are mean ± s.e.mean for 15 mice (doses 0 and 2 μg) or for five mice (all other doses). The 4 h-migration induced by CMC alone has been subtracted and it was as in Table 4. *$P < 0.05$ vs IL-8 0.3 μg alone (histamine dose 0).

Table 3 Effect of mepyramine on cytokine-induced PMN migration into mouse air-pouch

Treatment (i.p.)	*IL-8* PMN	*IL-8* % inhibition	*IL-1β* PMN	*IL-1β* % inhibition
PBS	5.55 ± 0.36 (8)	0	5.33 ± 0.33 (6)	0
Mepyramine	2.32 ± 0.33 (12)**	58	6.52 ± 0.75 (6)	− 22

Mepyramine (6 mg kg^{-1}) or PBS (10 ml kg^{-1}) were injected i.p. 10 min before treatment with IL-8 (1 μg) or with IL-1β (20 ng) and PMN migration measured 4 h later. Values (10^6 per mouse) are mean ± s.e.mean (n) and express net migration having been corrected for the carboxymethylcellulose (CMC)-induced migration (0.86 ± 0.12, $n = 7$).
**$P < 0.01$ vs appropriate PBS-treated group.

Table 4 Histamine potentiates interleukin-8 (IL-8)-induced PMN migration into mouse air-pouch

Treatment	*PMN migration* (10^6 per mouse)	Net migration	*Protein* (mg per mouse)
CMC	1.74 ± 0.26 (13)	0	2.50 ± 0.27
Histamine 2 μg	1.54 ± 0.30 (5)	0	2.23 ± 0.52
IL-8 0.3 μg	3.28 ± 0.27 (15)**	1.54	2.77 ± 0.10
IL-8 0.3 μg + Histamine 2 μg	4.34 ± 0.30 (14)**‡	2.60	3.18 ± 0.39

Histamine and IL-8 were injected concomitantly in 0.5 ml carboxymethylcellulose (CMC) and PMN migration was evaluated 4 h later. Values are mean ± s.e.mean (n).
**$P < 0.01$ vs CMC alone.
‡$P < 0.05$ vs. IL-8 alone.

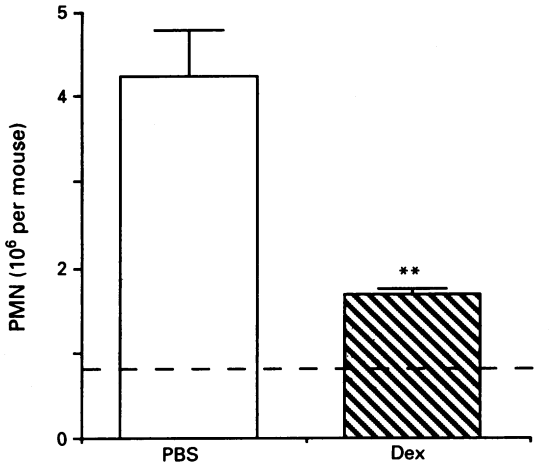

Figure 7 Effect of dexamethasone (Dex) on PMN migration induced by interleukin-8 (IL-8) in combination with histamine. Mice were given 0.5 ml of CMC containing histamine (2 μg) and IL-8 (0.3 μg), with or without 1 μg Dex, directly into the air-pouch. PMN influx was measured 4 h later. Values are mean ± s.e.mean for 6 mice per group. The dotted line indicates the migration observed with CMC alone ($0.8 \pm 0.3 \times 10^6$ PMN, $n = 6$). $^{**}P < 0.01$ vs PBS-treated group.

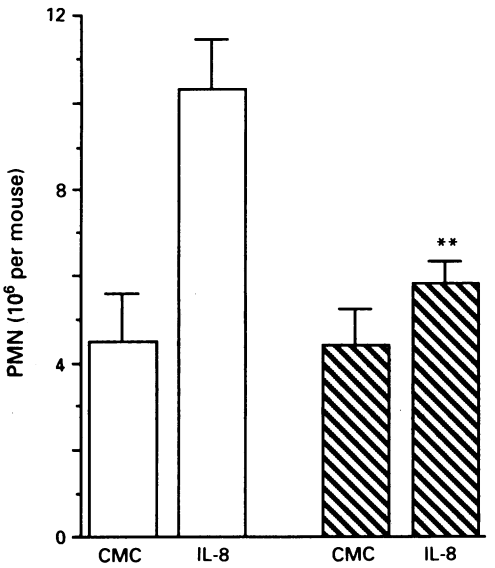

Figure 8 Effect of mast cell amine depletion on interleukin-8 (IL-8)-induced PMN migration. Mice received either PBS 5 ml kg^{-1}, i.p. (open columns) or compound 48/80 (hatched columns) as described in the Methods section for 5 days. IL-8 1 μg was then injected locally in 0.5 ml CMC, controls receiving CMC alone, and PMN migration measured 4 h later. Values are mean ± s.e.mean ($n = 4$ for CMC group and $n = 7$ for IL-8 group). $^{**}P < 0.01$ vs IL-8 group in PBS-treated mice.

the cell accumulation displayed the typical profile of an acute inflammatory process, with a maximal rate of influx between 2 and 4 h after injection of the cytokine. The IL-8 chemotactic response was not blocked by actinomycin D suggesting that continuing DNA-dependent RNA synthesis was not important for its action. Furthermore, the intense PMN accumulation was not paralleled by plasma protein extravasation. These characteristics, already observed after IL-8 intradermal injection in the rabbit skin (Colditz *et al.*, 1989; Foster *et al.*, 1989; Rampart *et al.*, 1989; Forrest *et al.*, 1992), confirmed that the murine air-pouch model was suitable for studying IL-8 chemotactic response and for assessing the effect of putative inhibitors.

Several conclusions can be drawn from the experiments performed with selective inhibitors of lipid mediator generation or action. Neither the cyclo-oxygenase inhibitor indomethacin (Higgs *et al.*, 1980) nor the selective 5'-lipoxygenase inhibitor BWA4C (Tateson *et al.*, 1988) had any effect upon the PMN migration into the pouch suggesting that eicosanoid formation is not a requisite for this response to occur. In contrast to IL-1-induced migration (Perretti *et al.*, 1993a), the IL-8 elicited response was insensitive to the selective NK_1 antagonist, RP-67580 (Garret *et al.*, 1991), which confirms the lack of effect of sensory neurone depletion obtained by pretreatment with capsaicin (J.G.H. and M.P., unpublished observation).

An interesting observation of this study is that the H_1 antagonist, mepyramine, inhibited IL-8-induced PMN migration into the air-pouch in a dose-dependent way. Its effect was not the consequence of a non-specific depression of cell function since this drug did not modify either CMC- or IL-1β-induced cell infiltration. The effect of two chemically distinct H_1 antagonists was also tested; we found that both diphenhydramine and triprolidine mimicked mepyramine, causing a similar reduction of cell accumulation in response to IL-8 (≈50–60%). The H_2 antagonist, ranitidine, was ineffective though tested at a dose reported to be active and specific (Del Soldato *et al.*, 1982). These data suggest a specific role for endogenous histamine and H_1 receptors in the response to IL-8. The specificity of its role is also highlighted by the ineffectiveness of methysergide treatment, which excludes the involvement of another endogenous amine, 5-HT.

It has been proposed that IL-8 may induce PMN infiltration *in vivo* through a haptotactic (i.e. migration induced by substrate bound chemoattractants) rather than a chemotactic (i.e. migration in response to soluble gradients of chemoattractants) action (Rot, 1993), and, that endothelial cells have specific binding sites for this cytokine (Rot, 1992; Tanaka *et al.*, 1993). According to this recently proposed model, the involvement of a co-factor(s) which facilitates leucocyte rolling on the endothelial wall (Rot, 1992), is a fundamental requisite for a subsequent firm adhesion via β_2-integrins (Lawrence & Springer, 1991; Von Andrian *et al.*, 1992). Previous studies have suggested that IL-8-induced PMN infiltration in the rat peritoneal cavity may require the presence of resident mast cells (Ribeiro *et al.*, 1991), and that a genetic strain of mice deficient of this cell type have an impaired response to IL-8 (reported in Rot, 1993). We have mimicked this situation by a subchronic treatment with compound 48/80, according to an accepted protocol (Di Rosa *et al.*, 1971), finding a marked reduction in the number of PMN migrated in response to IL-8 challenge. Migration due to CMC alone was not altered. The role of histamine was finally investigated in co-administration experiments. Exogenously added histamine, ineffective when given alone, dose-dependently potentiated PMN accumulation into the air-pouches in response to IL-8, with an optimal effect at the dose of 2 μg. Histamine potentiation of IL-8-induced migration was not accompanied by significant changes in protein extravasation. Histamine is well known to cause vascular leakage (Majno & Palade, 1961), however, the lack of effect on protein extravasation observed when administered into the pouch is unlikely to be due to the timing of the experiments, in that a single histamine injection causes significant plasma protein extravasation at the 4 h-time point (Collins *et al.*, 1993). Rather, the lack of effect is probably more related to the dose of histamine and/or the experimental model used. All these observations, together with the effect of the selective drug mepyramine and of compound 48/80 depletion experiments, suggest strongly that histamine, a mast cell product, is the co-factor required for manifestation of this important property of IL-8 *in vivo*. As stated above, PMN must roll on the endothelial wall before firmly adhering to these cells (Lawrence & Springer, 1991; Tanaka *et al.*, 1993), and the rolling process is brought about by selectins (Lasky, 1992),

one of which, P-selectin, is induced by histamine on endothelial cell membranes within minutes of the application (Lorant *et al.*, 1991; Lasky, 1992).

The anti-inflammatory glucocorticoid hormone, Dex, potently inhibited IL-8-induced PMN infiltration into the murine air-pouch, with an ED_{50} of approximately 5 μg per mouse ($\approx 150\ \mu g\ kg^{-1}$, i.v.). This inhibitory effect is likely to be brought about by endogenous LC1, since it was abrogated by passive immunisation of mice with a specific αLC1 polyclonal antibody. In this respect, this study confirms the observations obtained in IL-1-induced PMN migration (Perretti & Flower, 1993), and indicates that induction of LC1 can be a general mechanism by which systemic anti-inflammatory steroids can affect cytokine induced cell migration, and, more generally, the cellular response characteristic of the inflammatory process. Moreover, treatment with either the full length LC1 molecule, as well as with a N-terminal peptide of this protein, amino acids 2–26, resulted in a significant reduction of both IL-1- and IL-8-induced PMN accumulation (Cirino *et al.*, 1993; Perretti & Flower, 1993; Perretti *et al.*, 1993b), again strengthening the concept of LC1 as the mediator of the effect observed with systemic steroid treatment.

Local injection of Dex together with IL-8 resulted in a significant and consistent inhibitory effect of the action of this cytokine. The steroid had a similar action on IL-1-induced migration, with a mechanism which was LC1-independent (Perretti & Flower, 1993). The efficacy of Dex against IL-8 is, at a first glance, surprising because local administration of anti-inflammatory glucocorticoid hormones is well known to inhibit cell recruitment elicited by indirect agents, like IL-1, TNF and endotoxin, but not those induced by direct-acting agents, like C5a and formyl-Met-Leu-Phe (Ribeiro *et al.*, 1991; Perretti & Flower, 1993; Yarwood *et al.*, 1993). However, the Dex effect was specific and brought about by an interaction with the endogenous corticoid receptors, as evidenced by the antagonism exerted by RU38486 (Peers *et al.*, 1988). Moreover, another glucocorticoid, hydrocortisone, was similarly a potent inhibitor of cell infiltration. As discussed above, IL-8 should no longer be considered a classical direct-acting chemoattractant as we have reported here a role for endogenous histamine in its action. Dexamethasone inhibited the PMN influx induced by IL-8 plus histamine to a similar extent as the cell accumulation observed in response to IL-8 alone. It is likely that under these experimental conditions Dex and hydrocortisone are interfering with the action of histamine, rather than with its endogenous release. It is noteworthy that a recent study has shown Dex to be a potent inhibitor of histamine-induced PMN adherence to endothelial cells (Watanabe *et al.*, 1991). The exact molecular mechanism of the Dex effect, i.e. inhibition of histamine-induced P-selectin expression and/or of the biological effect of this adhesion molecule is at the moment a matter of speculation and requires further studies to be clarified.

In conclusion, this study identifies the mouse air-pouch model as a useful experimental system for the study of IL-8-induced PMN accumulation and suggests that this phenomenon may require endogenous histamine. The involvement of this biogenic amine may provide an explanation for the inhibitory action of locally injected Dex upon the cell response to the cytokine. As in other studies, the inhibition of PMN migration exerted by systemic Dex appears to be brought about by endogenous LC1.

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