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Microvascular mechanisms of histamine-induced potentiation of leukocyte adhesion evoked by chemoattractants

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1 Intravital microscopy of the rat mesentery was used to examine interactions between histamine and the chemoattractant leukotriene B_4 (LTB_4) with regard to leukocyte adhesion in postcapillary venules.

2 Topical administration of histamine caused a four fold potentiation of LTB_4-induced leukocyte adhesion.

3 Histamine significantly increased the rolling leukocyte flux by 25%, and this effect of histamine on rolling was strictly blood flow-dependent, *i.e.* we found significant positive correlations between both blood flow and total leukocyte flux and between total and rolling leukocyte flux, while no changes in leukocyte rolling fraction or rolling velocity were observed. Furthermore, histamine caused a clear-cut increase in venular plasma protein leakage.

4 The platelet-activating factor (PAF) receptor antagonist WEB 2086, which effectively inhibited adhesion of leukocytes evoked by exogenous PAF, did not reduce the potentiating effect of histamine on LTB_4-induced leukocyte adhesion.

5 The vasodilator acetylcholine (ACh) caused a moderate enhancement of LTB_4-induced leukocyte adhesion in proportion to its blood flow-dependent 40% increase in rolling leukocyte flux. In contrast to histamine, ACh did not provoke vascular leakage of plasma proteins.

6 Taken together, our findings suggest that histamine plays an important pro-inflammatory role in tissues where leukocyte rolling is already present, by potentiating chemoattractant-induced firm leukocyte adhesion through a combination of microcirculatory changes such as increased rolling leukocyte flux and vascular permeability.

Keywords: Histamine; leukotriene B_4; leukocyte adhesion; microcirculation; platelet-activating factor

Introduction

Acute inflammation is characterized by increased blood flow and vascular permeability, as well as recruitment of leukocytes into the extravascular space. The leukocyte response is a sequential multi-step process involving rolling along the venular endothelium, followed by firm adhesion and extravasation. In this cascade of adhesion receptor mediated events, the initial rolling of leukocytes has been shown to be a precondition for the subsequent steps in the leukocyte emigration process (Lawrence & Springer, 1991; von Andrian *et al.*, 1992; Lindbom *et al.*, 1992). In fact, a close relationship between the extent of rolling and the magnitude of firm adhesion induced by chemotactic stimuli has been demonstrated *in vivo* (Lindbom *et al.*, 1992).

The microcirculatory changes in inflammation are caused by locally released inflammatory mediators, some of which are known to act in a synergistic manner to amplify the inflammatory reaction. For example, vasodilating prostaglandins such as prostaglandin E_2 potentiate the tissue accumulation of both leukocytes and plasma proteins induced by chemotactic stimuli (Bray *et al.*, 1981; Issekutz 1981; Rampart & Williams, 1986; Raud *et al.*, 1988; Raud & Lindborn, 1994), most likely as a result of increased local blood flow. Moreover, there is evidence to indicate that enhanced vascular permeability may potentiate the recruitment of leukocytes to an inflammatory site independently of changes in blood flow (Issekutz, 1981). In this context, histamine is an interesting agent, because this mast cell-derived mediator is known to cause a range of effects in the microvasculature which may influence the leukocyte response during inflammation. Thus, histamine evokes arteriolar dilatation and increased local blood flow, as well as endothelial cell contraction in postcapillary venules resulting in plasma extravasation (Majno & Palade, 1961; Busse, 1979; Thureson-Klein *et al.*, 1987; Wu & Baldwin, 1992). Furthermore, it has been shown that histamine promotes leukocyte rolling through increased expression of endothelial P-selectin *in vitro* (Jones *et al.*, 1993) as well as *in vivo* (Asako *et al.*, 1994; Kubes & Kanwar, 1994). In addition, based on *in vitro* studies, histamine has been suggested to induce endothelial expression of platelet-activating factor (PAF), a chemotactic mediator with potential to activate leukocytes in a juxtacrine fashion (McIntyre *et al.*, 1985; Lorant *et al.*, 1991; Watanabe *et al.*, 1991).

Pretreatment with antihistamines has been demonstrated to reduce significantly allergen-induced leukocyte accumulation in some human and animal models of allergic inflammation (Woodward *et al.*, 1985; 1986; Ciprandi *et al.*, 1992; 1993). However, it is important to emphasize that histamine alone causes little or no recruitment of leukocytes into human or animal tissues (Atkins *et al.*, 1973; Issekutz, 1981; Arfors *et al.*, 1987), suggesting that endogenous histamine rather contributes to inflammatory leukocyte recruitment by acting in synergy with chemotactic mediators. Yet, the exact mechanisms by which histamine interacts with chemotactic factors in microvessels *in vivo* are still unclear. Therefore, the aim of this study was to examine the role of microvascular changes (*i.e.* blood flow, leukocyte rolling, vascular permeability) and PAF in the potentiation of chemoattractant-induced leukocyte adhesion by histamine. For this purpose, intravital fluorescence video-microscopy of the rat mesenteric microcirculation was used.

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Methods

Rat mesentery preparation

Adult female Wistar rats weighing 225–250 g were anaesthetized with equal parts of fluanison/fentanyl (Hypnorm, 10/0.2 mg ml^{-1}) and midazolam (Dormicum, 5 mg ml^{-1}) diluted 1:1 with sterile water (2 ml kg^{-1} i.m.). The trachea was cannulated to facilitate spontaneous breathing. A catheter was placed in the left femoral vein for i.v. administration of test substances and supplementary doses of anaesthesia. Body temperature was maintained at 37°C by a heating pad connected to a rectal thermistor. Laparotomy was performed by a midline incision and a segment of the ileum was exteriorized from the peritoneal cavity and placed on a heated transparent pedestal to allow microscopic observation of the mesenteric microcirculation. The exposed tissue was superfused with a warmed (37°C) bicarbonate-buffered saline solution (composition in mM: NaCl 132, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, $NaHCO_3$ 18) equilibrated with 5% CO_2 in nitrogen to maintain physiological pH.

Intravital microscopy

Observations of the mesenteric microcirculation were made by a Leitz Orthoplan microscope equipped with water immersion lenses (×25, NA 0.6 or ×55, NA 0.8). The microscopic image was televised (Panasonic WV-1550, WV-1900 cameras) and recorded on video tape (Panasonic NV-F100 S-VHS recorder) for subsequent off-line analysis. After positioning under the microscope, a 15 min equilibration period preceded quantitative measurements. Analysis of blow flow, leukocyte flux and leukocyte-endothelium interactions (rolling and adhesion) was made in small venules (inner diameter 15–25 μm) with stable resting blood flow.

The 'rolling leukocyte flux' was determined at indicated time points by counting the number of rolling leukocytes per min passing a reference point in the microvessel. The number of firmly adherent leukocytes (stationary for >60 s) after chemotactic stimulation was counted in 600 μm long vascular segments. In some experiments, the quantification of rolling leukocyte flux and adherent leukocytes was made under ordinary light transillumination. In order to detect the free-flowing fraction of leukocytes for determination of the ratio between rolling and total leukocyte flux, video-triggered stroboscopic (11360, Chadwick Helmuth) fluorescent light epi-illumination (Leitz Ploemopak, filter block I2) was used in combination with simultaneous i.v. infusion of the fluorochrome acridine orange (5 mg kg^{-1}). The latter compound labels the circulating leukocytes but not the erythrocytes (*i.e.* nuclear staining). This procedure also permitted, through frame-by-frame analysis of video-recordings, determinations of the velocity of individual free-flowing leukocytes. Values for vessel radius (r) and highest detected white blood cell velocity (V_{max}) were used to calculate blood flow (Q) using the following relation: $Q=(V_{max}/2)\ \pi r^2$. Wall shear rate (γ) was calculated from $\gamma=8V_{max}/(2r)$ (s^{-1}). Rolling velocity was determined by analyzing leukocytes rolling along the venular endothelium at an apparently constant velocity over a 200 μm distance. For each determination, the mean rolling velocity of 8–15 leukocytes was used.

Experimental design

Leukocyte adhesion was induced with leukotriene B_4 (LTB_4), platelet-activating factor (PAF) or f-Met-Leu-Phe (fMLP) added topically for 5 min via the superfusion buffer at final concentrations of 50 nM (LTB_4 and PAF) or 100 nM (fMLP). The PAF receptor antagonist WEB 2086 was administered both i.v. (5 mg kg^{-1}) and topically (final concentration 10 μM). At the end of the stimulation with LTB_4, fMLP or PAF, the number of adherent leukocytes was counted as described above. Histamine dihydrochloride was applied topically at final concentrations of 0.01, 0.1, and 1 mM. Acetylcholine was similarly applied at a final concentration of 1 mM. In experiments designed to examine synergistic effects of histamine (0.1 mM topically) and acetylcholine (1 mM topically) on chemoattractant-induced leukocyte adhesion, two consecutive stimulations with the chemoattractant were performed with a resting period of 30 min in between. Histamine or acetylcholine was administered 5 min before and throughout the second chemoattractant challenge. In control experiments with PAF and the PAF receptor antagonist WEB 2086, PAF was applied for 5 min. WEB 2086 was given i.v. 5 min before PAF administration and topically 5 min before and throughout PAF challenge.

Qualitative changes in microvascular permeability for circulating macromolecules in response to topical histamine or acetylcholine were examined using fluorescent light epi-illumination in combination with i.v. injection of FITC-conjugated human serum albumin (FITC-HSA, 50 mg kg^{-1}).

Materials

Acetylcholine, acridine orange, FITC-conjugated human serum albumin, f-Met-Leu-Phe, histamine dihydrochloride, and platelet-activating factor were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Leukotriene B_4 (LTB_4) was from Biomol, Plymouth Meeting, PA, USA. WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6H-thieno(3,2-f)(1,2,4)trizolo(4,3-a)(1,4)diazepine-2-yl)-1-(4-morpholinyl)-1-propanone) was from Boehringer Ingelheim, Ingelheim/Rhein, Germany. Dormicum was from Hoffman-La Roche, Basel, Switzerland. Hypnorm was from Janssen Pharmaceutica, Beerse, Belgium.

Statistical analysis

Statistical evaluations were performed by use of linear regression and the Wilcoxon signed-ranks test for paired samples. The results are presented as mean values ± s.e.mean. Unless stated otherwise, *n* represents number of animals.

Results

At the end of the equilibrium period, the basal rolling leukocyte flux along the venular endothelium was 8.5 ± 1.4 cells min^{-1} ($n=20$), constituting 37 ± 3% of the total leukocyte flux (*i.e.* rolling plus free-flowing leukocytes) in the observed venules. When unstimulated preparations were followed for up to 1 h, the rolling leukocyte flux remained largely constant and only occasional cells adhered spontaneously during this time period (not shown).

Potentiation by histamine of chemoattractant-induced firm leukocyte adhesion

Topical administration of LTB_4 to the mesentery resulted in a significant increase in firm adhesion of rolling leukocytes to the venular endothelium, *i.e.* a 5 min application of LTB_4 (50 nM) increased leukocyte adhesion from 1.8 ± 1.1 to 6.8 ± 1.1 cells per 600 μm vessel length ($P=0.016$ *vs.* control, $n=6$). Upon withdrawal of the chemoattractant after this short period of stimulation, the adherent cells dislodged from the endothelium and rendered the vessel virtually clear within 15–20 min. Repeated stimulation with LTB_4 after a 30 min resting period resulted in a response that was 73 ± 16% of the first challenge in the same venular segments (NS *vs.* first LTB_4 application). In the presence of histamine stimulation (0.1 mM given 5 min before and during the second LTB_4-stimulation) the increase in leukocyte adhesion was nearly four fold the first LTB_4 challenge (Figure 1a). The use of another chemoattractant, fMLP (100 nM) resulted in a similar potentiating effect of histamine as that found for the LTB_4-induced adhesion (Figure 1b). Histamine *per se* did not cause significant firm leukocyte adhesion during a 45 min stimulation period ($n=3$).

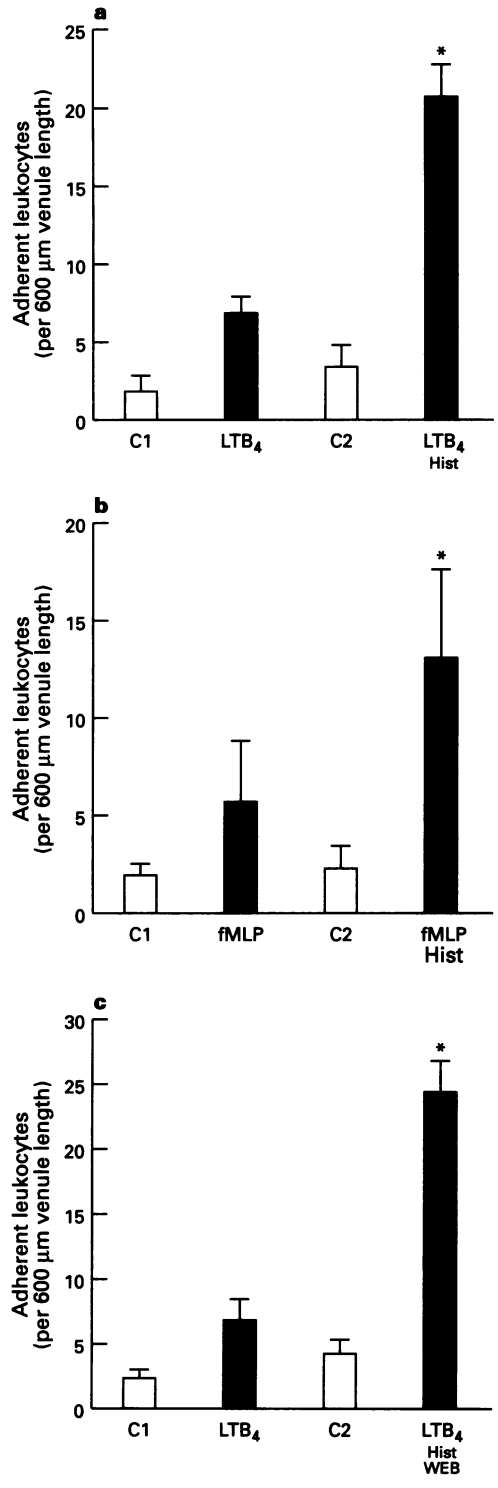

Figure 1 Effect of histamine (Hist, 0.1 mM) on leukotriene B_4 (LTB_4 50 nM)- and formyl-methionyl leucyl phenylalanine (fMLP, 100 nM)-induced firm leukocyte adhesion in small venules of the rat mesentery. The responses to topical LTB_4 (a) and fMLP (b) alone and in the presence of histamine are shown together with the control values (C1, C2) immediately before agonist challenge. Also, the effect of pretreatment with the PAF receptor antagonist WEB 2086 (WEB, 5 mg kg^{-1} i.v. and 10 μM topically) on challenge with LTB_4 and histamine is illustrated (c). Mean values ± s.e.mean are shown. Asterisks indicate significant difference *vs.* stimulation with chemoattractant alone (*$P<0.05$, $n=5-12$).

To investigate the possibility that histamine-dependent expression of PAF on the endothelial cells was involved in the potentiation of chemoattractant-induced adhesion, animals were pretreated with the PAF receptor antagonist WEB 2086 (5 mg kg^{-1} i.v. and 10 μM topically). This treatment rendered the tissue unresponsive to topically administered PAF (50 nM for 5 min), *i.e.* 0.25 ± 1.0 adherent leukocytes per 600 μm vessel length in the presence of WEB 2086 as compared with 16 ± 1.6 leukocytes in response to PAF alone ($P=0.016$ PAF *vs.* WEB 2036 and PAF, $n=6$). However, the potentiating effect of histamine on the LTB_4-induced adhesion was unchanged after WEB 2086 treatment (Figure 1c).

Effects of histamine on leukocyte rolling, blood flow, and vascular permeability

Topical application of histamine for 10 min increased the total leukocyte flux in a dose-dependent manner (Table 1). For example, at 0.1 mM histamine (the dose used in the experiments together with LTB_4) this increase was 25 ± 7%. The increase in total leukocyte flux occurred in parallel with increased blood flow (Tables 1 and 2), indicating that the increased delivery of leukocytes was related to the vasodilating properties of histamine. In fact, when data from unstimulated preparations and values after 5 and 10 min of histamine (0.1 mM) challenge were considered collectively, a significant positive correlation was found between blood flow and total leukocyte flux ($r=0.496$, $P<0.01$, $n=32$ observations in 16 animals/venules). Along with the increase in total leukocyte flux, histamine also increased the rolling leukocyte flux in a dose-dependent manner (Table 1). At histamine 0.1 mM, this increase was 39 ± 11%. However, the rolling fraction of the total leukocyte flux (*i.e.* rolling flux/rolling + free-flowing flux) and the leukocyte rolling velocity were not influenced by the different doses of histamine (Table 1). A strong positive correlation was found between total and rolling leukocyte flux (Figure 2), indicating that the effect of histamine on leukocyte rolling was mainly blood flow-dependent rather than related to a qualitative change of the leukocyte-endothelium interactions. Because venular diameters did not change, the histamine-induced increase in blood flow was entirely a result of increased blood flow velocity (most likely due to upstream arteriolar dilatation). Thus, the venular wall shear rate increased in parallel with the increased blood flow (Table 2).

Although the 30–50% increase in rolling leukocyte flux induced by histamine may have accounted for part of the potentiating effect of histamine on LTB_4-induced firm adhesion, this moderate increase in rolling was unlikely to be the full explanation for the synergistic four fold increase in leukocyte adhesion. This notion was further supported by experiments where LTB_4 was applied in the presence of acetylcholine, another vasodilator that by itself does not cause plasma or leukocyte extravasation in the microcirculation (Raud, 1990). Thus, acetylcholine (1 mM) increased blood flow and rolling leukocyte flux by 52 ± 15% and 40 ± 27%, respectively. However, in contrast to the effect of histamine, acetylcholine enhanced the LTB_4-induced adhesion by merely 37 ± 11% ($P=0.0016$ *vs.* 50 nM LTB_4 alone, $n=6$).

Finally, we confirmed that histamine (0.1 mM topically) evoked clear-cut extravasation of circulating macromolecules (FITC-HSA) in the mesenteric venules (not shown). In contrast, acetylcholine (1 mM topically) had no visible effect on the venular permeability (not shown).

Discussion

In this study, we have analysed the microvascular mechanisms of synergism between histamine, an important mediator of allergic inflammation, and the chemotactic factor LTB_4. By itself, histamine is a moderately potent vasodilator and an effective inducer of increased vascular permeability that lacks the ability to cause overt tissue accumulaiton of leukocytes (Williams & Peck, 1977; Issekutz, 1981; Arfors *et al.*, 1987). Yet, we found that histamine markedly enhanced the LTB_4-induced leukocyte adhesion in small venules of the rat mesentery prepared for intravital microscopy. One mode of ac-

Table 1 Effects of histamine on leukocyte-endothelium interactions in rat mesentery

	Total flux (Tf)	*Rolling flux* (Rf)	*Rolling fraction* (Rf/Tf x 100)	*Rolling velocity* ($\mu m\,s^{-1}$)
Control	23.0 ± 11.2	8.5 ± 1.4	37.1 ± 3.1	21.2 ± 1.6
Histamine		(% increase)		
10^{-5} M	16.1 ± 10.7	17.9 ± 7.6	3.3 ± 8.9	7.0 ± 3.8
10^{-4} M	25.0 ± 7.2*	39.0 ± 10.8*	13.0 ± 5.7	1.0 ± 2.9
10^{-3} M	48.9 ± 30.0*	52.1 ± 20.2*	6.9 ± 9.8	10.2 ± 6.6

Histamine data represent changes in % from baseline value (Control) prior to histamine stimulation for 10 min. *$P<0.05$ relative to control ($n=6-19$).

Table 2 Effects of histamine on microvascular haemodynamics in rat mesentery

	Venular diameter (μm)	*Blood flow velocity* ($mm\,s^{-1}$)	*Venular blood flow* ($nl\,s^{-1}$)	*Wall shear rate* (s^{-1})
Control	18.1 ± 2.8	1.03 ± 0.08	0.44 ± 0.13	260 ± 21.6
Histamine		(% increase)		
10^{-5} M	2.1 ± 1.7	8.2 ± 10.3	8.2 ± 10.3	8.2 ± 10.3
10^{-4} M	1.4 ± 1.3	15.1 ± 7.5*	15.1 ± 7.5*	14.9 ± 7.7*
10^{-3} M	3.3 ± 1.7	38.0 ± 17.4*	38.0 ± 17.4*	37.5 ± 17.1*

Histamine data represent changes in % from baseline value (Control) prior to histamine stimulation for 10 min. *$P<0.05$ relative to control ($n=6-19$).

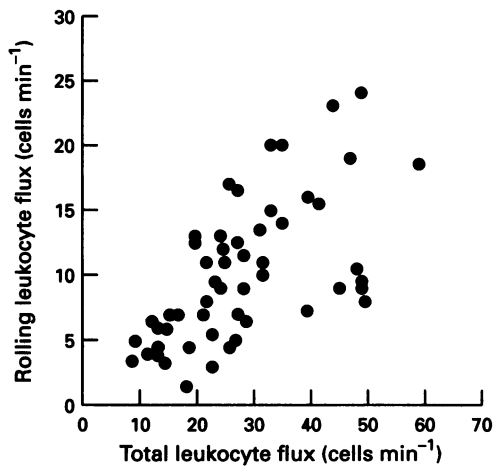

Figure 2 Relationship between total and rolling leukocyte flux in small venules of the rat mesentery. Data represent values from unstimulated tissue and from tissues stimulated with histamine (0.1 mM) for 5 or 10 min. $r=0.668$, $P<0.001$ for all values collectively ($n=56$ observations in 19 animals/venules).

tion by histamine in this respect was most likely related to the observed increase in rolling leukocyte flux. In other words, knowing that the frequency of leukocyte rolling in the rat mesenteric venules determines the magnitude of subsequent firm leukocyte adhesion evoked by chemotactic factors (Lindbom *et al.*, 1992), it is reasonable to suggest that the increased rolling observed after histamine application contributed to the enhancement of leukocyte adhesion evoked by LTB_4. This was supported by the finding that acetylcholine, a vasodilator without proinflammatory activity in the microcirculation (Raud, 1990; this paper), also increased rolling leukocyte flux and LTB_4-induced firm adhesion. However, in contrast to the proportional changes in rolling flux and evoked adhesion caused by acetylcholine, the interaction between histamine and LTB_4 was clearly synergistic, *i.e.* the increase in LTB_4-induced leukocyte adhesion caused by histamine was approximately ten fold greater than the histamine-induced increase in leukocyte rolling. This indicated that additional mechanisms were involved in the interaction between histamine and LTB_4. The potentiating effect of histamine on chemoattractant-induced leukocyte adhesion was also observed with fMLP, indicating that this effect of histamine is not specific for LTB_4.

Based on *in vitro* studies of cultured large vessel endothelium, it has been suggested that histamine may trigger leukocyte adhesion by inducing formation and surface expression of the chemoattractant PAF on endothelial cells, *i.e.* a potential mechanism for juxtracrine activation of rolling leukocytes (McIntyre *et al.*, 1985; Lorant *et al.*, 1991; Watanabe *et al.*, 1991). However, in our experiments, the PAF receptor antagonist WEB 2038, in doses effectively inhibiting the response to exogenously administered PAF, did not interfere with the synergistic effect of histamine on LTB_4-induced firm leukocyte adhesion, suggesting that the observed effect of histamine was not related to an expression of endothelial PAF. A lack of effect of histamine in this respect is in agreement with a recent *in vitro* study where the potential role of PAF in leukocyte binding to cultured endothelium was examined under flow conditions (Jones *et al.*, 1993). Thus, the physiological significance of juxtracrine activation of leukocytes by endothelial PAF remains to be determined.

It has been repeatedly shown *in vitro* that histamine upregulates endothelial P-selectin (McEver *et al.*, 1989; Hattori *et al.*, 1989; Geng *et al.*, 1990; Lorant *et al.*, 1991; Jones *et al.*, 1993), a lectin-like molecule demonstrated to support leukocyte rolling *in vitro* (Lawrence & Springer, 1991; Jones *et al.*, 1993) as well as *in vivo* (Doré *et al.*, 1993; Mayadas *et al.*, 1993). Moreover, increased expression/density of P-selectin has been shown to increase the degree of leukocyte rolling and/or to reduce the rolling velocity in flow chambers (with a constant leukocyte perfusion) *in vitro* (Lawrence & Springer, 1991; Jones *et al.*, 1993) and in small venules *in vivo* (Asako *et al.*, 1994; Ley, 1994; Kubes & Kanwar, 1994; Thorlacius *et al.*, 1994). Consequently, if histamine stimulation increased P-selectin expression also in our experiments, one would have expected an increase in the fraction of rolling leukocytes and/or a reduction in rolling velocity. However, we found that both the rolling fraction and the rolling velocity remained constant during stimulation with a range of histamine concentrations, indicating that the increase in rolling leukocyte flux was not related to a qualitative change in the leukocyte-endothelium interaction. This is also in line with recent studies by Ley

(1994) and Kubes & Kanwar (1994) showing that exogenous histamine cannot increase the fraction of rolling leukocytes in the rat mesentery once the preparation-induced 'spontaneous' rolling has been allowed to develop. In fact, the increase in leukocyte rolling due to histamine stimulation appeared to be strictly blood flow-dependent, because a significant positive correlation was found between blood flow and total leukocyte flux on one hand, and between total leukocyte flux and rolling leukocyte flux on the other. Although not sufficient to explain fully the synergism between histamine and LTB_4, the latter observation may, in view of the critical relationship between leukocyte rolling and subsequent firm adhesion, help explain the mechanism for previously documented enhancement of leukocyte recruitment by specific vasodilating mediators (Issekutz 1981; Raud *et al.*, 1988). Such a blood-flow dependent increase in leukocyte rolling thus represents a mechanism to promote further rolling in tissues where the rolling of leukocytes is already upregulated.

As shown previously (Majno & Palade, 1961; Ohya & Guth, 1985; Wu & Baldwin, 1992) and confirmed in this study, the concentrations of histamine used in our experiments also disrupt the integrity of the endothelial lining and cause increased vascular permeability in the rat mesenteric venules. Therefore, as indicated by Issekutz (1981) in rabbit skin, it is tempting to speculate that the enhanced permeability could facilitate diffusion of chemotactic agents from the extravascular tissue into the vessel lumen. Such a mechanism, together with the blood flow-dependent increase in leukocyte rolling, could well explain the observed synergistic effect of combined histamine and LTB_4 challenge on firm leukocyte adhesion. Because LTB_4 *per se* does not open up endothelial gaps (Thureson-Klein *et al.*, 1986; 1987), the latter notion is further supported by intravital microscopic findings that clear-cut venular leukocyte adhesion occurs after micropipette application of LTB_4 either directly into the lumen of venules or extravascularly in the upstream capillary exchange region, but not when LTB_4 is applied abluminally in the vicinity of venules (Lundberg *et al.*, 1988; Nagai & Katori, 1988). Furthermore, a permissive role of increased vascular permeability on diffusion of chemotactic factors from the extra- to the intravascular space is in line with our observation that, in contrast to histamine, acetylcholine did not increase the venular permeability.

The dose of histamine required to cause significant changes in microvascular haemodynamics and leukocyte rolling was relatively high (i.e. 0.1 mM), and it may be argued that this is outside the physiological dose-range for histamine. However, the same dose of histamine was required to increase leukocyte rolling in a similar rat model where spontaneous rolling had been reduced by stabilizing the mesenteric mast cells with disodium cromoglycate (Kubes & Kanwar, 1994). Moreover, in line with our observations, Asako *et al.* (1994) and Ley (1994) have shown that histamine at $\leqslant 10$ μM has no effect on the haemodynamics in the rat mesentery. This would also be in agreement with the rat being relatively insensitive to histamine (see Green *et al.*, 1979).

The observed synergism between histamine and the chemoattractant LTB_4 raises the question whether or not histamine contributes significantly to the recruitment of leukocytes seen in different allergic diseases. Unfortunately, the literature on this subject is rather complex and partly contradictory. For example, while some common H_1-receptor antagonists have been described as effective inhibitors of allergic leukocyte-accumulation in the human conjunctiva (Ciprandi *et al.*, 1992; 1993), treatment with the same or similar anti-histamines appears to offer no or relatively poor protection against cutaneous leukocyte infiltration evoked by intradermal allergen injection (Bierman *et al.*, 1991; Charlesworth *et al.*, 1992; Juhlin & Pihl-Lundin, 1992). However, in this context, it is important to emphasize that the lack of effect of anti-histamines on allergic leukocyte recruitment in human skin must be viewed in light of the fact that clinically used doses of anti-histamines only partially inhibit histamine-induced wheal and flare reactions (Charlesworth *et al.*, 1992; Juhlin & Pihl-Lundin, 1992). In fact, it cannot be excluded that allergen-induced dermal leukocyte recruitment may be profoundly reduced if only the microvascular actions of histamine are abolished. In this context, it is also of interest that inhibition of cellular components of allergic inflammation may be more pronounced during combined H_1- and H_2-receptor blockade than with inhibition of H_1-receptors alone (Woodward *et al.*, 1985; 1986). On the other hand, it is also worth noting that rapid leukocyte rolling and adhesion after mast cell activation is mediated to a significant extent via mediators other than histamine (Thorlacius *et al.*, 1994).

In conclusion, we have shown that histamine can greatly enhance acute chemoattractant-induced firm leukocyte adhesion in the microcirculation. Our data indicate that the mode of action by histamine in this regard involved increased rolling leukocyte flux and likely also increased microvascular permeability, but not increased synthesis of PAF. Moreover, the histamine-induced increase in leukocyte rolling was found to be blood flow-dependent, and independent of endothelial expression of adhesion molecules that could increase the rolling fraction or decrease rolling velocity. These findings demonstrate that histamine can promote leukocyte recruitment through a combination of microvascular actions, in addition to P-selectin-dependent upregulation of leukocyte rolling.

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