British Journal of Pharmacology (1998) 123, 381–389

An approach for studies of mediator-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery

[1]Kohji Yamaki, Lennart Lindbom, Henrik Thorlacius, Per Hedqvist & [2]Johan Raud

Department of Physiology & Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden

1 Although intravital microscopy is the method of choice for observation of inflammatory leukocyte rolling and adhesion in small venules *in vivo*, a problem with this technique is that surgical exposure of suitable tissues *per se* triggers the rolling mechanism. In this study, we describe an approach to investigate induction of rolling in undisturbed microvessels. For this purpose, intravital microscopic observation of leukocyte rolling and adhesion in the rat mesentery was combined with histological determination of the intravascular concentrations of polymorphonuclear and mononuclear leukocytes (PMNL and MNL).

2 By relating the histologically determined number of intravascular leukocytes to either microvessel volume or to the erythrocyte concentration, the baseline MNL and PMNL content was found to be 3–6 fold higher in venules than in systemic blood. This increase in microvessel leukocyte concentration did not seem to be related to leukocyte-endothelium interactions, because the leukocyte concentration was similarly elevated in arterioles where rolling and adhesion did not take place.

3 Preparation of the rat mesentery for intravital microscopy time-dependently increased the venular PMNL concentration to over 100 fold the systemic PMNL concentration 45 min after exteriorization of the small intestine. The MNLs were much less responsive to the preparative manipulation. By treatment with the polysaccharide fucoidin (inhibits rolling but not firm adhesion *per se*), or by use of intravital microscopy immediately before tissue fixation, approximately 90% of the accumulated venular PMNLs were found to represent rolling cells.

4 Intraperitoneal injection of 10^{-3}M histamine increased the venular PMNL (but not the MNL) concentration to almost 50 fold the systemic PMNL value. The histamine response did not vary with venular diameter, and the relative contribution of rolling vs firmly adherent cells to the PMNL, accumulation was again $\approx$90%. Intraperitoneal injection of leukotriene C_4, but not prostaglandin E_2, caused a significant increase in venular PMNL concentration.

5 Systemic treatment with the anti-P-selectin monoclonal antibody PB1.3 had no effect on the histamine-induced venular PMNL accumulation (i.e. rolling) in female Wistar or male Sprague-Dawley rats. On the other hand, identical treatment with PB1.3 very effectively inhibited the histamine-induced PMNL response in the mesentery of rabbits.

6 In conclusion, we have shown that a histologically determined increase in leukocyte concentration in rat mesenteric venules may be used as an index of mediator-induced leukocyte rolling if the relative contribution of rolling and firm leukocyte adhesion is first determined, for example by the means described in this study. This relatively simple approach may be very useful for studying various aspects of leukocyte rolling when the 'spontaneous' rolling triggered by preparation of tissues for intravital microscopy is undesirable.

Keywords: Histamine; intravital microscopy; leukocyte adhesion; leukocyte rolling; leukotriene C_4; microcirculation; P-selectin; prostaglandin E_2

Introduction

Recruitment of leukocytes during inflammation is known to be a multistep process where initial rolling of leukocytes along the endothelium is a precondition for subsequent firm adhesion and extravasation (Lawrence & Springer, 1991; Lindbom *et al*., 1992). The rolling mechanism, known to be mediated by the selectin family (e.g., Carlos & Harlan, 1994), serves to promote endothelial contact and reduce the speed of leukocytes entering the venular portion of the microcirculation in inflamed tissue, and there are several studies showing that inhibition of the rolling mechanism reduces inflammatory leukocyte recruitment (Carlos & Harlan, 1994). The three members of the selectin family, L-selectin on leukocytes and E- and P-selectin on the endothelium, have all been shown to mediate leukocyte rolling *in vitro* (Lawrence & Springer, 1991; Abbassi *et al*., 1993) as well as *in vivo* (Ley *et al*., 1991; von Andrian *et al*., 1991; Doré *et al*., 1993; Mayadas *et al*., 1993; Olofsson *et al*., 1994). However, although partially overlapping, their roles in promoting rolling are temporally dissociated. Thus, recent *in vivo* studies suggest that initial leukocyte rolling is mainly P-selectin-dependent (Doré *et al*., 1993; Mayadas *et al*., 1993; Ley *et al*., 1995), while E- and/or L-selectin appear to be responsible for rolling only after proinflammatory stimulation for an hour or more (Ley *et al*., 1993; 1995; Olofsson *et al*., 1994; Kunkel & Ley, 1996).

To study leukocyte rolling *in vivo*, the standard approach is to use intravital microscopy which allows detailed quantitative analysis of a wide range of microvascular parameters related to leukocyte-endothelium interactions. However, a problem with this method is that the most commonly used variants of intravital microscopy involve surgical preparation and/or exteriorization of the specimen (e.g. hamster cheek pouch, mesentery). This relatively mild

[1]Present address: Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku Tokyo, 108 Japan.
[2]Author for correspondence at: Present address: Astra Pain Control AB, Preclinical R&D, Novum Unit, S-141 57 Huddinge, Sweden.

stimulation of the tissue causes rapid and pronounced up-regulation of rolling (usually termed 'spontaneous' rolling) (Fiebig *et al*., 1991; Ley, 1994), apparently via partial degranulation of perivascular mast cells (Kubes & Kanwar, 1994; Ley, 1994) and endothelial expression of P-selectin (Doré *et al*., 1993; Mayadas *et al*., 1993). Consequently, it may be very difficult to determine whether or not a specific proinflammatory stimulus can trigger the rolling mechanism in tissues prepared for intravital microscopy, i.e. if 'spontaneous' rolling due to release of endogenous mediators is close to maximal in a given venule, it is obviously not possible to evoke a further increase with an exogenous mediator. To circumvent this problem, two intravital microscopic approaches with the rat mesentery have recently been described. Thus, by examining rat mesenteric venules immediately after exteriorization of the intestines, Ley (1994) showed that very few leukocytes roll during the first 2–3 min of observation, while a rapid increase in rolling leukocyte flux occurred during the next 15 min. Ley also showed that after a 20 min i.p. pretreatment with histamine, the leukocyte rolling during the first few minutes after exteriorization was equal to the maximal preparation-induced rolling. The other approach, described by Kubes & Kanwar (1994), is based on pharmacological reduction of the 'spontaneous' rolling. They showed that the rolling evoked by preparation was markedly reduced by inhibiting mast cell activation with systemic disodium cromoglycate (DSCG), and that topical histamine challenge caused a significant increase in rolling from the relatively low baseline rolling after DSCG treatment. Yet, for different reasons, neither of these procedures is optimal. Namely, it is technically difficult to examine rolling in the mesentery within a few minutes after tissue exteriorization. Moreover, with this approach, the leukocyte rolling evoked by mediators given i.p. before exteriorization will inevitably be a mixed response with contribution of preparation-induced rolling. For the same reason, intravital microscopic models do not permit reliable analysis of temporal aspects of rolling evoked by selected exogenous mediators. In addition, it is difficult to differentiate granulocyte responses from those of mononuclear cells by intravital microscopy. With regard to the DSCG pretreatment, results with this approach must also be interpreted with caution, because DSCG has been found not only to inhibit mast cell activation, but also different effector functions of eosinophils, neutrophils, macrophages, platelets, as well as sensory nerves (Edwards, 1994).

Taken together, there is clearly a need for simple and objective methods for studies of leukocyte rolling evoked by exogenous stimuli in intact microvascular beds. Therefore, our aim in this study was to design and characterize a non-intravital microscopic approach for studies of mediator-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery. For this purpose, we employed an approach previously described by Fiebig *et al*. (1991), who studied effects of intestinal exteriorization on leukocyte accumulation in mesenteric venules of the rabbit. We performed histological analysis of changes in leukocyte concentrations in rat mesenteric microvessels after intraperitoneal challenge with different proinflammatory stimuli. Moreover, a series of experiments, including intravital microscopic observations, were carried out in order to determine basic characteristics of the method, including the relative contribution of free-flowing, rolling and firmly adherent poly- and mononuclear cells to the evoked increases in microvascular leukocyte concentration.

Methods

Animals and anaesthesia

Adult female Wistar rats (180–210g) and male Sprague-Dawley rats (205–215g) 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.) Female New Zealand White rabbits (0.95–1.05 kg) were sedated with diazepam (Stesolid Novum, 5 mg ml^{-1}, 3.5 mg kg^{-1}, i.m.) and anaesthetized with fluanison/fentanyl (Hypnorm, 3/0.06 mg kg^{-1}, i.m.). The animal experiments were approved by the Regional Ethical Committee for Animal Experimentation.

Intravital microscopy

The microcirculatory behaviour of leukocytes was directly observed and quantified in the rat mesentery by the use of well established techniques for intravital microscopy. Thus, in the anaesthetized rats, the trachea was cannulated to facilitate spontaneous breathing and a polyethylene catheter was placed in the left jugular vein for administration of drugs and supplementary doses of anaesthetics. Body temperature was maintained at 37°C by a heating pad controlled by a rectal thermistor. Laparotomy was performed by a midline incision and a segment of the terminal ileum was exteriorized from the peritoneal cavity and placed on a warmed transparent pedestal to allow transillumination and microscopic observation of the mesenteric microcirculation. The exposed tissue was superfused with a thermostated (37°C) bicarbonate-buffered saline solution (composition in mM: NaCl 132, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2 and $NaHCO_3$ 18) equilibrated with 5% CO_2 in nitrogen to maintain physiological pH. In order to reduce tissue dehydration, the exteriorized gut adjacent to the mesenteric window under examination was covered in with gauze soaked in superfusion buffer.

Observation of the mesenteric microcirculation was made with a Leitz Orthoplan microscope equipped with water immersion lenses (x4, NA 0.12 or x55, NA 0.8). The microscopic image was televised (Panasonic WV-1550 or WV-1900 cameras) and recorded on video tape (Panasonic NV-F100 S-VHS recorder) for subsequent off-line analysis. Analysis of leukocyte-endothelium interactions (rolling and adhesion) and behaviour of i.v. injected fluorescein isothiocyanate (FITC)-labelled erythrocytes was made in arterioles and venules with stable blood flow. The rolling leukocyte flux was determined by counting the number of rolling leukocytes min^{-1} passing a reference point in the microvessel. The number of leukocytes adhering firmly for >30 s was counted in vascular segments of defined length. Leukocyte rolling velocity was determined as the average value (mm s^{-1}) of 10 leukocytes rolling along the venular endothelium with a stable velocity over a 100 μm distance. The leukocyte rolling index was calculated as the rolling leukocyte flux divided by the rolling velocity. In some experiments, the rats were injected i.v. with a bolus of FITC-labelled erythrocytes (3×10^8 cells per animal). To visualize the free-flowing FITC-labelled red cells in the microcirculation, fluorescent light epi-illumination (Leitz Ploemopak, filter block I2) was used.

After the intravital microscopic measurements, the circulation in the observed mesenteric vessels was instantaneously arrested with a few drops of fixative for later histological analysis (see below). With this approach, the degree of directly

observed leukocyte rolling and adhesion could be compared with histologically determined vascular leukocyte concentrations in the same venules. In order to help identify the vascular segments observed *in vivo* in the fixed and stained samples, drawings and low magnification video recordings were made of the microvascular networks. Some rats received an i.p. injection of histamine before the surgery for intravital microscopy. In these animals, exteriorization of the mesentery was performed rapidly and directly under the microscope, permitting observation of leukocyte rolling and adhesion immediately after tissue exposure.

Arterial blood samples were collected (tail artery) for systemic PMNL and MNL counts in a Bürker chamber after staining with Türk's solution.

Intraperitoneal challenge with mediators

Anaesthetized and thermocontrolled rats were challenged i.p. with histamine, prostaglandin E_2 (PGE_2) or leukotriene C_4 (LTC_4) dissolved in 5 ml sterile PBS warmed to 37°C. Control rats received either no treatment or 5 ml PBS alone (room temperature or warmed to 37°C). Fifteen minutes after challenge, the animals were killed by an intracardiac injection of 0.2 ml pentobarbitone (60 mg ml^{-1}). The subsequent rapid cardiac arrest was confirmed by palpation before laparotomy for tissue sampling. The corresponding experiments in rabbits were performed according to the same protocol but with 15 ml i.p. and 1 ml intracardiac injections. In order to evaluate the potential role of P-selectin in the venular leukocyte responses to histamine, the animals were pretreated with the monoclonal anti-P-selectin antibody PB1.3 5 min before intraperitoneal challenge. Systemic treatment with the sulphated polysaccharide fucoidin was performed 5 min before the overdose of pentobarbitone i.e. 10 min after the start of i.p. histamine. In rabbits, PBS alone or purified mouse IgG_1 (iso- and subtype matched) were used as negative controls to PB1.3. Blood samples were taken as described above (ear artery in rabbits).

Sampling of mesenteric tissue and histological procedures

After the animals had been killed 15 min after i.p. challenge with PBS/mediators, or at different time-points after preparation for intravital microscopy, samples of intact mesenteric microvascular networks were harvested according to Fiebig *et al.* (1991), with some modifications. Thus, 1–3 pieces of mesenteric tissue from each animal were collected by gently spreading them between two plastic rings made from the top of Eppendorf tubes with a hole cut in the lid. The tissue samples were then fixed with 2% paraformaldehyde and 5% glutardialdehyde in PBS, initially warmed to 37°C to avoid vasoconstriction. After continued fixation for 24–48 h at 4°C, the samples were rinsed in 3% ethanol in PBS and stained for 1 h at room temperature with Giemsa stain (0.6% azur II-eosin, 0.16% azur II, and 50% glycerol in methanol) diluted 1:20 in deionized water. After being rinsed briefly in water, the specimens were next differentiated for 10–15 min in 0.01% acetic acid and, after removal of the outer plastic ring, attached to glass slides coated with a 1% gelatin solution. After being dried for 1 h, the bottom ring was removed. Following further differentiation in 0.01% acetic acid for 2–3 min on the slides, the samples were left to dry overnight at room temperature. Finally, Canada balsam was used for mounting a cover glass.

Calculation of intravascular leukocyte concentration in histological samples

The intravascular PMN and MN leukocytes were easily identified in stained mesenteric arterioles and venules with a Leitz Laborlux D microscope and a x100 (NA 1.25) oil immersion lens. In each preparation, leukocytes were counted in 2–6 vessels generally between 1–4 mm in length. For calculation of leukocyte concentrations we assumed cylindrical vascular shape and used the inner vessel diameter to estimate microvascular volume. Mean values of intravascular leukocyte concentrations in the different groups of animals were based on the mean value of the different vessels in each animal. The inner diameters of the vessels examined ranged between 18.3–58.4 μm (median 30.2 μm) for venules and 10.9–21.6 μm (median 15.1 μm) for arterioles.

In the intravital microscopic experiments where FITC-labelled rat erythrocytes were administered systemically, the ratio of leukocytes to labelled red cells (number of leukocytes/100 erythrocytes) was calculated as follows: immediately after arresting the mesenteric circulation with a few drops of fixative, the number of FITC-labelled erythrocytes was counted in a defined venular segment. Thereafter, the MNLs and PMNLs were counted in the same vascular segment after fixation and staining as described above. A corresponding ratio of leukocytes to labelled red cells was calculated for systemic arterial blood. Here, the erythrocytes and MNLs/PMNLs were counted separately in Bürker chambers by use of fluorescent light epi-illumination (with some background normal light transillumination) for the FITC-labelled red cells (in PBS) and normal light alone for the leukocytes stained with Türk's solution.

A Leitz Vario Ortomat 2 automatic microscope camera and Fujichrome 400 ASA professional daylight film were used for micrographs.

Preparation of FITC-labelled erythrocytes

Heparin-treated rat blood (2 ml) was diluted 1:3 in saline, centrifuged at $400 \times g$ for 10 min at room temperature, thereafter the buffy coat and supernatant were discarded. The red cell pellet was resuspended in 5 ml saline and the following solutions were added: 0.3 ml dextrose (0.3 M in saline), 0.5 ml NaH_2PO_4 (0.18 M in deionized water), 3 ml Na_2HPO_4 (0.1 M in deionized water), 2 ml FITC (5.1 M in the 0.1 M Na_2HPO_4 solution). The pH of the cell suspension was adjusted to 7.8 with NaOH and incubated overnight at 4°C. Before use, the labelled red cells were washed several times until a clear supernatant was obtained. The final cell suspension was adjusted to 10^9 erythrocytes ml^{-1} PBS. Control experiments showed that the systemic concentration of injected FITC-labelled erythrocytes remained stable as measured at 6, 12 and 18 min after injection (data not shown, $n=3$). Moreover, as examined by intravital microscopy, the labelled red cells did not adhere or roll in the mesenteric microcirculation.

Materials

Histamine dihydrochloride, fucoidin and bovine skin gelatin type B were purchased from Sigma Chemical Co (St. Louis, MO, U.S.A.). PGE_2 and LTC_4 were from Cascade Biochem Ltd (Berkshire, U.K.). The monoclonal anti-P-selectin antibody PB1.3 (lots P92-344 and P92-344B) was a kind gift from Laurie Philips, Cytel Corp. (La Jolla, CA, U.S.A.). Purified mouse IgG_1 was from The Binding Site, Birmingham (U.K.). Fluorescein isothiocyanate was from Molecular Probes Inc.

(Eugene, OR, U.S.A.). Dormicum was from Hoffman-La Roche (Basel, Switzerland). Hypnorm was from Janssen Pharmaceutica (Beerse, Belgium). Stesolid Novum was from A/S Dumex (Copenhagen, Denmark). Mebumal was from Nordvacc Läkemedel AB, (Stockholm, Sweden). Giemsa stain was from Karolinska Apoteket (Stockholm, Sweden). Canada balsam was from BDH Laboratory Supplies (Poole, U.K.).

Statistical evaluation

For statistical analysis, two-tailed nonparametric tests were used throughout. Thus, analysis of paired observations was performed by use of the Wilcoxon signed ranks test, and independent samples were analysed by the Mann-Whitney rank sum test or Kruskal-Wallis one way analysis of variance on ranks. Where appropriate, correlations between different parameters were sought by calculating the Spearman rank-order correlation coefficient (r_s). P values <0.05 were considered significant. All values are expressed as mean $\pm$ s.e.mean. Unless stated otherwise, n represents number of animals per group.

Results

Microvascular leukocyte concentrations in undisturbed rat mesentery

All mesenteric samples chosen for histological examination contained networks of microvessels with obvious differences between venules and arterioles, e.g., as compared to the venules, the arterioles had a thicker muscular wall with characteristic nuclei (Figure 1).

In undisturbed tissue, where the animal was killed and the circulation stopped before laparotomy, the concentrations of MNLs and PMNLs were higher in microvessels than in systemic arterial blood, i.e. the PMNL concentrations in systemic arterial blood, arterioles, and venules were 3.6 ± 0.5, 15.4 ± 2.3, and 20.3 ± 3.3 cells nl^{-1}, respectively, and the corresponding values for MNLs were 0.73 ± 0.12, 2.40 ± 0.89, and 1.74 ± 0.37, respectively ($P<0.05$ for all arteriolar and venular values vs systemic blood values, $n=12$). One possible explanation for this apparent discrepancy between the systemic and microvascular leukocyte concentrations could have been fixation-induced distortion of the microvessels, leading to errors in the calculation of microvessel volume. However, in rats given an i.v. bolus injection of FITC-labelled rat erythrocytes, the ratio of leukocytes to labelled red cells also revealed higher concentrations in venules (60.7 ± 13.1 MNLs and 8.9 ± 2.7 PMNLs per 100 red cells) as compared to systemic blood (8.9 ± 1.3 MNLs and 1.8 ± 0.3 PMNLs per 100 red cells, $n=6$, $P<0.05$ vs venular values). In these experiments, the number of FITC-labelled red cells nl^{-1} systemic blood (32.8 ± 4.4 cells nl^{-1}) and the number of red cells per calculated nl of venular blood (23.2 ± 3.5 cells nl^{-1}) did not differ significantly ($P=0.094$, $n=6$). An additional possibility for errors when intravascular leukocytes were counted was if endothelial or smooth muscle cell nuclei in the vessel wall were mistaken for those of leukocytes. However, the nuclei in the vessel wall were larger, had a more oval shape and stained lighter than the leukocytic nuclei (Figure 1). In addition, the intravascular position was easily confirmed by the presence of erythrocytes in the same focal plane.

The systemic leukocyte concentration (PMNL + MNL) in the Wistar rats ranged from a minimum of 1.33 to a high of 6.33 cells nl^{-1} arterial blood, with a median value of 3.25 cells nl^{-1} and no significant differences between the different treatment groups described below. Because the systemic leukocyte concentration will be reflected in the microcirculation (see also Yamaki *et al*., 1998), the induced changes in venular leukocyte concentration to be described were normalized to the systemic leukocyte count in each individual animal. For the group of untreated animals described above, such normalization revealed the venular/systemic leukocyte concentration-ratios shown in Figure 2 ('Baseline'. Note that vascular concentration exceeds systemic concentration when ratio >1).

Microvascular leukocyte concentrations in exteriorized rat mesentery

After preparation for intravital microscopy, the venular PMNL concentration rose strikingly during the first 45 min after preparation (Figure 2), reaching a venular/systemic PMNL concentration ratio of ≈ 100 at 30 and 45 min. In contrast, the MNLs were much less responsive in this respect (Figure 2). The venular PMNL accumulation did not appear to differ significantly between venules of different size because there was no correlation between venular diameter (range 18–60 μm) and the venular/systemic PMNL concentration-ratio in mesenteries exteriorized for 30 min ($r_s=-0.406$, $P=0.227$, $n=10$ venules).

As documented further below, the microvascular accumulation of leukocytes in the surgically or pharmacologically manipulated mesentery was a consequence of adhesive leukocyte-endothelium interactions (i.e. rolling and/or firm adhesion). To quantify the relative contribution of rolling *vs* firmly adherent leukocytes to the preparation-induced rise in venular PMNL concentration, intravital microscopy was used to count the number of firmly adherent leukocytes in a given venular segment 30 min after preparation. Immediately thereafter, the circulation was instantaneously arrested with a few drops of topical fixative, and the subsequent histological analysis revealed that the firmly adherent leukocytes represented $11.0 \pm 2.3\%$ ($n=9$) of the total number of intravascular leukocytes in the same venular segment. Thus, the histologically observed leukocyte accumulation was to a major extent dependent on increased leukocyte rolling, a contention that was further strengthened by the significant positive correlation between the rolling leukocyte flux and the venular PMNL concentration ($r_s=0.709$, $P=0.022$, $n=10$). In the latter experiments, the histologically measured number of PMNLs nl^{-1} in a given venular segment was related to the mean rolling leukocyte flux measured by intravital microscopy at three locations in the same venular segment 15–30 min after preparation (immediately before fixation). In addition to the rolling leukocyte flux, the leukocyte rolling velocity is likely to contribute to the degree of venular leukocyte accumulation. However, the degree of correlation was almost the same when the rolling leukocyte index (rolling leukocyte flux divided by the mean rolling velocity) was related to the venular PMNL concentration ($r_s=0.721$, $P=0.019$, $n=10$), indicating that rolling velocity was more constant than the rolling flux.

In contrast to the venules, the surgical preparation did not evoke significant leukocyte accumulation in arterioles. Thus, the arteriolar/systemic MNL and PMNL concentration-ratios were 5.07 ± 1.94 and 2.34 ± 0.50, respectively, 30 min after preparation ($n=7$), as compared to 4.92 ± 0.92 and 3.97 ± 1.75, respectively, in undisturbed mesenteric tissue ($n=12$). This is in line with the intravital microscopic observation that not even one rolling (or adherent) leukocyte was observed in arterioles 20–30 min into the experiments, as compared to a rolling

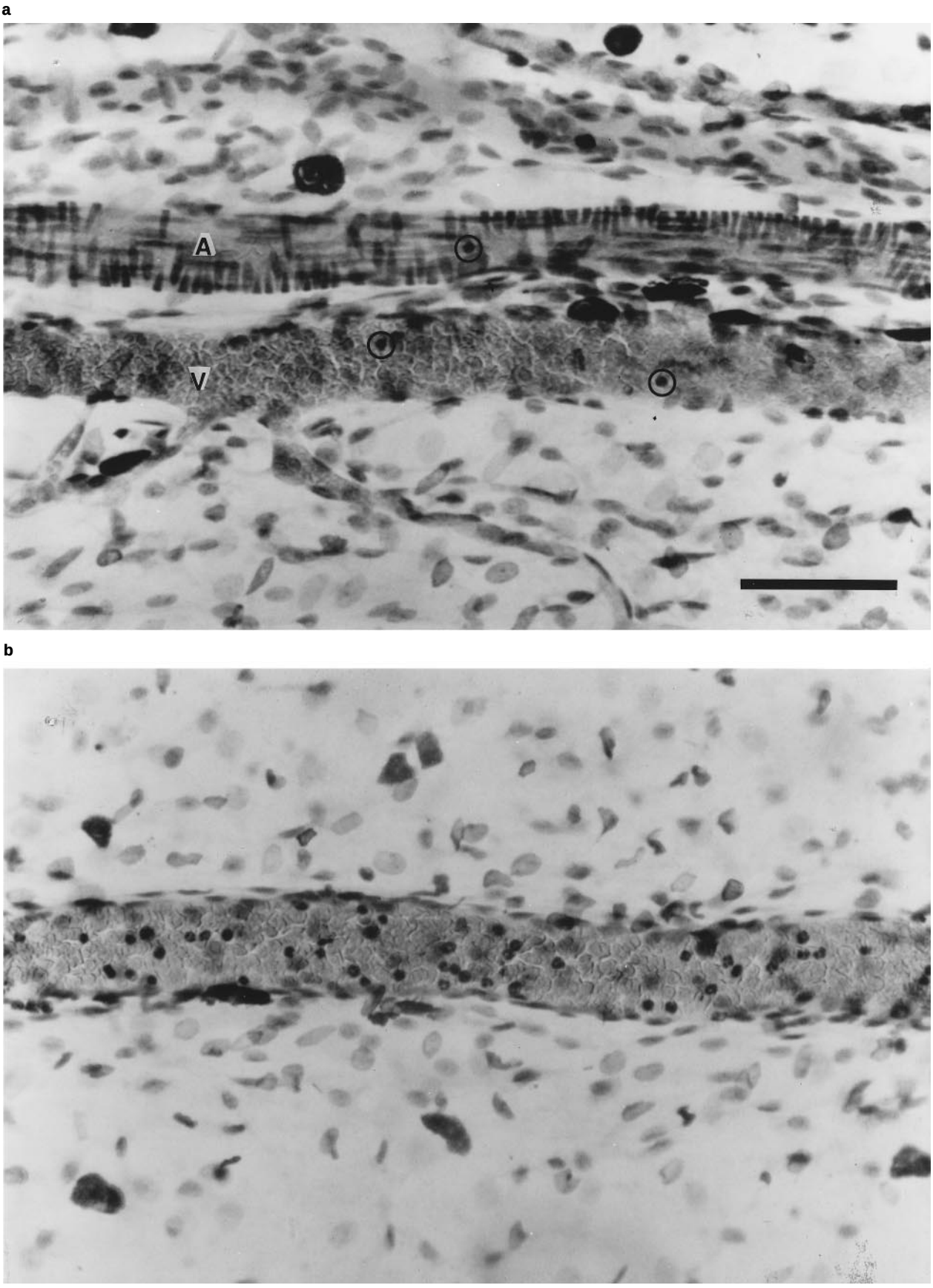

Figure 1 (a) An arteriole (A) and a venule (V) in a Giemsa-stained mesenteric whole-mount of an untreated rat. Note great number of smooth muscle nuclei in the arteriolar wall and a few encircled intravascular MNLs. (b) Illustrates increase in predominantly PMNLs in a venule 15 min after i.p. challenge with histamine (10^{-3} M). Note that the PMNLs may appear as MNLs unless strictly in the plane of focus. Bar = 70 μm.

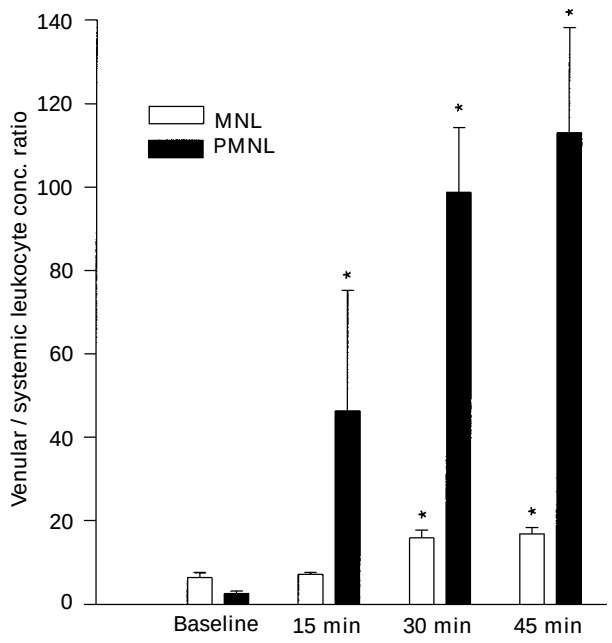

Figure 2 Ratio of mesenteric intravenular to systemic concentration of MNLs and PMNLs in undisturbed tissue (baseline, $n=12$), and 15 min ($n=5$), 30 min ($n=10$) or 45 min ($n=4$) after intestinal exteriorization for intravital microscopy in the rat. Mean values ± s.e.mean are shown. $^*P<0.05$ vs corresponding baseline values.

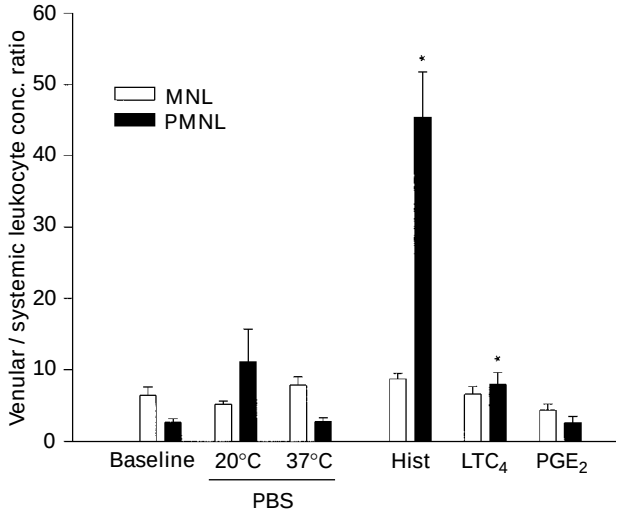

Figure 3 Ratio of mesenteric intravenular to systemic concentration of MNLs and PMNLs in undisturbed tissue (baseline, $n=12$), 15 min after i.p. challenge with 20°C PBS ($n=10$), 37°C PBS ($n=9$), histamine (10^{-3} M, $n=6-15$), LTC_4 (10^{-7} M, $n=9$), or PGE_2 (10^{-7} M, $n=6$) in the rat. Mean values ± s.e.mean are shown. $^*P<0.05$ vs corresponding baseline values.

leukocyte flux of 31.6 ± 7.5 cells min^{-1} in nearby venules ($n=10$).

With regard to systemic leukocytes, the MNL and PMNL counts in blood from tail artery 15, 30 and 45 min after preparation did not differ significantly from the corresponding values in the control rats exposed to anaesthesia alone (data not shown).

Mediator-induced leukocyte accumulation in mesenteric microvessels

We noted that i.p. injection of 5 ml PBS at room temperature (≈20°C) tended to increase the venular PMNL accumulation (Figure 3). Although this effect did not reach statistical significance due to a large variation, 4 animals out of 10 responded with a pronounced increase in venular PMNs (317, 1055, 1227, and 1312%, respectively, of the mean baseline value in the untreated group). In contrast, when the PBS solution was warmed to 37°C, the PMNL concentration remained at the baseline level in all 9 animals tested (Figure 3). Therefore, all subsequent experiments were performed with solutions warmed to 37°C.

Fifteen minutes after i.p. injection of 10^{-3} M histamine the PMNL concentration in mesenteric venules was increased 16 fold compared to untreated animals, whereas the MNL concentration was unchanged (Figure 3). In contrast, histamine had no effect on the PMNL concentration on the arteriolar side of the microcirculation, i.e. the arteriolar/systemic PMNL concentration-ratio was 2.04 ± 0.89 in undisturbed tissue and 2.74 ± 0.54 after i.p. challenge with histamine 10^{-3} M for 15 min.

The lipid mediator LTC_4 (10^{-7} M), which has a proinflammatory potency in the microcirculation exceeding that of histamine by 2–4 orders of magnitude (Dahlén *et al*., 1981), also caused a significant increase in venular PMNL recruitment (Figure 3). On the other hand, i.p. injection of the vasodilator PGE_2 did not increase the venular PMNL concentration (Figure 3). In animals given PBS i.p., the systemic leukocyte count was $0.66\pm0.06\times10^6$ ml^{-1} blood ($n=9$), and this parameter did not differ significantly in animals challenged i.p. with histamine, LTC_4 or PGE_2 (data not shown).

The relative contribution of rolling *vs* firmly adherent leukocytes to the histamine-induced increase in venular PMNL concentration was tested by use of two separate approaches. Firstly, a combination of intravital microscopic observation and histology (as described above) showed that the firmly adherent leukocytes in response to i.p. histamine (10^{-3} M, 15 min) represented $12.1\pm4.5\%$ ($n=5$) of the total number of intravascular leukocytes. Secondly, we examined the effect of fucoidin on the venular leukocyte accumulation evoked by i.p. histamine. The sulphated polysaccharide fucoidin has been shown to inhibit rapidly and effectively early leukocyte rolling in the rat mesentery without interfering with firm leukocyte adhesions *per se* (Lindbom *et al*., 1992). When fucoidin (10 mg kg^{-1}) was administered i.v. for 5 min, starting 10 min after i.p. histamine, the venular PMNL response to histamine 10^{-3} M was dramatically reduced (Figure 4). The systemic fucoidin treatment did not significantly change the circulating PMNL count, i.e. the number of PMNLs nl^{-1} systemic blood 5 min after i.v. fucoidin was 0.72 ± 0.11 ($n=4$) as compared 0.65 ± 0.07 ($n=9$) in vehicle-treated rats.

Effect of anti-P-selectin antibody treatment on histamine-induced PMNL accumulation in mesenteric venules

In contrast to fucoidin treatment, the commonly used anti-P-selectin monoclonal antibody (mAb) PB1.3 (2 mg kg^{-1}, i.v.) did not influence the histamine-induced venular PMNL accumulation in the female Wistar rats (Figure 4). Because PB1.3 was completely inactive in the first group of 4 animals tested, we repeated the experiments with a new lot of PB1.3 in additional 3 rats. However, with the same negative results (the two groups of animals were combined in Figure 4). In previous studies, PB1.3 has been shown to inhibit leukocyte responses in rat strains other than female Wistar (e.g. male Sprague-

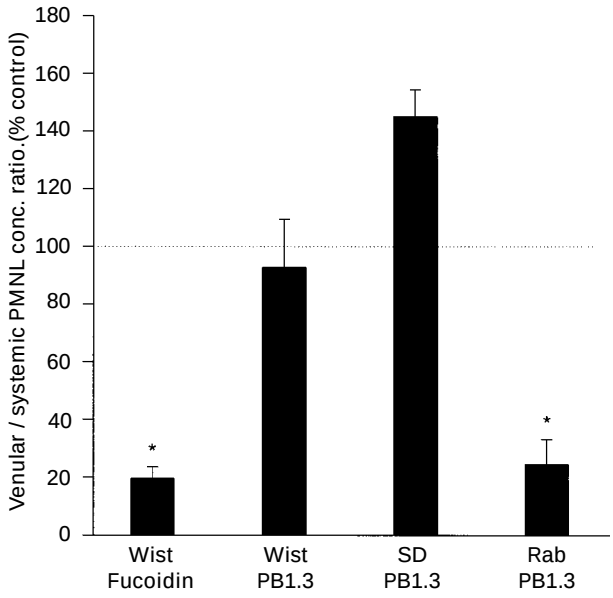

Figure 4 Effects of fucoidin (10 mg kg^{-1}, i.v.) in female Wistar rats (Wist, $n=4$) and anti-P-selectin mAb PB1.3 (2 mg kg^{-1}, i.v.) in female Wistar rats ($n=7$), male Sprague-Dawley rats (SD, $n=3$), and rabbits (Rab, $n=3$) on histamine-induced increase in ratio of intravenular to systemic PMNL concentration. Dashed line indicates control response to i.p. histamine (10^{-3} M) in Wistar rats ($n=15$), SD rats ($n=5$), and rabbits ($n=5$). Mean values$\pm$s.e.mean are shown. *$P<0.05$ vs control.

Dawley; SD) (Mulligan *et al*., 1992; Asako *et al*., 1994; Kubes & Kanwar, 1994). Therefore, we tested the antibody in a few male SD rats. In this rat strain, the venular/systemic PMNL concentration-ratio after i.p. histamine (10^{-3} M) alone was 30.31 ± 7.09 ($n=5$), as compared to a ratio of 2.32 ± 1.90 ($n=3$) in unstimulated rats, and the response to histamine was not reduced by PB1.3 treatment (2 mg kg^{-1}, i.v.) (Figure 4). Leukocyte concentration-ratios in PB1.3-treated rats were initially compared with saline-treated controls. Because no positive effect of PB1.3 was seen, negative control experiments with isotype matched control antibodies were not performed. We also tested PB1.3 in three rabbits, in which the venular/systemic PMNL concentration-ratio was 0.60 ± 0.09 in undisturbed mesentery ($n=2$) and 16.18 ± 2.72 after i.p. histamine 10^{-3} M ($n=5$). In contrast to the two rat strains, PB1.3 (2 mg kg^{-1}, i.v.) now very effectively inhibited the venular PMNL response to histamine (Figure 4). In one rabbit treated with negative control mouse IgG_1 (2 mg kg^{-1}, i.v.), the venular/systemic PMNL concentration-ratio after histamine challenge was 22.36 (i.e. unchanged). The systemic treatment with PB1.3 did not significantly change the circulating PMNL count in the rats (data not shown) or rabbits (1.17 ± 0.15 and 1.99 ± 0.36 PMNLs nl^{-1} systemic blood after i.p. histamine alone and histamine+PB1.3, respectively). Similar to the female Wistar rats, the venular MNL concentration in male SD rats and rabbits did not change in response to i.p. histamine challenge (data not shown).

Discussion

We have described the detailed characteristics of a histological approach for studies of leukocyte rolling in the microcirculation of the undisturbed rat mesentery. A series of experiments, including intravital microscopic observations, was performed to document that an increased venular leukocyte concentration may be used as an objective and reproducible index of leukocyte rolling triggered by inflammatory stimuli. The basis for the histological experiments is that the velocity of rolling leukocytes is up to 50 times slower than that of cells flowing freely with the axial blood stream (Ley & Gaehtgens, 1991; Mayrovitz, 1992). Consequently, the rapid influx of free-flowing leukocytes and the slow discharge of rolling cells will lead to an accumulation of leukocytes in microvessels where the rolling mechanism has been triggered.

The procedure of exposing the rat mesenteric microcirculation for intravital microscopy time-dependently increased the venular PMNL concentration, from a low baseline to approximately 40 and 100 fold the systemic PMNL concentration at 15 and 45 min, respectively. On the other hand, the MNLs were much less responsive to the preparative manipulation. Thus, the venular MNL concentration was unchanged 15 min after intestinal exteriorization and reached a maximum of only 16 fold the systemic MNL value 45 min after preparation. The apparent delay in MNL accumulation is in line with findings based on more complicated intravital microscopic approaches for differentiating MNL and PMNL rolling (Lindbom *et al*., 1994; Tangelder *et al*., 1995) and clearly illustrates that MNL and PMNL interactions with microvascular endothelium occur by distinct mechanisms.

With regard to the venular increase in PMNL concentration, our observations revealed that this reponse was caused by leukocyte rolling rather than firm adhesion. Thus, there was a highly significant correlation between the increased PMNL concentration (determined by histology) and the rolling leukocyte flux (measured by intravital microscopy immediately before fixation) in individual venules. Moreover, using the same type of combined intravital microscopy and histology, we were able to document that only $\approx10\%$ of the histologically determined increase in venular PMNL concentration (at 30 min) could be attributed to firm leukocyte adhesion. In addition, the intestinal exteriorization neither caused leukocyte rolling nor increased the PMNL concentration in arterioles running parallel to the venules under investigation.

As determined by histological estimation of microvessel volume, as well as by relating the number of leukocytes to the number of circulating FITC-labelled erythrocytes, the baseline concentration of MNLs and PMNLs was 3–6 fold higher in microvessels than in systemic blood. While this may suggest a certain degree of baseline rolling and/or adhesion of leukocytes in undisturbed tissue, the similarly elevated leukocyte concentration in arterioles, together with complete absence of rolling and adhesion in the latter vessels, indicated that the relatively higher microvascular leukocyte count was related to haemodynamic factors rather than to adhesive leukocyte-endothelium interactions. This is in line with previous intravital microscopic estimations of leukocyte concentration (calculated from leukocyte flux and blood flow) in rat mesenteric microvessels by Gaehtgens and co-workers (Gaehtgens *et al*., 1985; Ley *et al*., 1988). They showed that the local leukocyte concentration in the mesenteric vessels was up to six times the systemic leukocyte count and suggested that this may be related to disproportional distribution of leukocyte flux and blood volume at arteriolar bifurcations. Our observations that rolling is virtually non-existent in undisturbed rat mesenteric venules and is triggered during tissue preparation confirm and extend similar observations in other models/species (Fiebig *et al*., 1991; Ley, 1994). This is in contrast to the significant spontaneous rolling observed in intact untraumatized skin

(Mayrovitz, 1992), indicating the presence of organ specificity with regard to mechanisms of leukocyte rolling.

Our data on preparation-induced leukocyte accumulation in rat mesenteric venules are basically in agreement with observations by Fiebig *et al*. (1991), who studied venular leukocyte accumulation in the exteriorized mesentery of rabbits. Yet, there appears to exist significant differences between rats and rabbits in this regard. Firstly, while the venular leukocyte concentration 30 min after mesenteric exteriorization was 20 times the systemic leukocyte count in rabbits, our corresponding value in rats was as much as 5 fold higher still. Secondly, rat MNLs appear to be less responsive than rabbit MNLs, because in rabbits the increase in venular MNL concentration 5 and 30 min after preparation was approximately 50% of the PMNL value, while we observed no increase in MNL concentration at 15 min and only a minor rise at 30 min after exteriorization of the rat mesentery. Thirdly, Fiebig and co-workers found that systemic treatment with an anti-CD18 mAb, which blocks firm leukocyte adhesion but not rolling in rabbit tissues (Arfors *et al*., 1987; von Andrian *et al*., 1991), reduced the preparation-induced total venular leukocyte concentration by 60%. This suggests that mesenteric exteriorization in the rabbit results in a relatively large number of firmly adherent leukocytes, as compared to only $\approx$10% firmly adherent cells in the rat mesentery, where the fraction of these non-rolling leukocytes was directly documented by intravital microscopy immediately before fixation for histology.

A main objective of this study was to utilize the above histological technique for studies of leukocyte rolling evoked by exogenous mediators in otherwise undisturbed rat mesenteric venules. In these experiments, we first noted that i.p. injection of sterile room-temperature, but not body-temperature, PBS greatly increased the venular PMNL concentration in several animals. This illustrates that the experimental system is sensitive to even minor changes in the physical environment and suggests that peritoneal challenge should be performed with 37°C solutions to avoid triggering of 'unspecific' proinflammatory effector functions. Injection of body-tempered histamine for 15 min increased the venular PMNL (but not the MNL) concentration up to approximately the same extent as that caused by mesenteric exteriorization for 15 min. The almost 50 fold increase in PMNL concentration in response to histamine did not appear to vary with venular diameter and was 90% the result of triggered PMNL rolling (i.e. 10% due to firm adhesion). In further support of the histamine response being dependent on rolling, systemic treatment with the sulphated polysaccharide fucoidin, an effective anti-rolling agent which does not block firm leukocyte adhesion *per se* (Lindbom *et al*., 1992), almost abolished the histamine-induced increase in venular PMNLs. These findings are also in line previous intravital microscopic observations that topical application of histamine for up to 30 min does not evoke significant firm leukocyte adhesion in the rat mesentery (Kubes & Kanwar, 1994; Thorlacius *et al*., 1995).

Because histamine is a vasodilator in the rat mesentery (e.g. Thorlacius *et al*., 1995), it may be argued that haemodynamic changes *per se* rather than adhesive leukocyte-endothelium interactions were responsible for the PMNL response to histamine. However, histamine failed to affect the PMNL concentration on the arteriolar side of the microcirculation and did not change the systemic leukocyte counts. Moreover, i.p. injection of PGE_2, at a dose known to cause maximal arteriolar dilatation in the microcirculation (Raud, 1990), did not increase the venular PMNL concentration. The observations that PGE_2 had no effect on leukocyte accumulation and that LTC_4, a potent inflammatory mediator with a histamine-like mode of action at the endothelium (Majno & Palade, 1961; Hedqvist *et al*., 1987), caused a significant histamine-like increase in venular PMNL recruitment also demonstrate clear-cut mediator specificity with regard to the initial leukocyte response in microvessels.

It is obviously difficult to obtain reliable data on mediator-induced leukocyte rolling in intravital microscopic systems, where the preparation-induced 'spontaneous' rolling has been allowed to develop and this has been clearly documented in several laboratories, where local histamine challenge of the rat mesentery failed to increase rolling above baseline values (Kubes & Kanwar, 1994; Ley, 1994; Thorlacius *et al*., 1994). In this context, the histological index of evoked changes in leukocyte rolling described above is a useful, reproducible, relatively simple, and possibly even a more objective alternative for characterizing mode of action of proinflammatory stimuli (including time-course studies) and effects of different drugs on rolling. In addition, the method allows easy analysis of the apparently distinct rolling mechanisms of MNLs and PMNLs.

We also wanted to determine the nature of the adhesion molecule(s) responsible for histamine-induced PMNL accumulation. In this respect, the strongest candidate is P-selectin, because histamine is known to upregulate P-selectin rapidly on cultured endothelial cells and thus to support leukocyte rolling under flow conditions *in vitro* (Jones *et al*., 1993; Carlos & Harlan, 1994). Moreover, based on intravital microscopic findings, Asako *et al*. (1994) and Kubes & Kanwar (1994) have shown that the anti-human-P-selectin monoclonal antibody PB1.3 (claimed to cross-react with rat P-selectin; Mulligan *et al*., 1992) blocks histamine-induced rolling in the rat mesentery. However, we were unable to inhibit the mesenteric leukocyte response to histamine with mAb PB1.3 in Wistar and Sprague-Dawley rats, although the same mAb effectively reduced the corresponding histamine response in the mesentery of rabbits. We cannot explain these discrepancies in effect by PB1.3 in the basically very similar rat mesentery systems for histamine-induced rolling. However, knowing that 'spontaneous' rolling in, for example, mouse and dog is almost entirely a P-selectin-dependent event (Doré *et al*., 1993; Mayadas *et al*., 1993; Ley *et al*., 1995), it must be noted in this context that systemic treatment with PB1.3, as used in the present study, has been found to have no inhibitory effect on preparation-induced 'spontaneous' rolling in the rat mesentery (Thorlacius *et al*., 1994; Granger & Kubes, 1994). Thus, based on (i) our present positive findings with mAb PB1.3 in the rabbit, (ii) the current understanding of histamine and P-selectin function in systems other than the rat mesentery, and (iii) the possibility that mAb PB1.3 may express limited blocking activity against rat P-selectin, it remains likely that the presently observed PMNL response to histamine in the rat is indeed P-selectin-dependent. Yet, confirmation of the role of P-selectin in this regard requires additional experiments with specific anti-rat-P-selectin mAbs (presently not available).

In conclusion, we have shown that an increase in leukocyte concentration in mesenteric venules in the rat (or other animals) may be used as an index of mediator-induced leukocyte rolling, if the relative contribution of rolling and firm leukocyte adhesion is first determined, for example by the means described in this study. This relatively simple approach may be very useful for studying various aspects of leukocyte rolling when the 'spontaneous' rolling triggered by preparation for intravital microscopy is undesirable. As shown in the

following paper, we have used this method to characterize further histamine-induced leukocyte rolling (Yamaki *et al*., 1998).

This study was supported by the Swedish Medical Research Council (14X-4342, 04P-10738), The Swedish Foundation for Health Care Sciences and Allergy Research (A95093), the Swedish Asthma and Allergy Association, and Karolinska Institutet.

References

ABBASSI, O., KISHIMOTO, T.K., MCINTIRE, L.V., ANDERSON, D.C. & SMITH, C.W. (1993). E-selectin supports neutrophil rolling *in vitro* under conditions of flow. *J. Clin. Invest.*, **92,** 2719–2730.

ARFORS, K.-E., LUNDBERG, C., LINDBOM, L., LUNDBERG, K., BEATTY, P.G. & HARLAN, J.M. (1987). A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage *in vivo*. *Blood,* **69,** 338–340.

ASAKO, H., KUROSE, I., WOLF, R., DEFREES, S., ZHENG, Z.L., PHILLIPS, M.L., PAULSON, J.C. & GRANGER, D.N. (1994). Role of H1 receptors and P-selectin in histamine-induced leukocyte rolling and adhesion in postcapillary venules. *J. Clin. Invest.,* **93,** 1508–1515.

CARLOS, T.M. & HARLAN, J.M. (1994). Leukocyte-endothelial adhesion molecules. *Blood,* **84,** 2068–2101.

DAHLÉN, S.E., BJÖRK, J., HEDQVIST, P., ARFORS, K.E., HAMMARSTRÖM, S., LINDGREN, J.Å. & SAMUELSSON, B. (1981). Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: *in vivo* effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci. U.S.A.,* **78,** 3887–3891.

DORÉ, M., KORTHUIS, R.J., GRANGER, D.N., ENTMAN, M.L. & SMITH, C.W. (1993). P-selectin mediates spontaneous leukocyte rolling *in vivo*, *Blood,* **82,** 1308–1316.

EDWARDS, A.M. (1994). Sodium cromoglycate (Intal®) as an anti-inflammatory agent for the treatment of chronic asthma. *Clin. Exp. Allergy,* **24,** 612–623.

FIEBIG, E., LEY, K. & ARFORS, K.-E. (1991). Rapid leukocyte accumulation by "spontaneous" rolling and adhesion in the exteriorized rabbit mesentery. *Int. J. Microcirc. Clin. Exp.,* **10,** 127–144.

GAEHTGENS, P., LEY, K., PRIES, A.R. & MÜLLER, R. (1985). Mutual interaction between leukocytes and microvascular blood flow. *Prog. Appl. Microcirc.,* **7,** 15–28.

GRANGER, D.N. & KUBES, P. (1994). The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. *J. Leukocyte Biol.,* **55,** 662–675.

HEDQVIST, P., THURESON-KLEIN, Å., ÖHLÉN, A., RAUD, J., LINDBOM, L. & DAHLÉN, S.-E. (1987). Neuropeptides and arachidonic acid derivatives as messengers in microvascular function. In *Neuronal Messengers in Vascular Function*. ed. Nobin, A., Owman, C. & Arneklo-Nobin, B. pp. 435–446. Amsterdam: Elsevier.

JONES, D.A., ABBASSI, O., MCINTIRE, L.V., MCEVER, R.P. & SMITH, C.W. (1993). P-selectin mediates neutrophil rolling on histamine-stimulated endothelial cells. *Biophys. J.,* **65,** 1560–1569.

KUBES, P. & KANWAR, S. (1994). Histamine induces leukocyte rolling in post-capillary venules. A P-selectin-mediated event. *J. Immunol.,* **152,** 3570–3577.

KUNKEL, E.J. & LEY, K. (1996). Distinct phenotype of E-selectin-deficient mice. E-selectin is required for slow leukocyte rolling *in vivo*. *Circ. Res.* **79,** 1196–1204.

LAWRENCE, M.B. & SPRINGER, T.A. (1991). Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell,* **65,** 859–873.

LEY, K. (1994). Histamine can induce leukocyte rolling in rat mesenteric venules. *Am. J. Physiol.,* **267,** H1017–H1023.

LEY, K., BULLARD, D.C., ARBONÉS, M.L., BOSSE, R., VESTWEBER, D., TEDDER, T.F. & BEAUDET, A.L. (1995). Sequential contribution of L- and P-selectin to leukocyte rolling *in vivo*. *J. Exp. Med.,* **181,** 669–675.

LEY, K. & GAEHTGENS, P. (1991). Endothelial, not hemodynamic, differences are responsible for preferential leukocyte rolling in rat mesenteric venules. *Circ. Res.,* **69,** 1034–1041.

LEY, K., GAEHTGENS, P., FENNIE, C., SINGER, M.S., LASKY, L.A. & ROSEN, S.D. (1991). Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules *in vivo*. *Blood,* **77,** 2553–2555.

LEY, K., PRIES, A.R. & GAEHTGENS, P. (1988). Preferential distribution of leukocytes in rat mesentery microvessel networks. *Pflugers Arch.,* **412,** 93–100.

LEY, K., TEDDER, T.F. & KANSAS, G.S. (1993). L-selectin can mediate leukocyte rolling in untreated mesenteric venules *in vivo* independent of E- or P-selectin. *Blood,* **82,** 1632–1638.

LINDBOM, L., XIE, X., RAUD, J. & HEDQVIST, P. (1992). Chemoattractant-induced firm adhesion of leukocytes to vascular endothelium *in vivo* is critically dependent on initial leukocyte rolling. *Acta Physiol. Scand.,* **146,** 415–421.

LINDBOM, L., XIE, X., RAUD, J. & HEDQVIST, P. (1994). *In vivo* rolling of mononuclear leukocytes is distinct from that of polymorphonuclear cells. *FASEB J.,* **8,** A332 (abstract).

MAJNO, G. & PALADE, G.E. (1961). Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscope study. *J. Biophys. Biochem. Cytol.,* **11,** 571–605.

MAYADAS, T.N., JOHNSON, R.C., RAYBURN, H., HYNES, R.O. & WAGNER, D.D. (1993). Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell,* **74,** 541–554.

MAYROVITZ, H.N. (1992). Leukocyte rolling: a prominent feature of venules in intact skin of anesthetized hairless mice. *Am. J. Physiol.,* **262,** H157–H161.

MULLIGAN, M.S., POLLEY, M.J., BAYER, R.J., NUNN, M.F., PAULSON, J.C. & WARD, P.A. (1992). Neutrophil-dependent acute lung injury. Requirement for P-selectin (GMP-140). *J. Clin. Invest.,* **90,** 1600–1607.

OLOFSSON, A.M., ARFORS, K.E., RAMEZANI, L., WOLITZKY, B.A., BUTCHER, E.C. & VON ANDRIAN, U.H. (1994). E-selectin mediates leukocyte rolling in interleukin-1-treated rabbit mesentery venules. *Blood,* **84,** 2749–2758.

RAUD, J. (1990). Vasodilatation and inhibition of mediator release represent two distinct mechanisms for prostaglandin modulation of acute mast cell-dependent inflammation. *Br. J. Pharmacol.,* **99,** 449–454.

TANGELDER, G.J., JANSSENS, C.J., SLAAF, D.W., OUDE EGBRINK, M.G. & RENEMAN, R.S. (1995). *In vivo* differentiation of leukocytes rolling in mesenteric postcapillary venules. *Am. J. Physiol.,* **268,** H909–H915.

THORLACIUS, H., RAUD, J., XIE, X., HEDQVIST, P. & LINDBOM, L. (1995). Microvascular mechanisms of histamine-induced potentiation of leukocyte adhesion evoked by chemoattractants. *Br. J. Pharmacol.,* **116,** 3175–3180.

THORLACIUS, H., RAUD, J., ROSENGREN-BEEZLEY, S., FORREST, M.J., HEDQVIST, P. & LINDBOM, L. (1994). Mast cell activation induces P-selectin-dependent leukocyte rolling and adhesion in postcapillary venules *in vivo*. *Biochem. Biophys. Res. Commun.,* **203,** 1043–1049.

VON ANDRIAN, U.H., CHAMBERS, J.D., MCEVOY, L.M., BARGATZE, R.F., ARFORS, K.-E. & BUTCHER, E.C. (1991). Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and the leukocyte $\beta 2$ integrins *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 7538–7542.

YAMAKI, K., THORLACIUS, H., XIE, X., LINDBOM, L., HEDQVIST, P. & RAUD, J. (1998). Characteristics of histamine-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery. *Br. J. Pharmacol.,* **123,** 390–399.

(Received June 16, 1997
Revised September 12, 1997
Accepted October 17, 1997)