Contribution of selectins to leucocyte sequestration in pulmonary microvessels by intravital microscopy in rabbits

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1. Sequestration of leucocytes in the lung is the net result of leucocyte rolling and sticking in pulmonary arterioles and venules and their retention in alveolar capillaries.

2. In order to investigate whether adhesion molecules of the selectin family contribute to these phenomena the effects of fucoidin (an inhibitor of L- and P-selectin) on microhaemodynamics and leucocyte kinetics were studied in pulmonary arterioles, capillaries and venules by means of intravital fluorescence microscopy in a rabbit model.

3. Fucoidin reduced leucocyte rolling in pulmonary arterioles and venules by 75 and 83%, respectively, without affecting leucocyte sticking. In alveolar capillaries, fucoidin reduced leucocyte retention and accelerated leucocyte passage, thus reducing the alveolar transit time of leucocytes by 62%.

4. It is concluded that rolling of leucocytes in pulmonary microvessels is mediated by selectins, whereas sticking relies on selectin-independent mechanisms.

5. Leucocyte retention in alveolar capillaries is not due solely to mechanical hindrance of leucocyte passage through narrow vessel segments, as previously hypothesized, but also depends on interaction of leucocytes with the capillary endothelium.

Under physiological conditions, a large pool of leucocytes is sequestered in the pulmonary microcirculation, exceeding the total amount of circulating leucocytes 2- to 4-fold (Doerschuk, Allard, Martin, Mackenzie, Autor & Hogg, 1987). In the systemic circulation, intravascular accumulation of leucocytes is due to sticking, i.e. firm adhesion of leucocytes to the vascular endothelium, and rolling, i.e. transient interaction of leucocytes with the endothelium, resulting in slow movement along the vessel wall, and is predominantly restricted to venular vessel segments. Rolling of leucocytes is mediated by interaction of selectins with their ligands, namely L-selectin constitutively expressed on the surface of leucocytes (Ley, Gaehtgens, Fennie, Singer, Lasky & Rosen, 1991), E-selectin expressed by endothelial cells upon stimulation (Lawrence & Springer, 1993) and P-selectin on platelets and endothelial cells. The latter has recently been shown to mediate spontaneous leucocyte rolling in postcapillary venules of mesentery (Dore, Korthuis, Granger, Entman & Smith, 1993) and unstimulated skin (Nolte *et al.* 1994). In contrast, sticking of leucocytes in venules of the systemic circulation depends on β_2-integrins (CD11/CD18) on leucocytes, and their ligand (intercellular adhesion molecule 1; ICAM-1), on endothelial cells (Springer, 1990).

Though selectins and integrins have been shown to mediate intravascular leucocyte accumulation in systemic circulation, the contribution of these adhesion molecules to physiological sequestration of leucocytes in pulmonary circulation has not been investigated so far. In recent intravital microscopic studies (Kuebler, Kuhnle, Groh & Goetz, 1994; Kuhnle, Kuebler, Groh & Goetz, 1995), it has been demonstrated that rolling and sticking of leucocytes can be observed also in pulmonary arterioles and venules, thereby contributing to leucocyte pooling in the lung. However, the predominant site of leucocyte sequestration appears to be the capillary bed (Kuebler *et al.* 1994). Previously, leucocyte retention in alveolar capillaries has been attributed solely to mechanical hindrance of neutrophil transit, as a substantial percentage of capillary segments exhibit diameters that are distinctly smaller than mean neutrophil diameter (Doerschuk, Beyers, Coxson, Wiggs & Hogg, 1993).

The present study investigates the role of selectins in leucocyte sequestration in the lung. The objective was to determine whether leucocyte rolling observed in pulmonary arterioles and venules depends on selectins, as shown for venular segments of the systemic circulation, and whether leucocyte retention in capillaries is independent of these adhesion molecules. Microhaemodynamics and leucocyte

The authors wish to dedicate this paper to Professor Konrad Messmer on the occasion of his 60th birthday.

kinetics were investigated in pulmonary arterioles, venules and capillary networks prior to and following I.V. infusion of fucoidin, which has previously been reported as being a potent inhibitor of both L- and P-selectin (Ley, Linnemann, Meinen, Stoolman & Gaehtgens, 1993).

METHODS

Animal preparation

All animals received care in accordance with the 'Guide for the Care and Use of Laboratory Animals' (NIH publication no. 85-23, revised 1985). The study was approved by the local animal care and use comittee. Anaesthesia protocol, surgical preparation and experimental setup have recently been described in detail (Kuebler *et al.* 1994; Kuhnle *et al.* 1995). In brief, following induction of anaesthesia by thiopental sodium (50 mg I.V.), seven male New Zealand White rabbits (weighing 2·7–3·1 kg) were tracheotomized and ventilated mechanically (infant ventilator model IV-100, Sechrist Industries, Inc., Anaheim, USA) at an inspired O_2 fraction (F_{I,O_2}) of 0·33. Inspiratory airway pressure was set to 8 mmHg, and postive end-expiratory pressure at 2 mmHg. Respiratory rate was adjusted to maintain end-tidal P_{CO_2} at 35 mmHg. Anaesthesia was maintained by α-chloralose (50 mg (kg body wt)$^{-1}$ I.V.), and for analgesia and neuromuscular blockade piritramide (0·5 mg (kg body wt)$^{-1}$ I.V.) and pancuronium bromide (0·3 mg (kg body wt)$^{-1}$ I.V.) were administered. Adequacy of anaesthesia throughout the experiments was ensured by continuous monitoring of heart rate and arterial blood pressure. For continuous monitoring of macrohaemodynamic pressures and heart rate, catheters were introduced into the aorta (for measurement of arterial pressure and heart rate) and inferior vena cava (for measurement of central venous pressure) via femoral artery and vein, respectively. Following left thoracotomy, catheters were introduced via the left auricle into the left atrium (to measure left atrial pressure) and via the right ventricle into the pulmonary artery (to measure pulmonary arterial pressure). Cardiac output was measured continuously by means of an electromagnetic flow probe (Hellige, Freiburg, Germany) implanted around the pulmonary artery. Following partial resection of the right fourth and fifth ribs, a transparent window was implanted into the right thoracic wall, providing visual access to the surface of the right lung (Kuebler *et al.* 1994).

Cell labelling and intravital fluorescence microscopy

Autologous erythrocytes were labelled *in vitro* by fluorescein isothiocyanate (FITC) and reinjected, and leucocytes were stained *in vivo* by bolus injection of rhodamine 6G (0·06 μmol (kg body wt)$^{-1}$; Merck, Darmstadt, Germany). A Ploemopak illuminator (Leica, Wetzlar, Germany) fitted with an L3 filter block for FITC and an N2 filter block for rhodamine 6G (Leitz, Munich, Germany) enabled sequential visualization of FITC-labelled erythrocytes and rhodamine 6G-stained leucocytes in pulmonary microvessels by means of intravital videofluorescence microscopy. Kinetics of red and white blood cells in subpleural pulmonary arterioles, capillaries and venules were monitored by a silicon-intensified video camera (C2400-08, Hamamatsu, Herrsching, Germany) and recorded on videotape (video recorder AG-7350, Panasonic, Munich, Germany). In order to exclude respiratory movements, video recordings were performed during inspiratory plateau periods prolonged to 5 s each.

Quantification of microhaemodynamics and leucocyte kinetics

Microhaemodynamics and leucocyte kinetics were quantified in pulmonary arterioles and venules with diameters ranging from 19 to 26 μm, and in capillary networks of subpleural alveolar wall areas as previously described (Kuebler *et al.* 1994; Kuhnle *et al.* 1995). Microscopic investigations were performed on the lower margin of the right middle lung lobe. Only well perfused vessels of which wall margins depicted clearly in the focus plain and which did not show signs of oedema formation, i.e. widening of interalveolar septa, prior to baseline assessment, were included for analysis. Analysis of video recordings was performed by the frame-to-frame technique, using a digital image analysis system (Optimas, Bioscan, Edmonds, USA).

For determination of microhaemodynamics and leucocyte kinetics in pulmonary arterioles and venules, single vessel segments were analysed. Diameter (D) and length (L) of each investigated vessel segment were quantified. The passage of at least thirty labelled erythrocytes was analysed for determination of mean erythrocyte velocity ($\overline{V}_{RBC}$) and erythrocyte flux ($\dot{N}_{RBC}$), as described previously (Kuhnle *et al.* 1995). Blood flow velocity ($\overline{V}_b$) was calculated by correcting $\overline{V}_{RBC}$ for the Fåhraeus effect: the ratio of tube haematocrit (H_T) and discharge haematocrit (H_D) equals the ratio $\overline{V}_b/\overline{V}_{RBC}$ and can be calculated as follows (Pries, Neuhaus & Gaehtgens, 1992):

$$H_T/H_D = H_D + (1 - H_D)(1 + 1{\cdot}7\,e^{-0{\cdot}415D} - 0{\cdot}6\,e^{-0{\cdot}011D}). \quad (1)$$

H_D was assumed to correspond to systemic haematocrit as measured in blood drawn from the aorta. Microvascular blood flow (Q_b) and apparent wall shear rate (γ) were calculated as described previously (Kuebler *et al.* 1994; Kuhnle *et al.* 1995).

The velocity of each of at least thirty leucocytes (V_{WBC}) passing a predefined vessel cross-section was assessed as the distance cells travelled in axial direction of the vessel per time. Mean leucocyte velocity ($\overline{V}_{WBC}$) was calculated as the harmonic mean of the velocities of all passing leucocytes. Leucocyte flux ($\dot{N}_{WBC}$) was determined as the number of cells passing a predefined vessel cross-section per time. Rolling leucocytes, i.e. passing leucocytes temporarily interacting with the vascular endothelium and thus travelling distinctly slower than erythrocytes, were identified by means of a velocity criterion as white blood cells passing with a velocity below a critical velocity V_{crit}. V_{crit} was calculated from $\overline{V}_b$, assuming a parabolic velocity profile in the microvessel (Gaehtgens, Ley, Pries & Mueller, 1985):

$$V_{crit} = \overline{V}_b\,\epsilon\,(2 - \epsilon), \quad (2)$$

where ϵ is the ratio of leucocyte diameter (assumed to be 7 μm; Schmid-Schönbein, Shih & Chien, 1985) to vessel diameter. The flux of rolling leucocytes ($\dot{N}_R$) was expressed as a percentage of $\dot{N}_{WBC}$. Sticking leucocytes were defined as cells not moving during one entire observation period of 5 s, and expressed as density of cells adhering to the inner vascular wall surface (N_{ST}). The discharge concentration of leucocytes in a microvessel ($[WBC]_D$), representing the concentration of leucocytes in blood exiting the vessel segment, was calculated as:

$$[WBC]_D = \dot{N}_{WBC}/Q_b. \quad (3)$$

The tube concentration of leucocytes ($[WBC]_T$) representing the concentration of leucocytes within the vessel, was estimated as:

$$[WBC]_T = [WBC]_D\,(\overline{V}_b/\overline{V}_{WBC}) + N_{ST}/(L\,\pi\,(D/2)^2). \quad (4)$$

For determination of microhaemodynamics and leucocyte kinetics in alveolar capillaries, the subpleural wall of a single alveolus was investigated. The alveolar wall area (A_A) was measured planimetrically and the passage of at least thirty labelled erythrocytes and thirty leucocytes through the alveolar capillary

Table 1. Effect of fucoidin on macrohaemodynamics, blood gases and blood cell count

Parameter	Baseline	Fucoidin
AP (mmHg)	82·3 ± 5·3	77·3 ± 4·7
CVP (mmHg)	4·9 ± 0·9	5·4 ± 0·9
PAP (mmHg)	15·6 ± 1·0	15·6 ± 1·5
LAP (mmHg)	3·3 ± 0·5	3·7 ± 0·7
HR (beats min^{-1})	243 ± 10	225 ± 7
CO (ml min^{-1})	201 ± 26	193 ± 33
P_{a,O_2} (mmHg)	149 ± 4	151 ± 5
P_{a,CO_2} (mmHg)	33·8 ± 2·2	35·4 ± 2·5
pH	7·35 ± 0·04	7·32 ± 0·03
Hct (%)	33·0 ± 1·2	31·7 ± 1·0
RBC (10^6 μl^{-1})	5·01 ± 0·24	4·82 ± 0·21
WBC (10^3 μl^{-1})	7·98 ± 1·84	2·31 ± 0·56*
PLT (10^3 μl^{-1})	250 ± 51	23 ± 3*

Definition of abbreviations: AP, arterial pressure; CVP, central venous pressure; PAP, pulmonary arterial pressure; LAP, left atrial pressure; HR, heart rate; CO, cardiac output; P_{a,O_2}, arterial O_2 tension; P_{a,CO_2}, arterial CO_2 tension; Hct, haematocrit; RBC, red blood cell count; WBC, white blood cell count; PLT, platelet count. Values are means ± S.E.M., $n = 7$ animals. * $P < 0·05$ *versus* baseline.

network was analysed. The total length (L_A) of all perfused capillary segments was assessed by superimposing the pathways of all erythrocytes passing through the capillary network. The capillary perfusion index (CPI) representing the density of erythrocyte-perfused capillaries in the alveolar wall was calculated according to Wagner & Latham (1975):

$$\mathrm{CPI} = L_A / A_A \times 10^4. \quad (5)$$

Alveolar erythrocyte flux ($\dot{N}^A_{RBC}$) and leucocyte flux ($\dot{N}^A_{WBC}$) were determined as the number of cells passing a predefined cross-section perpendicular to the major flow direction in the network per time. The velocity of each erythrocyte (V^A_{RBC}) and leucocyte (V^A_{WBC}) passing the alveolar area was measured by tracing the entire passage through the capillary network and dividing the distance travelled by the time required. Mean erythrocyte velocity ($\bar{V}^A_{RBC}$) and mean leucocyte velocity ($\bar{V}^A_{WBC}$) were calculated as the harmonic mean of single-cell velocities. As leucocytes were traced from entering until leaving the capillary network of the alveolus, the time required for passage represents the alveolar capillary transit time of leucocytes (TT_{WBC}). Travelling the capillary networks leucocytes frequently stop for distinct time periods before moving again in order to continue their passage (Kuebler *et al.* 1994). Leucocytes stopping for 0·1 s or more, but moving again within the observation interval of 5 s, were defined as temporarily retained leucocytes (N_{TS}) and expressed as a percentage of $\dot{N}^A_{WBC}$. Time elapsed during each of these stops (t_{TS}) was determined, and subtracted from the alveolar capillary transit time of the respective leucocyte, thus enabling calculation of the *flow velocity* ($V^A_{WBC,flow}$) of each individual leucocyte and the mean leucocyte flow velocity ($\bar{V}^A_{WBC,flow}$). In contrast to V^A_{WBC} representing the *average velocity* of a single leucocyte during its entire passage through the capillary network *including temporary stops*, the retention of leucocytes in distinct capillary sites is *omitted* for calculation of $V^A_{WBC,flow}$, thus representing the velocity of the *moving leucocytes*. Leucocytes sticking within the alveolar wall area (N^A_{ST}), defined as cells not moving during one entire observation period of 5 s, were quantified as the number of cells per alveolar wall area. Assuming a mean capillary diameter (D_C) of 5·78 μm (Guntheroth, Luchtel & Kawabori, 1982), the tube concentration of leucocytes in the vascular segments of the capillary network ($[WBC]^A_T$) can be estimated as (Kuhnle *et al.* 1995):

$$[\mathrm{WBC}]^A_T = (\dot{N}^A_{WBC} TT_{WBC} + N^A_{ST}) / (L_A \pi (D_C/2)^2). \quad (6)$$

Experimental protocol

Under baseline conditions, in each arteriole, venule or capillary network erythrocyte kinetics were video-recorded during three inspiratory plateau periods using the filter block for FITC-fluorescence, and leucocyte kinetics during six inspiratory plateau periods using the filter block for rhodamine 6G. Following completion of video recordings, arterial blood samples were obtained for blood gas analysis (ABL 300, Radiometer, Copenhagen, Denmark) and peripheral blood cell count (T540, Coulter Electronics Inc., Krefeld, Germany). After a stabilization interval of 15 min, 20 mg (kg body wt)$^{-1}$ of the sulphated fucose polymer fucoidin (Sigma) was administered I.V. via the central venous catheter. Five minutes prior to application of fucoidin, a 0·3 mg (kg body wt)$^{-1}$ bolus of monovalent dextran (mol. wt 1000; Promit®, Schiwa, Glandorf, Germany) was infused I.V. as hapten in order to minimize anaphylactoid reactions due to infusion of polysaccharides (Hedin & Richter, 1982). Following I.V. injection of fucoidin, video recordings of the identical pulmonary microvessels were repeated, again followed by arterial blood drainage for blood gas analysis and peripheral blood cell count. At the end of the experiment, animals were killed by I.V. injection of saturated potassium chloride.

Statistics

All data are given as means ± S.E.M. Data were analysed statistically with the Wilcoxon matched-pairs signed-rank test for related samples. The Kolmogorov–Smirnov test was applied for testing frequency distributions for normal or lognormal distribution, as well as for detection of differences between two distributions. Skewness was calculated according to Pearson. Statistical significance was assumed at $P < 0·05$.

Table 2. Effect of fucoidin on microhaemodynamics and leucocyte kinetics in pulmonary arterioles and venules

	Arterioles		Venules	
Parameter	Baseline	Fucoidin	Baseline	Fucoidin
D (μm)	22·6 ± 0·8	21·7 ± 0·7	23·9 ± 0·7	22·6 ± 1·1
$\bar{V}_{RBC}$ (μm s^{-1})	756 ± 76	859 ± 69	1062 ± 156	1007 ± 92
$\dot{N}_{RBC}$ (10^3 s^{-1})	1·02 ± 0·17	1·15 ± 0·19	1·12 ± 0·31	1·15 ± 0·08
Q_b (nl min^{-1})	12·6 ± 2·0	13·0 ± 2·0	20·3 ± 3·7	17·2 ± 3·0
γ (s^{-1})	178 ± 18	213 ± 18	242 ± 32	238 ± 15
$\bar{V}_{WBC}$ (μm s^{-1})	354 ± 49	666 ± 92*	89 ± 12	405 ± 147*
$\dot{N}_{WBC}$ (s^{-1})	1·71 ± 0·26	0·80 ± 0·20*	1·56 ± 0·40	1·47 ± 0·80
$\dot{N}_R$ (%)	13·0 ± 4·6	3·3 ± 1·9*	75·8 ± 4·0	12·9 ± 3·5*
N_{ST} (mm^{-2})	181 ± 62	221 ± 93	527 ± 74	475 ± 135
$[WBC]_D$ (10^9 l^{-1})	10·1 ± 2·7	4·4 ± 1·4*	5·2 ± 1·2	5·1 ± 2·5
$[WBC]_T$ (10^9 l^{-1})	48·1 ± 15·7	44·9 ± 16·1	126 ± 14	102 ± 29

Definition of abbreviations: D, inner vessel diameter; $\bar{V}_{RBC}$, mean erythrocyte velocity; $\dot{N}_{RBC}$, erythrocyte flux; Q_b, microvascular blood flow; γ, apparent wall shear rate; $\bar{V}_{WBC}$, mean leucocyte velocity; $\dot{N}_{WBC}$, leucocyte flux; $\dot{N}_R$, percentage of rolling leucocytes; N_{ST}, number of sticking leucocytes per vessel wall surface; $[WBC]_D$, leucocyte discharge concentration; $[WBC]_T$, leucocyte tube concentration. Values are means ± S.E.M., $n = 7$ vessels within seven different animals. * $P < 0·05$ *versus* baseline.

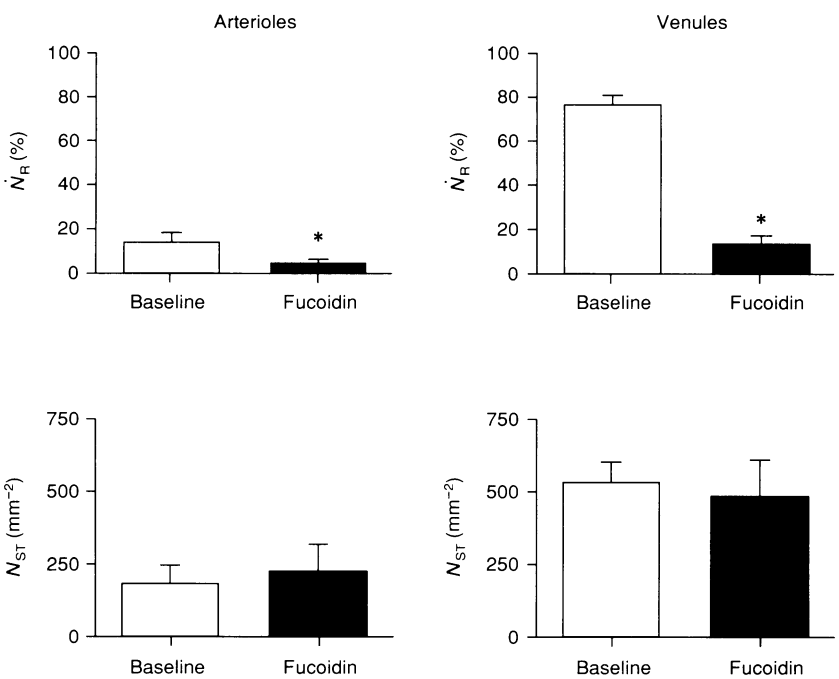

Figure 1. Effect of fucoidin on leucocyte endothelium interaction in pulmonary arterioles and venules

Percentage of rolling leucocytes ($\dot{N}_R$) and number of sticking leucocytes per vessel wall surface (N_{ST}) in pulmonary arterioles (left) and venules (right) under baseline conditions (open bars) and following I.V. infusion of fucoidin (filled bars). Means ± S.E.M., $n = 7$ vessels within seven different animals. * $P < 0·05$ *versus* baseline.

RESULTS

Macrohaemodynamics, blood gases and blood cell count

Macrohaemodynamic parameters and arterial blood gases did not change following I.V. infusion of 20 mg (kg body wt)$^{-1}$ fucoidin (Table 1). However, fucoidin induced a significant decrease in white blood cell count (WBC) and platelet count (PLT) in peripheral arterial blood, whereas red blood cell count (RBC) and haematocrit (Hct) remained unchanged (Table 1).

Microhaemodynamics and leucocyte kinetics in pulmonary arterioles and venules

Microhaemodynamics and leucocyte kinetics were quantified in seven pulmonary arterioles and seven venules, one per animal each, $22{\cdot}6 \pm 0{\cdot}8$ and $23{\cdot}9 \pm 0{\cdot}7$ μm in diameter, respectively, each prior to and following I.V. infusion of 20 mg (kg body wt)$^{-1}$ fucoidin. Fucoidin did not change vascular diameters or microhaemodynamics in pulmonary arterioles or venules (Table 2). However, fucoidin elicited significant effects on leucocyte kinetics (Table 2 and Fig. 1). Following I.V. infusion of fucoidin, mean leucocyte velocity increased significantly both in pulmonary arterioles and venules. Leucocyte flux and concomitantly the discharge concentration of leucocytes ($[WBC]_D$) decreased significantly in arterioles; however, both parameters did not change in pulmonary venules. Leucocyte rolling was observed under baseline conditions in pulmonary venules and to a smaller extent in pulmonary arterioles. Intravenous infusion of fucoidin reduced leucocyte rolling in arterioles by 75% and in venules by 83%, whereas it did not affect leucocyte sticking (Fig. 1). Finally, the tube concentration of leucocytes ($[WBC]_T$) decreased slightly without reaching significance in arterioles or venules.

Frequency distributions of single leucocyte velocities relative to mean erythrocyte velocity ($V_{WBC}/\bar{V}_{RBC}$) were established for pulmonary arterioles (Fig. 2) and venules (Fig. 3) under baseline conditions and following I.V. infusion of fucoidin. Despite the increase of V_{WBC}, the frequency distribution of $V_{WBC}/\bar{V}_{RBC}$ did not change in pulmonary arterioles following I.V. infusion of fucoidin. However, it changed significantly ($P < 0{\cdot}001$) in pulmonary venules: whereas under baseline conditions, $V_{WBC}/\bar{V}_{RBC}$ revealed a lognormal distribution approximately described by the equation:

$$y = e^{(-5{\cdot}4x+4{\cdot}7)},$$

following fucoidin both mode and median of the distribution revealed a 6- to 7-fold increase, while skewness decreased by 75%. These changes resulted in a right shift of $V_{WBC}/\bar{V}_{RBC}$, creating a non-lognormal frequency distribution of single leucocyte velocities relative to mean erythrocyte velocity. As a result from this shift in venules the frequency distributions of $V_{WBC}/\bar{V}_{RBC}$, which differed significantly between arterioles and venules under baseline conditions ($P < 0{\cdot}001$), were similar in both vessel compartments after fucoidin.

Microhaemodynamics and leucocyte kinetics in alveolar capillaries

In seven alveoli, one per animal each, with wall areas ranging from $4{\cdot}1 \times 10^3$ to $19{\cdot}7 \times 10^3$ μm^2, capillary length, capillary perfusion index, microhaemodynamics and leucocyte kinetics were quantified under baseline conditions and following I.V. infusion of fucoidin (Table 3). Total length of perfused

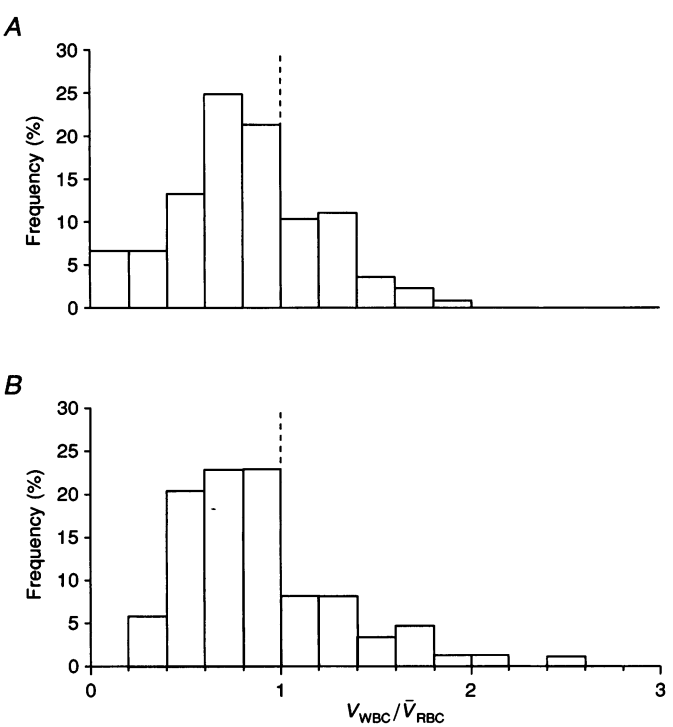

Figure 2. Effect of fucoidin on leucocyte kinetics in pulmonary arterioles

Frequency distribution of leucocyte velocity relative to mean erythrocyte velocity ($V_{WBC}/\bar{V}_{RBC}$) in pulmonary arterioles under baseline conditions (*A*) and following I.V. infusion of fucoidin (*B*). $\bar{V}_{RBC}$ is depicted as the interrupted line $x = 1$ in each panel. *A*, baseline, $n = 241$ cells, mode = 0·7, median = 0·79, skewness = 0·28. *B*, fucoidin, $n = 234$ cells, mode = 0·8, median = 0·81, skewness = 0·48. Seven vessels within seven different animals.

Table 3. Effect of fucoidin on microhaemodynamics and leucocyte kinetics in alveolar capillaries

	Alveolar capillaries	
Parameter	Baseline	Fucoidin
A_A (10^3 μm^2)	9·5 ± 1·8	9·3 ± 1·4
L_A (μm)	641 ± 73	781 ± 98*
CPI (μm)	781 ± 122	884 ± 95*
$\bar{V}^A_{RBC}$ ($\mu m\ s^{-1}$)	501 ± 47	591 ± 62*
$\dot{N}^A_{RBC}$ ($10^3\ s^{-1}$)	0·32 ± 0·05	0·37 ± 0·05
$\bar{V}^A_{WBC}$ ($\mu m\ s^{-1}$)	116 ± 23	224 ± 34*
$\bar{V}^A_{WBC,flow}$ ($\mu m\ s^{-1}$)	196 ± 30	296 ± 25*
$\dot{N}^A_{WBC}$ (s^{-1})	0·65 ± 0·17	0·50 ± 0·12
TT_{WBC} (s)	1·01 ± 0·37	0·38 ± 0·07*
N_{TS} (%)	37·6 ± 8·1	27·9 ± 7·2*
t_{TS} (s)	0·55 ± 0·22	0·22 ± 0·03*
N^A_{ST} (mm^{-2})	305 ± 83	289 ± 75
$[WBC]^A_T$ ($10^9\ l^{-1}$)	242 ± 69	141 ± 35*

Definition of abbreviations: A_A, alveolar wall area; L_A, total length of perfused capillary segments; CPI, capillary perfusion index; $\bar{V}^A_{RBC}$, mean erythrocyte velocity; $\dot{N}^A_{RBC}$, erythrocyte flux; $\bar{V}^A_{WBC}$, mean leucocyte velocity; $\bar{V}^A_{WBC,flow}$, mean leucocyte flow velocity; $\dot{N}^A_{WBC}$, leucocyte flux; TT_{WBC}, alveolar transit time of leucocytes; N_{TS}, percentage of temporarily stopping leucocytes; t_{TS}, time of temporary leucocyte stops; N^A_{ST}, number of sticking leucocytes normalized to alveolar wall area; $[WBC]^A_T$, leucocyte tube concentration. Values are means ± S.E.M., $n = 7$ capillary networks within seven different animals. * $P < 0·05$ *versus* baseline.

capillaries as well as capillary perfusion index and mean erythrocyte velocity increased significantly by 21·8, 13·2 and 18·0%, respectively, after I.V. infusion of fucoidin. However, both erythrocyte and leucocyte flux in alveolar wall areas remained constant.

Leucocyte kinetics in alveolar capillaries differ from those observed in pulmonary arterioles and venules. Leucocytes enter the capillary network with a relatively high velocity, but a certain percentage of cells stop at distinct sites of the capillary bed for a variable time period before moving again

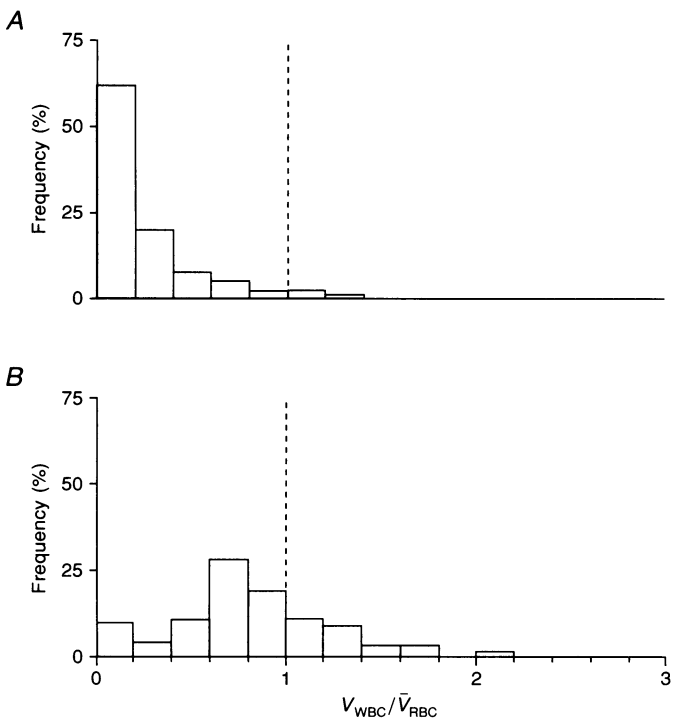

Figure 3. Effect of fucoidin on leucocyte kinetics in pulmonary venules

Frequency distribution of leucocyte velocity relative to mean erythrocyte velocity ($V_{WBC}/\bar{V}_{RBC}$) in pulmonary venules under baseline conditions (*A*) and following I.V. infusion of fucoidin (*B*). $\bar{V}_{RBC}$ is depicted as the interrupted line $x = 1$ in each panel. *A*, baseline, $n = 237$ cells, mode = 0·1, median = 0·12, skewness = 1·33. *B*, fucoidin, $n = 229$ cells, mode = 0·7, median = 0·78, skewness = 0·33. Seven vessels within seven different animals.

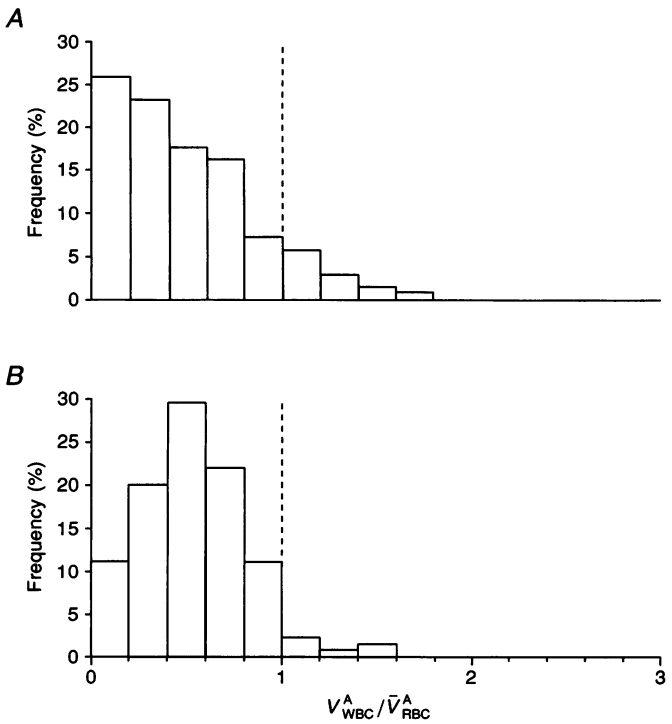

Figure 4. Effect of fucoidin on leucocyte kinetics in alveolar capillaries: leucocyte velocity

Frequency distribution of average velocity of single leucocytes relative to mean erythrocyte velocity ($V^{A}_{WBC}/\bar{V}^{A}_{RBC}$) in alveolar capillary networks under baseline conditions (*A*) and following I.V. infusion of fucoidin (*B*). $\bar{V}^{A}_{RBC}$ is depicted as the interrupted line $x = 1$ in each panel. *A*, baseline, $n = 219$ cells, mode = 0·1, median = 0·42, skewness = 0·63. *B*, fucoidin, $n = 226$ cells, mode = 0·5, median = 0·52, skewness = 0·11. Seven capillary networks within seven different animals.

and continuing their passage through the pulmonary microvasculature. Intravenous infusion of fucoidin changed leucocyte behaviour present under baseline conditions. As in arterioles and venules, fucoidin increased mean leucocyte velocity in alveolar capillaries significantly and thus reduced alveolar capillary transit time of leucocytes by 62%. This effect was on one hand due to an increase of leucocyte flow velocity in alveolar capillaries, and on the other hand due to a rarification of temporary leucocyte retention (reduction of N_{TS}) and shortening of retention time (decrease of t_{TS}). Despite this marked decrease of temporary leucocyte retention within alveolar capillaries, sticking of leucocytes in the alveolar wall area did not change during I.V. infusion of fucoidin. However, reduction of temporary leucocyte

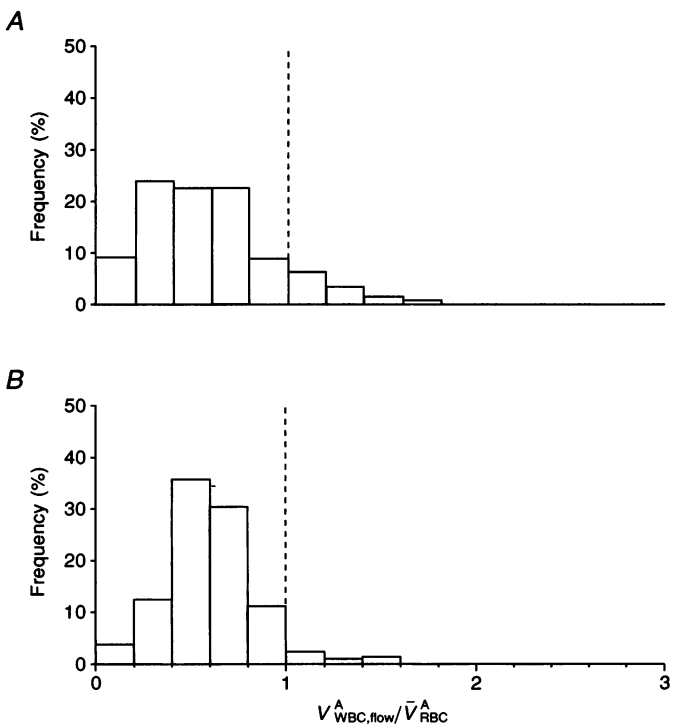

Figure 5. Effect of fucoidin on leucocyte kinetics in alveolar capillaries: leucocyte flow velocity

Frequency distribution of flow velocity of single leucocytes relative to mean erythrocyte velocity ($V^{A}_{WBC,flow}/\bar{V}^{A}_{RBC}$) in alveolar capillary networks under baseline conditions (*A*) and following I.V. infusion of fucoidin (*B*). $\bar{V}^{A}_{RBC}$ is depicted as the interrupted line $x = 1$ in each panel. *A*, baseline, $n = 219$ cells, mode = 0·3, median = 0·58, skewness = 0·26. *B*, fucoidin, $n = 226$ cells, mode = 0·5, median = 0·58, skewness = 0·25. Seven capillary networks within seven different animals.

retention by fucoidin significantly diminished the tube concentration of leucocytes in the vascular segments of the capillary network ($[WBC]^{A}_{T}$).

As in pulmonary arterioles and venules, frequency distributions of single leucocyte velocities relative to mean erythrocyte velocity were created for alveolar capillaries both for the *average velocity* of a single leucocyte (V^{A}_{WBC}) *including temporary stops* (Fig. 4) and for the *flow velocity* of each individual leucocyte ($V^{A}_{WBC,flow}$) *excluding temporary stops* (Fig. 5). Under baseline conditions, $V^{A}_{WBC}/\overline{V}^{A}_{RBC}$ exhibited a continuous left-shifted distribution, as indicated by dissociation of mode and median and positive skewness. Intravenous infusion of fucoidin changed this distribution significantly ($P < 0{\cdot}05$) and shifted the median and in particular the mode of the distribution to the right, thus abolishing the dissociation of mode and median and creating a normal distribution of $V^{A}_{WBC}/\overline{V}^{A}_{RBC}$. Left shift of frequency distribution by dissociation of mode and median was less expressed under baseline conditions for $V^{A}_{WBC,flow}/\overline{V}^{A}_{RBC}$ than for $V^{A}_{WBC}/\overline{V}^{A}_{RBC}$. Fucoidin also changed the frequency distribution of $V^{A}_{WBC,flow}/\overline{V}^{A}_{RBC}$ significantly ($P < 0{\cdot}05$) towards a normal distribution and resolved the interval between mode and median.

DISCUSSION

A large pool of physiologically sequestered leucocytes resides within the microvasculature of the lung (Doerschuk *et al.* 1987). In recent studies it was possible to demonstrate that rolling and sticking of leucocytes in pulmonary arterioles and venules as well as temporary cell retention in alveolar capillaries contribute to leucocyte sequestration in the pulmonary microcirculation (Kuebler *et al.* 1994; Kuhnle *et al.* 1995). The present study was designed to investigate whether these phenomena rely on selectin-dependent interactions of leucocytes with the vascular endothelium. As leucocyte sequestration in the lung is rather a dynamic process than a static phenomenon, intravital microscopy in particular lends itself to the study of microhaemodynamics and leucocyte kinetics in the pulmonary microvasculature.

Experiments were performed using a recently described model (Kuebler *et al.* 1994) for assessment of microhaemodynamics and leucocyte kinetics in pulmonary microvessels using fluorescently labelled erythrocytes and leucocytes. *In vivo* labelling of leucocytes by rhodamine 6G excludes cell activation by separation procedures and stains all circulating leucocytes for at least 90 min (Kuhnle *et al.* 1995). However, as the dye labels all leucocytes, this method does not permit differentiation between leucocyte subcategories (lymphocytes, neutrophils, basophils or monocytes) and caution must be taken not to treat leucocytes as a single homogenous population.

Surgical procedures as well as anaesthetic regimen and intravital microscopy have previously been shown by a myeloperoxidase assay not to induce pulmonary accumulation of neutrophils in the applied model (Kuebler *et al.* 1994). The stability of the preparation throughout the observation interval has also been demonstrated previously by means of intravital microscopy (Kuhnle *et al.* 1995).

For inhibition of both P- and L-selectin, 20 mg (kg body wt)$^{-1}$ of the sulphated fucose polymer fucoidin was administered intravenously. According to Handa, Nudelman, Stroud, Shiosawa & Hadomori (1991), sulphated glycans like fucoidin may interact with EGF-like domains or complement-regulatory sequence repeats of selectins, thereby inducing conformational changes of the lectin domain and thus inhibiting the binding of this domain to its ligand. In contrast, Ley *et al.* (1993) proposed that fucoidin might inhibit leucocyte rolling through a non-specific increase in charge density on the leucocyte surface. The reduction of leucocyte rolling by sulphated polysaccharides is dependent on the number of sulphate groups per monosaccharide (Tangelder & Arfors, 1991), and fucoidin was shown to be a potent inhibitor of leucocyte rolling *in vivo* (Granert, Raud, Xie, Lindquist & Lindbom, 1994) by blocking L- and P-selectin in a dose-dependent manner (Ley *et al.* 1993). However, in a recent study, Kubes, Jutila & Payne (1995) also proposed the existence of a fucoidin-sensitive, L- and P-selectin-independent leucocyte rolling. The authors observed a $> 90\%$ inhibition of leucocyte rolling in post-ischaemic mesenteric venules of cats by 25 mg (kg body wt)$^{-1}$ fucoidin, whereas a combination of an anti-L- and an anti-P-selectin antibody only reduced leucocyte rolling by 60%. The possible existence of selectin-unspecific effects of fucoidin has therefore to be kept in mind when inhibition of leucocyte rolling by fucoidin is interpreted.

Microhaemodynamics and leucocyte kinetics in pulmonary arterioles and venules

Microhaemodynamic data reported in this study under baseline conditions compare well with previously reported values (Kuebler *et al.* 1994; Kuhnle *et al.* 1995). Throughout the experiments, microhaemodynamics did not change. Rolling and sticking of leucocytes were observed in both pulmonary arterioles and venules and thus contribute to leucocyte sequestration in the lung under baseline conditions. Leucocyte rolling was more pronounced in pulmonary venules despite higher shear rates in these vessel segments.

Intravenous infusion of fucoidin inhibited leucocyte rolling in arterioles and venules by approximately 80%, without affecting leucocyte sticking. These data suggest that rolling of leucocytes in pulmonary arterioles and venules is predominantly mediated by L- and/or P-selectin, whereas sticking relies on adhesion molecules other than selectins.

As leucocyte rolling is a relatively scarce phenomenon in pulmonary arterioles, blocking of selectins by fucoidin does not influence leucocyte kinetics in these vessels decisively as indicated by constant frequency distributions of $V_{WBC}/\overline{V}_{RBC}$ (Fig. 2). In contrast, rolling is the predominant behaviour of leucocytes passing through pulmonary venules. In consequence, inhibition of leucocyte rolling significantly

changes leucocyte kinetics in these segments. Whereas $V_{WBC}/\overline{V}_{RBC}$ exhibited a lognormal distribution under baseline conditions resulting from multiplicative interaction of random variables, blocking of L- and P-selectin changed $V_{WBC}/\overline{V}_{RBC}$ to an almost normal distribution, indicating qualitative elimination of one of these variables (Fig. 3) and thus identifying selectin-mediated rolling as a major determinant of leucocyte kinetics in pulmonary venules.

Fucoidin abolished the difference in frequency distributions of $V_{WBC}/\overline{V}_{RBC}$ seen between pulmonary arterioles and venules under baseline conditions. This finding indicates that retardation of leucocyte transit in pulmonary venules compared with arterioles is due to a higher fraction of rolling cells in venules and thus to preferential expression of selectins and/or selectin ligands on the venular endothelium, but not to rheological properties. Despite inhibition of leucocyte rolling, mean leucocyte velocity did not reach mean erythrocyte velocity in arterioles or venules. This can be attributed to low flow rates in the pulmonary microvasculature displacing leucocytes to the marginal layers of the vessel cross-section, as a result of shear-dependent red cell aggregation.

Despite blocking of L- and P-selectin by fucoidin, rolling of leucocytes was not completely abolished in pulmonary arterioles or venules. This finding is in accordance with previous data from Ley *et al.* (1993) and Gaboury & Kubes (1994), who reported a 90% reduction of leucocyte rolling in mesenteric venules by fucoidin. Gaboury & Kubes (1994) demonstrated that the remaining rolling cells could be further reduced by 50% by additional administration of an anti-CD18 antibody, but increased 4-fold when shear rates were reduced by 50%. CD18-dependent rolling of leucocytes may well account for fucoidin-insensitive leucocyte rolling in pulmonary arterioles and venules, in particular as shear rates in the low-pressure system of the pulmonary circulation are small.

An important point of discussion is whether leucocyte rolling observed in pulmonary arterioles and venules is a physiological phenomenon or results from surgical preparation required for microcirculatory studies (Fiebig, Ley & Arfors, 1991). Spontaneous leucocyte rolling in unstimulated tissue is absent in the intact bat wing (Mayrovitz, Tuma & Wiedmann, 1980), but can be observed in skin venules of mice (Nolte *et al.* 1994) without prior stimulation or surgical trauma. Measurement of myeloperoxidase activity did not reveal pulmonary accumulation of neutrophils due to surgical trauma in the applied model (Kuebler *et al.* 1994). Moreover, leucocyte accumulation in pulmonary microvessels as calculated from our data (Kuhnle *et al.* 1995) compares well with values obtained by histomorphological studies, where surgical stimulation can be excluded (Hogg, McLean, Martin & Wiggs, 1988).

In a recent intravital microscopic study on dogs, Gebb *et al.* (1995) were able to confirm the previous finding (Kuebler *et al.* 1994; Kuhnle *et al.* 1995) that leucocyte rolling is a prominent feature in pulmonary microvessels. As the authors were unable to block this phenomenon by anti-P-selectin antibody, they concluded that rolling leucocytes in the pulmonary microvasculature adhere via L-selectin. However, studies by Nolte *et al.* (1994) showed that spontaneous leucocyte rolling in unstimulated ear skin of hairless mice is exclusively mediated by P- and not by L-selectin. Furthermore, von Willebrand factor (vWf) is found predominantly in pulmonary venules, but is scarce in arterioles (Simionescu, 1991). As P-selectin is stored together with vWf in the endothelial Weibel–Pallade bodies and is expressed during vWf release (Hattori, Hamilton, Fugate, McEver & Sims, 1989), it may be predominantly present on pulmonary venular compared with arteriolar endothelium, a finding that fits the distribution of leucocyte rolling in the different segments of the pulmonary microcirculation. Finally, it has to be taken into account that fucoidin might also block leucocyte rolling by L- and P-selectin-independent pathways, as proposed by Kubes *et al.* (1995). In conclusion, the question whether L- or P-selectin mediates leucocyte rolling in pulmonary arterioles and venules or the identity of a possible additional fucoidin-sensitive pathway remains unclear, and should be the subject of future studies.

Microhaemodynamics and leucocyte kinetics in alveolar capillaries

In alveolar capillaries, leucocytes travel with a velocity of approximately 40% of mean red cell velocity. At distinct sites of the capillary bed, leucocytes stop abruptly for variable periods before moving again in order to continue their passage through the capillary network. Doerschuk *et al.* (1993) attributed this retention of leucocytes to the large fraction of alveolar capillary segments that were narrower than spherical neutrophils and to differences between the time required for deformation of neutrophils and that required for deformation of erythrocytes. Gebb *et al.* (1995) observed that leucocytes have to deform into elongated shapes in order to pass through the pulmonary capillary bed, and Wiggs, English, Quinlan, Doyle, Hogg & Doerschuk (1994) suggested that neutrophils can undergo a rapid deformation from 6·4 to 5·0–5·1 μm, whereas larger deformations require longer time periods.

In contrast to this mechanical concept, the results of this study suggest that, in addition to pure cell size-dependent hindrance of leucocyte transit as the underlying cause for leucocyte sequestration in alveolar capillaries, interaction of leucocytes with the capillary endothelium may contribute to this phenomenon. Intravenous infusion of fucoidin significantly reduced frequency and duration of temporary leucocyte retention in alveolar capillaries, and furthermore increased flow velocity of moving leucocytes. As a result, capillary transit of leucocytes accelerated drastically.

Fucoidin significantly changed the frequency distributions of both average velocity (V^{A}_{WBC}) and flow velocity ($V^{A}_{WBC,flow}$) of single leucocytes relative to mean erythrocyte velocity

($\overline{V}^{A}_{RBC}$). These findings confirm the retarding effect of fucoidin-sensitive leucocyte–endothelial interaction on leucocyte retention and leucocyte flow in alveolar capillaries independent of microhaemodynamic factors. Blocking of both L- and P-selectin by fucoidin changed $V^{A}_{WBC}/\overline{V}^{A}_{RBC}$ and $V^{A}_{WBC,flow}/\overline{V}^{A}_{RBC}$ to simple normal distributions typical of rheological patterns of free-flowing particles not interacting with the vascular wall.

The results of this study indicate for the first time that leucocyte retention in alveolar capillaries does not solely result from mechanical hindrance of leucocyte transit through narrow capillary segments, but is in addition prolonged by fucoidin-sensitive interaction of leucocytes with the capillary endothelium. By means of immunogold labelling for L-selectin, Burns & Doerschuk (1994) were able to demonstrate that elongated neutrophils within small alveolar capillaries show small clusters of L-selectin at discrete sites along the flattened neutrophil surface, which may promote selectin-mediated interaction of retained leucocytes with the capillary endothelium. In addition to the amplifying effect on leucocyte retention, fucoidin-sensitive leucocyte–endothelium interaction also retards flow of moving leucocytes in alveolar capillary networks and thus contributes quantitatively and qualitatively to leucocyte accumulation in alveolar capillaries. Again the results of this study do not allow discrimination between L- and P-selectin-mediated leucocyte–endothelial interaction, and as no specific antibodies were used, selectin-unspecific effects of fucoidin must also be considered (Kubes *et al.* 1995). However, sequestration of leucocytes is also attributable to mechanical events, as fucoidin did not completely prevent leucocyte retention in alveolar capillaries. It may be speculated that initial tethering of leucocytes is due to cell size-dependent hindrance, whereas selectin-mediated interaction with the endothelium prolongs leucocyte retention, as this could account for the observed shortening and rarification of temporary leucocyte retention following fucoidin.

In contrast to temporary leucocyte retention, sticking of leucocytes in alveolar capillary networks, defined as firm adhesion of cells for an entire observation period of 5 s, was not affected by fucoidin. It may therefore be concluded that sticking of leucocytes, as observed in our model, differs qualitatively from temporary leucocyte retention, and relies on mechanisms independent of selectins, similar to leucocyte sticking in pulmonary arterioles and venules. In a recent study using ^{125}I-labelled anti-ICAM-1 monoclonal antibody in rats, Panés *et al.* (1995) were able to demonstrate that constitutive expression of ICAM-1 in lung tissue exceeds values from other organs at least 30-fold. It may therefore be speculated that sticking of leucocytes in pulmonary microvessels is mediated by ICAM-1 and β_2-integrins.

In contrast to pulmonary arterioles and venules, blocking of L- and P-selectin revealed significant effects on microhaemodynamic parameters in alveolar capillary networks. Following fucoidin, the total length of perfused capillaries and thus capillary perfusion index increased as well as mean erythrocyte velocity in alveolar capillaries. This can be attributed to the close connection between CPI, $\overline{V}^{A}_{RBC}$ and leucocyte retention in alveolar capillary networks (Kuebler *et al.* 1994; Kuhnle *et al.* 1995). Fucoidin mobilizes leucocytes from alveolar capillary networks, and thus reopens capillary segments previously obstructed by retained leucocytes and therefore increases CPI. In addition, fucoidin accelerates leucocyte transit through alveolar capillaries, thus preventing erythrocytes from accumulating as red cell trains behind a slowly moving leucocyte within a small vessel tube, and therefore also increases $\overline{V}^{A}_{RBC}$. As a result of increasing CPI and $\overline{V}^{A}_{RBC}$, total capillary blood flow rises and thus blood flow in the feeding arteriolar vessels must augment. In the experiments of this study, $\overline{V}_{RBC}$ exhibited a tendency to increase in pulmonary arterioles following fucoidin, without reaching significance. However, blood flow in the draining pulmonary venules remained constant, as under zone 2 conditions investigated in our model, it is independent of capillary blood flow due to the pulmonary vascular waterfall (Permutt & Riley, 1963; Kuebler *et al.* 1994) dissociating hydraulic gradients for flow. These findings demonstrate that capillary recruitment and derecruitment in the lung are not only functions of blood flow and ventilation/perfusion pressures, but are also governed by leucocyte retention and mobilization in alveolar capillaries. Though the latter phenomenon does not alter pulmonary vascular resistance due to the density of the alveolar capillary network, it may affect gas exchange capacity without affecting gas exchange under physiological conditions.

Though I.V. infusion of fucoidin induced mobilization of leucocytes from all compartments of the pulmonary microvasculature, peripheral leucocyte count dropped by approximately 70%. This adverse effect might be due to an anaphylactoid reaction to the sulphated polysaccharide fucoidin. Dextrans used clinically for replacement of blood loss are known to induce both anaphylactoid and anaphylactic reactions (Hedin & Richter, 1982). The latter are immune-complex-mediated reactions caused by naturally occurring dextran-reactive antibodies predominantly of the IgG class and can be effectively prevented by hapten prophylaxis (Ljungstroem, Renck, Hedin, Richter & Wilholm, 1988). In contrast, anaphylactoid reactions are mild non-immunogenic reactions and may thus be observed despite preventive administration of monovalent dextran.

A potential anaphylactoid reaction to fucoidin might reduce leucocyte counts in rabbits significantly, though macrohaemodynamic parameters are not influenced. However, it remains unclear whether this depletion of leucocytes is due to lysis of cells or to sequestration in organs other than the lung.

Nevertheless, the observed depletion of leucocytes does not compromise the results of this study. In the case of leucocyte activation by fucoidin, expression of adhesion molecules and increased cell rigidity would enhance leucocyte sequestration in the lung (Worthen, Schwab, Elson & Downey, 1989). Even shedding of L-selectin (Kishimoto, Jutila & Butcher, 1990) by fucoidin would only confirm the conclusion that leucocyte rolling in pulmonary arterioles and venules and in part leucocyte retention in alveolar capillaries depend on engagement of selectins. However, leucocyte depletion by sulphated glycans may explain previous findings on the contribution of selectins to leucocyte sequestration in the lung. Tangelder & Arfors (1991) investigated mesenteric venules of anaesthetized rabbits and were able to inhibit leucocyte rolling in these vessels by I.V. infusion of sulphated dextrans. Yet, as systemic leucocyte counts did not change during their experiments, the authors concluded that rolling is not a mechanism responsible for leucocyte sequestration in the lung. The results of this study indicate that conclusions on leucocyte kinetics in the lung can hardly be drawn from peripheral cell counts in the presence of potentially anaphylactoid agents such as sulphated glycans. Under these conditions, only direct observation of leucocyte kinetics in pulmonary microvessels by intravital microscopy provides insight into the dynamic process of leucocyte sequestration and release in the pulmonary microvasculature.

Mediation of leucocyte sequestration in the lung by selectins has previously been reported for a variety of pathophysiological conditions. In various models of acute lung injury, involvement of selectins has been shown (Mulligan, Polley, Bayer, Nunn, Paulson & Ward, 1992; Mulligan, Miyasaka, Tamatani, Jones & Ward, 1994). Mulligan, Watson, Fennie & Ward (1993) were able to demonstrate protective effects of a P- and L-selectin–Ig chimera in a complement activation model and of a E- and L-selectin–Ig chimera in an immune complex model of acute lung injury. Using fucoidin, Shimaoka, Ikeda, Iida, Taenaka, Yoshiya & Honda (1996) were able to prevent neutrophil extravasation in phorbol myristate acetate-induced lung inflammation. Moore, Khimenko, Adkins, Miyasaka & Taylor (1995) showed that inhibition of P-selectin completely blocked ischaemia/reperfusion injury in rat lungs, whereas the anti-L-selectin antibody HRL3 had no effect. In contrast, the findings of this study suggest that selectins contribute to leucocyte sequestration in pulmonary arterioles, venules and even capillaries already under physiological conditions. Selectin-mediated interaction of leucocytes with the capillary endothelium may be the first step of an adhesion cascade promoting leucocyte accumulation in the lung under pathophysiological conditions and finally resulting in leucocyte emigration into interstitium and air spaces. This concept may explain why in pulmonary inflammation 97% of migrating leucocytes move across the capillary wall (Lien *et al.* 1991), in contrast to organs of the systemic circulation, where post-capillary venules were shown to be the predelection site for leucocyte emigration.

In summary, the present study has shown that sequestration of leucocytes in pulmonary arterioles and venules is due to selectin-mediated rolling and selectin-independent sticking of leucocytes. Leucocyte retention in alveolar capillaries is not due solely to mechanical hindrance of their transit through the narrow segments of the pulmonary capillary network, but also implies the interaction of leucocytes with the capillary endothelium.

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