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Direct in vivo observations of P-selectin glycoprotein ligand-1-mediated leukocyte-endothelial cell interactions in the pulmonary microvasculature in abdominal sepsis in mice

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Running Head: PSGL-1 and septic lung injury

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Abstract

Objective P-selectin glycoprotein ligand-1 (PSGL-1) has been shown to play a significant role in septic lung injury. However, the detailed role of PSGL-1 in the pulmonary leukocyte recruitment remains elusive. We have developed a method based on intravital fluorescence microscopy of the lung microcirculation to examine the role of PSGL-1 in the extravasation process of leukocytes in septic lung damage.

Methods Male C57BL/6 mice were treated with a control antibody or an anti-PSGL-1 antibody prior to cecal ligation and puncture (CLP). Leukocyte-endothelium interactions and microvascular hemodynamics were studied in pulmonary arterioles, capillaries and venules 4 hours after CLP.

Results Immunoneutralization of PSGL-1 decreased CLP-induced leukocyte rolling in pulmonary arterioles and venules significantly. Inhibition of PSGL-1 had no effect on leukocyte adhesion in venules, whereas the number of adherent leukocytes in lung arterioles and the number of trapped leukocytes in capillaries were markedly decreased. Moreover, immunoneutralization of PSGL-1 improved microvascular perfusion in the lung of septic animals.

Conclusions Taken together, these results document that PSGL-1 mediates leukocyte rolling in arterioles and venules. However, inhibition of PSGL-1 only decreases leukocyte adhesion in arterioles, suggesting that leukocyte rolling is not a prerequisite for pulmonary venular adhesion of leukocytes in sepsis. In addition, our data show that capillary trapping of leukocytes is dependent on PSGL-1 function.

Key Words: Adhesion, Inflammation, Leukocyte, Lung, Rolling and Selectins

Introduction

Intestinal perforation and dissemination of bacteria in the abdominal cavity trigger local synthesis of numerous pro-inflammatory substances, which subsequently leak into the circulation. Once in the blood, these pro-inflammatory substances activate circulating neutrophils and cause a systemic inflammatory response [1]**.** Abdominal sepsis is a major cause of mortality in intensive care units and poses a significant challenge for clinicians despite aggressive surgical interventions and antibiotic therapies [2-3]. The lung is the most sensitive and critical target organ in abdominal sepsis. It is well established that pulmonary infiltration of neutrophils is a rate-limiting step in septic lung injury. For example, depletion of neutrophils or immunoneutralization of specific adhesion molecules, including P-selectin glycoprotein ligand-1 (PSGL-1), LFA-1 and Mac-1 are effective ways to protect against sepsis-induced lung injury [4-5]. Although the therapeutic potential of inhibiting certain adhesion molecules is well documented, the detailed role of these molecules in regulating leukocyte-endothelium interactions in the lung microcirculation in sepsis is not known. This is most probably due to the difficulty of studying such adhesive interactions in the lung in vivo. Recently, a new model of intravital fluorescence microscopy of the lung microcirculation has been developed, which could help elucidating the detailed mechanisms of pulmonary accumulation of leukocytes [6].

In general, leukocyte recruitment is a multistep process initiated by a rolling adhesive interaction followed by firm adhesion and transendothelial migration. Numerous studies have shown that leukocyte rolling is a precondition for subsequent firm adhesion [7-8]. Leukocyte rolling is mediated by the selectin family of adhesion molecules, including P-, E- and L-selectins, which interact with their glycoprotein counter-ligands [9-10]. PSGL-1 is the best-characterized selectin counter-receptor, which preferentially binds to P-selectin but can also bind to E-selectin with low affinity [11]. It is well-documented that inhibition of PSGL-1 effectively inhibits leukocyte recruitment in different models of inflammation [12-16], including septic lung damage [5]. However, the recruitment process of leukocytes in the lung is more complex and less studied than in other organs. Under homeostatic conditions, most

neutrophils, which have a diameter larger than that of pulmonary capillaries, must deform in order to pass through the pulmonary microcirculation [17-18]**.** Upon activation neutrophil stiffness increases. This may promote mechanical sequestration of neutrophils in the lung capillaries [19]**.** These observations have raised questions related to the role of PSGL-1 and a rolling adhesive interaction for the recruitment of neutrophils in the lung.

Based on these considerations, the aim of this study was to define the detailed role of PSGL-1 in regulating sepsis-induced leukocyte rolling and adhesion in the pulmonary microvasculature. For this purpose, we used a model of polymicrobial sepsis based on intestinal perforation in mice and intravital fluorescence microscopy of the lung microcirculation.

Materials and Methods

Animals

Male C57BL/6 mice weighting 20 to 25 g were used. All experimental procedures were performed in accordance with the legislation on the protection of animals and were approved by the Regional Ethical Committee for Animal Experimentation at Lund University, Sweden. Animals were anesthetized by administration of 7.5 mg (i.p.) ketamine hydrochloride (Hoffman-La Roche, Basel, Switzerland) and 2.5 mg (i.p.) xylazine (Janssen Pharmaceutica, Beerse, Belgium) per 100 g body weight.

Experimental protocols

Polymicrobial sepsis was induced by cecal ligation and puncture (CLP) as described previously [4]. In brief, the abdomen was opened, the exposed cecum was filled with feces by milking stool backwards from the ascending colon, and a ligature was placed below the ileocecal valve. The cecum was soaked with phosphate-buffered saline (PBS; pH 7.4) and punctured twice with a 21-gauge needle. The cecum was then placed back into the abdominal cavity and the incision was sutured. Seven animals were included in each group. One group of animals underwent CLP alone. Other animals were pretreated with a control antibody (0.16 mg per 100 g body weight, clone R3-34, rat IgG_1, BD Biosciences Pharmingen, San Jose, CA, USA) or an antibody directed against PSGL-1 (0.16 mg per 100 g body weight, clone 2PH1, rat IgG_1, BD Biosciences Pharmingen) intravenously immediately prior to CLP. Sham mice underwent the same surgical procedures, that is, laparotomy and resuscitation, but the cecum was neither ligated nor punctured. Intravital fluorescence microscopy of the lung microcirculation was performed 4 h after CLP induction. One mouse died in the sham group and one the CLP alone group. Two mice died in the groups of septic animals pretreated with the control antibody and the anti-PSGL-1 antibody. All mice died during the preparation for the intravital fluorescence microscopy.

Intravital fluorescence microscopy

The right diaphragm was incised to create a right-sided pneumothorax under transient lowering of the stroke volume to 100 µL. A parasternal thoracotomy was performed up to the level of the fourth intercostal space. By this, the main part of the right thorax wall could be averted to the side. During the preparation, great care was taken not to manipulate the lung tissue directly, and the lung surface was rinsed intermittently with saline (37°C). A micromanipulator was used to fix a coverslip horizontally. The surface of the right lung was gently attached to the lower coverslip surface. Horizontal movements of the lung tissue could be minimized by modulating a positive-end expiratory pressure between 5 and 7 cm H_2O and adjusting stroke volume (minimum: 150 µL) and stroke frequency (minimum: 100 strokes/min) by use of a ventilator (Minivent type 845, Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany). Immediately after the surgical preparation, the mice were put on the microscope stage. Intravital fluorescence microscopy was performed after a retrobulbar injection of 0.1 mL 0.1% rhodamine 6G (Sigma-Aldrich, Taufkirchen, Germany) for direct staining of leukocytes and 0.1 mL 5% FITC-dextran (MW 150,000, Sigma Chemical) for contrast enhancement. The subpleural pulmonary microvasculature was visualized by means of a modified Olympus microscope (BX50WI, Olympus Optical Co. GmbH, Hamburg, Germany), equipped with a 100-W mercury lamp and filter sets for blue- (450–490 nm excitation and >520 nm emission wavelength) and green- (530–560 nm excitation; >580 nm emission) light epi-illumination. Microscopic images were televised by using a charge-coupled device video camera and recorded digitally. With this setup, all parts of the subpleural pulmonary microvasculature, i.e., arterioles, venules, and capillaries, could be identified. Pulmonary venules and arterioles could be distinguished by the direction of the blood flow, i.e. venules have convergent blood flow pattern and arterioles have a divergent blood flow pattern. For measurements, five arterioles, venules, and capillaries were selected randomly in each animal. Leukocyte rolling was determined by counting the number of such cells passing a reference point in the arteriole or venule/20 s and multiplying by three to get the total number per minute. Adherent leukocytes were defined as cells that did not move or detach from the arteriolar or venular endothelium within

a 20 s observation period and are given as number of cells per square millimetre of endothelial surface. Leukocyte adhesion in capillaries was determined in five regions of interest (ROI). Functional capillary density served as a measure of the quality of microvascular perfusion and was defined as the length of all red blood cell-perfused nutritive capillaries per observation area and is given in cm/cm^2. Diameters (d) were measured in micrometer perpendicularly to the vessel path. Flow velocity (v) was analyzed by the computer-assisted image analysis system using the line shift method. Wall shear rate was calculated based on the Newtonian definition: y=8 * v/d [20]. All quantitative analysis of microhemodynamic parameters in the lung microcirculation was performed by means of the computer-assisted image analysis system CapImage (Zeintl, Heidelberg, Germany).

Flow cytometry

For analysis of the number of binding platelets on circulating neutrophils, blood was collected into syringes prefilled with 1:10 acid citrate dextrose at 4 hours post-CLP induction. Immediately after collection, blood samples were incubated with an anti-CD16/CD32 antibody blocking FcγIII/II receptors to reduce nonspecific labeling for 10 minutes at room temperature and then incubated with fluorescein isothiocyanate-conjugated CD41 (Clone MWReg30, rat $IgG_{1,k}$) and PE-conjugated anti-Gr-1 (Clone RB6-8C5, rat IgG_{2b}) antibodies to detect the percentage of neutrophil-platelet aggregates by considering neutrophils as cells positive for Gr-1, and platelets as $CD41^+$ cells. Flow-cytometric determination of neutrophil-platelet aggregates was performed by first gating the neutrophil population of cells based on forward and side scatter characteristics. Then the percentage of neutrophils ($Gr\text{-}1^+$) binding platelets ($CD41^+$) was analyzed in this population on a FACSort flow cytometer (Becton Dickinson, Mountain View, CA), and a viable gate was used to exclude dead and fragmented cells.

Systemic leukocyte count

Blood was collected from the tail vein and was mixed with Turks solution (0.2 mg gentian violet in 1 mL glacial acetic acid; 6.25% vol/vol) in a 1:20 dilution. Leukocytes were counted as monomorphonuclear (MNL) and polymorphonuclear (PMNL) leukocytes in a Burker chamber.

Statistics

Data are presented as mean values ± SD (standard deviation). Statistical evaluations were performed by using Kruskal-Wallis one-way analysis of variance on ranks followed by multiple comparisons versus control group (Dunnett's method). $P < 0.05$ was considered significant and *n* represents the number of animals in each group.

Results

In sham animals leukocytes were rarely observed to interact with pulmonary endothelial cells. CLP caused a significant increase in leukocyte rolling and adhesion in arterioles and venules as well as trapping in capillaries in the lung. In response to CLP Leukocyte rolling increased by 5-fold and 10-fold in arterioles and venules, respectively (Figs. 1a and 1b, $P < 0.05$ vs. sham, $n = 6$). Immunoneutralization of PSGL-1 reduced CLP-induced leukocyte rolling by 77% in arterioles and by 90% in venules (Figs. 1a and 1b, $P < 0.05$ vs. Control antibody + CLP, $n = 5$). Moreover, we found that CLP enhanced the number of firmly adherent leukocytes in arterioles by 2-fold and in venules by 3-fold (Figs. 2 and 3, $P < 0.05$ vs. sham, $n = 6$). Pretreatment with the anti-PSGL-1 antibody decreased CLP-induced leukocyte adhesion in pulmonary arterioles by 48% (Fig. 2, $P < 0.05$ vs. Control antibody + CLP, $n = 5$). In contrast, we observed that immunoneutralization of PSGL-1 had no effect on CLP-evoked leukocyte adhesion in venules of the lung (Fig. 3, $P > 0.05$ vs. Control antibody + CLP, $n = 5$).

Next, we analyzed the number of trapped leukocytes in pulmonary capillaries. It was found that CLP increased the number of trapped leukocytes from 23 cells/ROI to 34 cells/ROI (Fig. 4, $P < 0.05$ vs. sham, $n = 6$). Notably we found that inhibition of PSGL-1 decreased CLP-induced leukocyte trapping in lung capillaries down to 30 cells/ROI, corresponding to a 17% reduction (Fig. 4, $P < 0.05$ vs. Control antibody + CLP, $n = 5$). Further, we determined the flow velocity and shear rate in lung arterioles and venules. It was found that CLP significantly decreased flow velocity and shear rate in pulmonary arterioles and venules (Table 1; $P < 0.05$ *vs*. sham, $n = 6$). Interestingly, we observed that pretreatment with the anti-PSGL-1 antibody restored flow velocity in lung arterioles and shear rate in both arterioles and venules of septic mice to levels observed in sham animals (Table 1, $n = 5$). In addition, we observed that CLP significantly decreased functional capillary density in the lung microcirculation (Fig. 5, $P < 0.05$ vs. sham, $n = 6$). Immunoneutralization of PSGL-1 significantly improved lung functional capillary density in septic animals (Fig. 5, $P < 0.05$ vs. Control antibody + CLP, $n = 5$). There was no difference in diameters between the different

experimental groups (Table 1). Analysis of systemic leukocyte counts revealed that CLP decreased the total number of leukocytes significantly (Table 2, $P < 0.05$ vs. sham, $n = 6$). However, no changes in the total number of systemic leukocytes could be observed between all CLP-treated groups (Table 2). Flow-cytometric analysis of the percentage of $CD41^+$ neutrophils revealed that CLP increased the number of neutrophil-platelet aggregates significantly (Fig. 6; $P < 0.05$ *vs*. sham, $n = 5$). Notably, animals treated with the anti-PSGL-1 antibody presented with significantly decreased numbers of neutrophil-platelet aggregates (Fig. 6, $P < 0.05$ vs. Control antibody + CLP, $n = 5$).

Discussion

The present study defines the adhesive role of PSGL-1 in leukocyte-endothelial cell interactions in the pulmonary microvasculature in an early phase of sepsis. Our data show that PSGL-1 is a dominant molecule in supporting leukocyte rolling in arterioles and venules in septic lung inflammation. Inhibition of PSGL-1 decreases sepsis-induced leukocyte adhesion in lung arterioles but not in venules, suggesting that leukocyte adhesion is independent of rolling in lung venules. Our data also demonstrate that capillary trapping of leukocytes is facilitated by PSGL-1 in septic lung injury. Moreover, inhibition of PSGL-1-dependent accumulation of leukocyte in the pulmonary microcirculation improves microvascular perfusion in the lung.

Generalized activation of the innate immune system is a key feature in abdominal sepsis. The systemic inflammatory response causes tissue damage in the lung, which is the most insidious component in sepsis due to compromised gas exchange [4, 21]. Convincing evidence has demonstrated that neutrophil recruitment is a critical component in the pathophysiology of septic lung injury [22-23]. However, the details of the recruitment process of leukocytes in the lung has been much less studied and seems to be more complex than that in peripheral organs. Thus, the common paradigm of leukocyte recruitment established in the peripheral tissues has not been studied in detail in the lung. This is most probably due to the difficulty of developing methods to analyze leukocyte-endothelium interactions in a spatial and temporal manner in the lung. Herein, we used a recent technique allowing detailed analysis of leukocyte rolling and adhesion in the pulmonary microvasculature in vivo [6]. We demonstrate for the first time that inhibition of PSGL-1 reduces sepsis-evoked leukocyte rolling in arterioles and venules in the lung. Moreover, we found that immunoneutralization of PSGL-1 attenuates CLP-induced leukocyte firm adhesion in arterioles by ~50%. These findings suggest that PSGL-1 not only mediates leukocyte rolling but also that PSGL-1-dependent rolling is a necessary prerequisite for the subsequent pulmonary arteriolar leukocyte adhesion in sepsis. On the other hand, we observed that inhibition of PSGL-1 had no effect on CLP-induced leukocyte adhesion in venules,

suggesting that firm adhesion in lung venules is not dependent on an initial rolling adhesive interaction. Nonetheless, our findings support the concept that leukocyte accumulation in the pulmonary microvasculature is more complex and that a rolling adhesive mechanism is not compulsory in the recruitment process of leukocytes.

Leukocyte accumulation in capillaries is considered to be related to mechanical factors. One reason is related to the fact that the diameter of pulmonary capillaries is smaller (6 μm) than that of neutrophils (7 μm) [24]. Moreover, pulmonary capillaries have lower perfusion pressures and greater lengths than capillaries in the peripheral circulation. All these properties force neutrophils to deform and make them susceptible to mechanical trapping when passing the narrow capillaries in the lung [25]. In the present study, we found that inhibition of PSGL-1 significantly decreased sepsis-evoked leukocyte trapping in pulmonary capillaries. It is not likely that PSGL-1 mediates direct adhesion to endothelial cells in the capillaries knowing that the ligands of PSGL-1, i.e. P- and E-selectin are not expressed in capillaries [26]. Instead, the anti-PSGL-1 antibody should interfere with some other mechanisms important for mechanical trapping in the lung capillaries. One such possibility is aggregate formation between leukocytes and platelets. Leukocyte-platelet complexes are larger in size and have been suggested to be susceptible for size-restricted microvascular trapping [27]. We observed that the number of leukocyte-platelet aggregates increased in septic mice, which is in line with previous studies showing increased generation of leukocyte-platelet complexes in inflammatory diseases [28-30]. Interestingly, we found that immunoneutralization of PSGL-1 completely inhibited sepsis-triggered formation leukocyte-platelet aggregates, which might help to explain the inhibitory effect of anti-PSGL-1 antibody on the accumulation of leukocytes in pulmonary capillaries in abdominal sepsis. In this context, it also interesting to note that sepsis significantly reduced pulmonary microvascular perfusion and that inhibition of PSGL-1 restored microcirculatory hemodynamics in septic animals. Considering the significant reduction in leukocyte accumulation in the lung arterioles and capillaries after inhibition of PSGL-1, it may be suggested that the improved pulmonary microvascular perfusion in mice treated with the anti-PSGL-1 antibody might be related to

attenuated microvascular obstruction exerted by intraluminal leukocytes.

In conclusion, our novel data demonstrate that PSGL-1 mediates leukocyte rolling in lung arterioles and venules in abdominal sepsis. Inhibition of PSGL-1-dependent rolling reduces leukocyte adhesion in arterioles but not in venules, suggesting that the importance of rolling varies in the pulmonary microcirculation. Moreover, inhibition of PSGL-1 also decreased sepsis-triggered leukocyte trapping in pulmonary capillaries potentially via inhibition of leukocyte-platelet aggregate formation in the systemic circulation. Taken together, these findings show that PSGL-1 plays different roles in the lung microcirculation and that targeting PSGL-1 is an effective way to inhibit leukocyte accumulation and improve microvascular perfusion in septic lung injury.

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Figure and Table legends

Table 1 Diameter, flow velocity and shear rate of pulmonary arterioles and venules as assessed in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) directly prior to CLP. Values are given as means ± SD. ∗*P* < 0.05 vs. Sham; # *P*< 0.05 vs. Con Ab + CLP.

Table 2 Systemic leukocyte counts as assessed in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) directly prior to CLP. Values are given as means ± SD. ∗*P* < 0.05 vs. Sham.

Fig. 1 a and b Number of rolling leukocytes (cells/min) in pulmonary arterioles (a) and venules (b) as assessed in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) directly prior to CLP. Values are given as means ± SD. ∗*P* < 0.05 vs. Sham; # *P*< 0.05 vs. Con Ab + CLP.

Fig. 2 a-d Intravital fluorescence microscopy of adherent leukocytes (arrows) in pulmonary arterioles. Note, that CLP enhanced the number of firmly adherent leukocytes in arterioles by 2-fold (b, arrows) compared to Sham-treated animals (a, arrows). Immunoneutralization of PSGL-1 reduced CLP-induced leukocyte adhesion in pulmonary arterioles by almost 50% (c and d). Green-light epi-illumination with direct staining of leukocytes by rhodamine 6 G. Scale bars: 35µm. **e:** Numbers of adherent leukocytes in pulmonary arterioles (cells/mm²) as assessed in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) directly prior to CLP. Values are given as means ± SD. ∗*P* < 0.05 vs. Sham; # *P*< 0.05 vs. Con Ab + CLP.

Fig. 3 a-d Intravital fluorescence microscopy of adherent leukocytes (arrows) in pulmonary venules. Note, that CLP enhanced the number of firmly adherent leukocytes in venules by 3-fold (b, arrows) compared to sham-treated animals (a, arrows). However, pretreatment with the anti-PSGL-1 antibody had no effect on CLP-evoked leukocyte adhesion (c and d). Green-light epi-illumination with direct staining of leukocytes by rhodamine 6 G. Scale bars: 35µm. **e:** Numbers of adherent leukocytes in pulmonary venules (cells/mm²) as assessed in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) directly prior to CLP. Values are given as means ± SD. $*P < 0.05$ vs. Sham.

Fig. 4 a-d Intravital fluorescence microscopy of trapped leukocytes (arrows) in pulmonary capillaries. Note, that in sham-treated animals only a few leukocytes can be observed (a, arrows), while CLP increased the number of trapped leukocytes significantly (b, arrows). Inhibition of PSGL-1 decreased CLP-induced leukocyte trapping in lung capillaries significantly compared to control antibody-treated animals. Green-light epi-illumination with direct staining of leukocytes by rhodamine 6 G. Scale bars: 35 µm. **e:** Numbers of trapped leukocytes in pulmonary capillaries (cells/ROI) as assessed in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) immediately prior to CLP. Values are given as means ± SD. $*P < 0.05$ vs. Sham; $^{\#} P< 0.05$ vs. Con Ab + CLP.

Fig. 5 Functional capillary density in the lung in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) directly prior to CLP. Values are given as means ± SD. $*P < 0.05$ vs. Sham; $^{\#} P< 0.05$ vs. Con Ab + CLP.

Fig. 6 Flow-cytometric analysis of the percentage of $CD41^+$ neutrophils, indicating neutrophil-platelet aggregates, as assessed in sham-treated animals (Sham), after 4h CLP (PBS + CLP) and in animals that were pretreated with a control antibody (Con Ab + CLP) or an antibody directed against PSGL-1 (Anti-PSGL-1Ab + CLP) directly prior to CLP. Values are given as means ± SD. $*P < 0.05$ vs. Sham; $^{\#}P < 0.05$ vs. Con Ab + CLP.

Table 1

	Arterioles			Venules		
	Diameter (μm)	Flow velocity (mm/s)	Shear rate (s^{-1})	Diameter (μm)	Flow velocity (mm/s)	Shear rate (s^{-1})
Sham	28.2±1.3	1.4±0.3	393.4±97.0	22.1±2.5	1.8±0.2	674.4±108.5
PBS + CLP	29.6±3.5	0.9±0.1*	247.2±32.7*	20.4±4.2	1.2±0.3*	522.0±100.1*
Con Ab + CLP	29.5±2.2	0.9±0.2*	232.2±44.2*	20.3±2.2	1.7±0.2	593.2±52.5
Anti-PSGL-1 Ab + CLP	31.6±3.9	1.3±0.3#	375.0±52.9#	20.9±2.8	1.9±0.2	723.0±82.9#

Table 2

	MNL	PMNL	Total
Sham	7.3±1.8	2.5±0.6	9.8±2.1
PBS + CLP	3.6±0.9*	1.4±0.5*	5.0±1.0*
Con Ab + CLP	4.2±0.4*	1.2±0.7*	5.4±1.0*
Anti-PSGL-1 Ab + CLP	4.6±0.8	1.4±0.4	6.0±1.1

Fig. 1

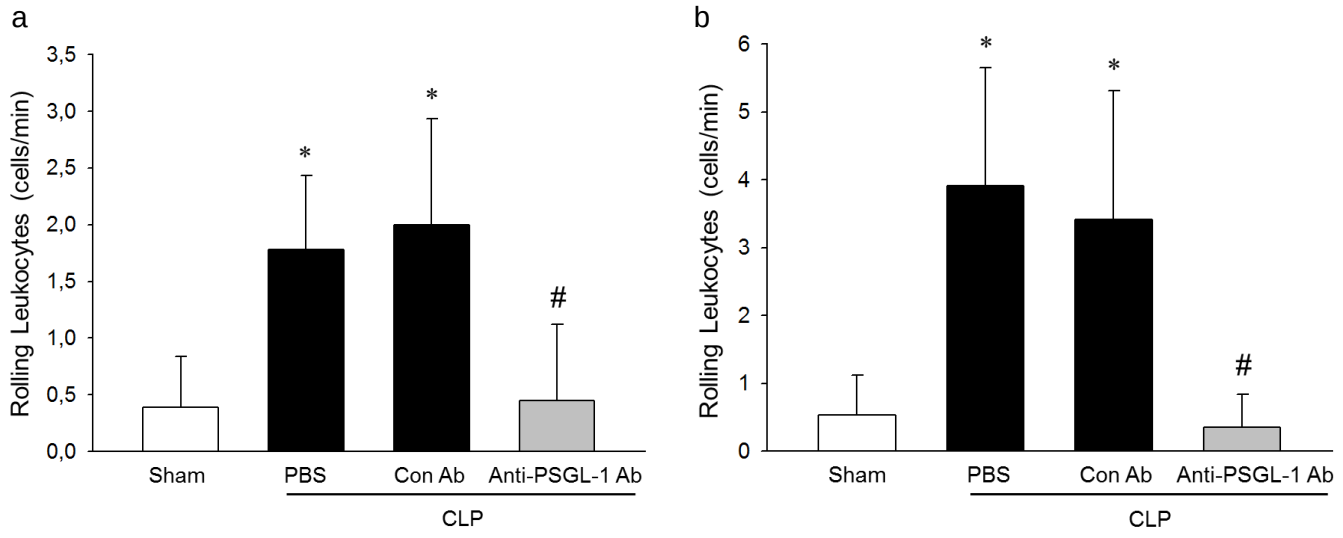

a
Rolling Leukocytes (cells/min)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
*
*
#
Sham
PBS
Con Ab
Anti-PSGL-1 Ab
CLP
b
Rolling Leukocytes (cells/min)
6
5
4
3
2
1
0
*
*
#
Sham
PBS
Con Ab
Anti-PSGL-1 Ab
CLP

Fig. 2

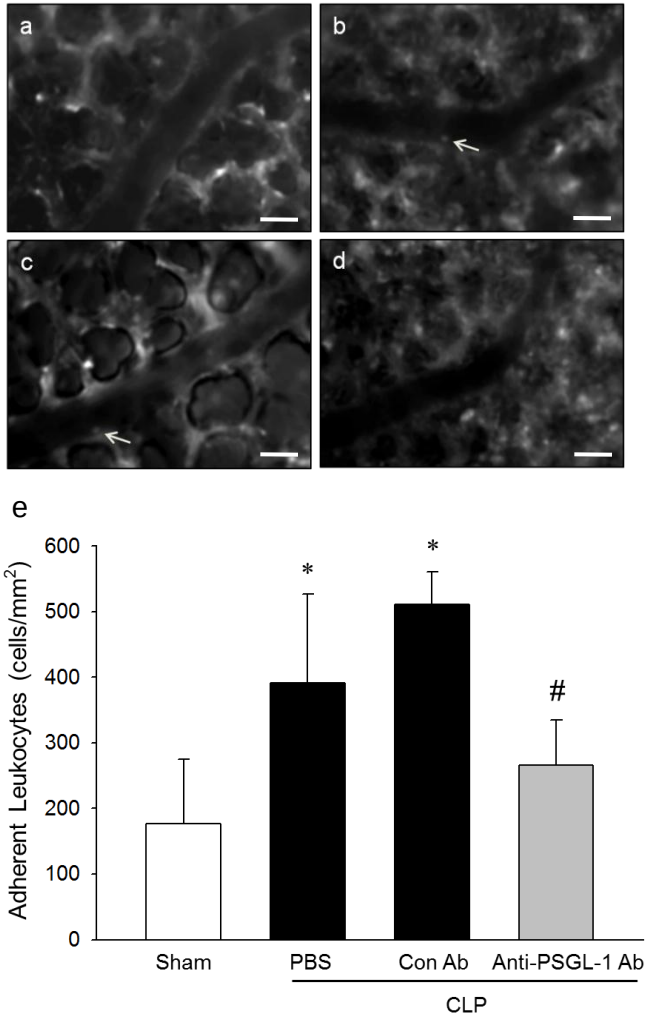

a
b
c
d
e
Adherent Leukocytes (cells/mm2)
600
500
400
300
200
100
0
*
*
#
Sham
PBS
Con Ab
Anti-PSGL-1 Ab
CLP

Fig. 3

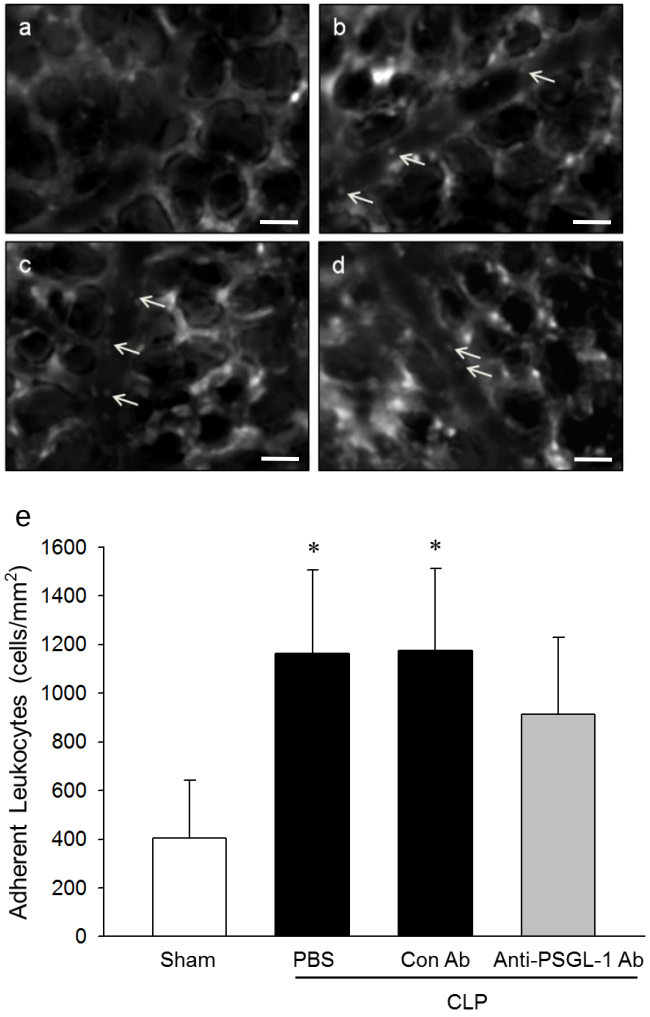

a
b
c
d
e
Adherent Leukocytes (cells/mm2)
1600
1400
1200
1000
800
600
400
200
0
*
*
Sham
PBS
Con Ab
Anti-PSGL-1 Ab
CLP

Fig. 4

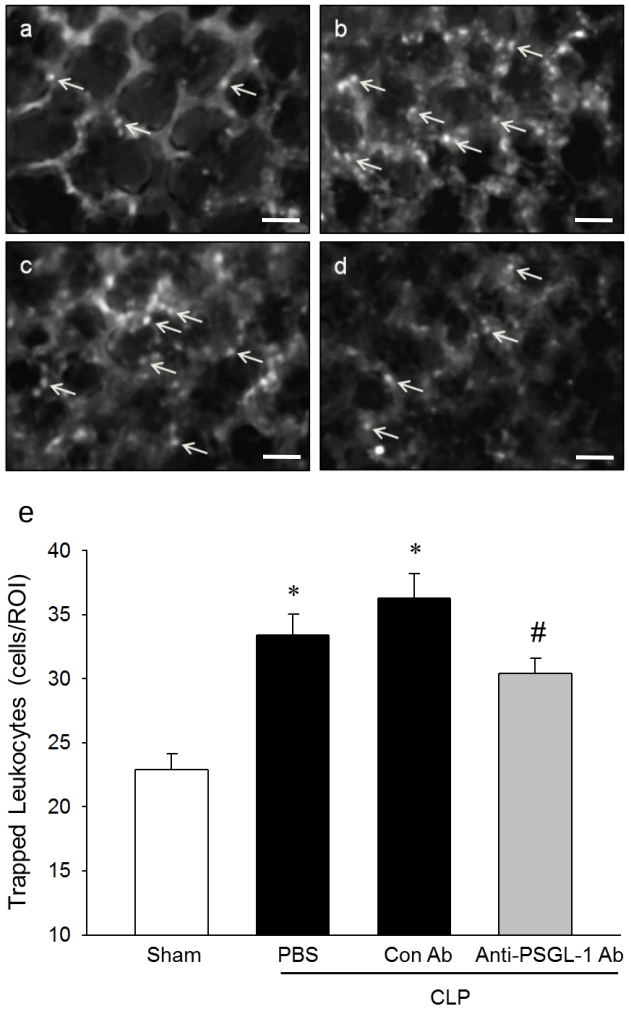

a
b
c
d
e
Trapped Leukocytes (cells/ROI)
40
35
30
25
20
15
10
*
*
#
Sham
PBS
Con Ab
Anti-PSGL-1 Ab
CLP

Fig. 5

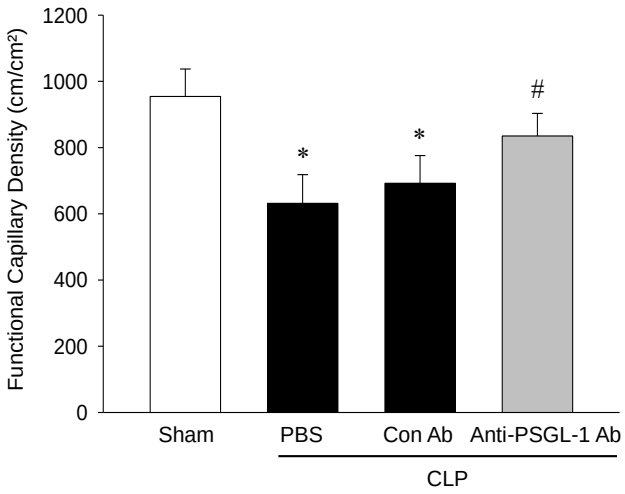
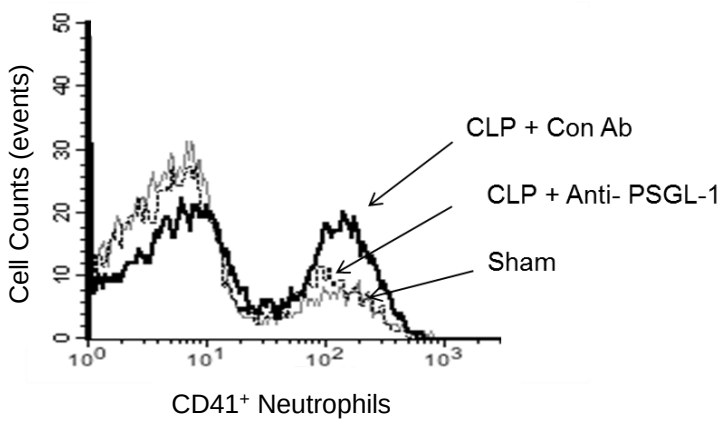
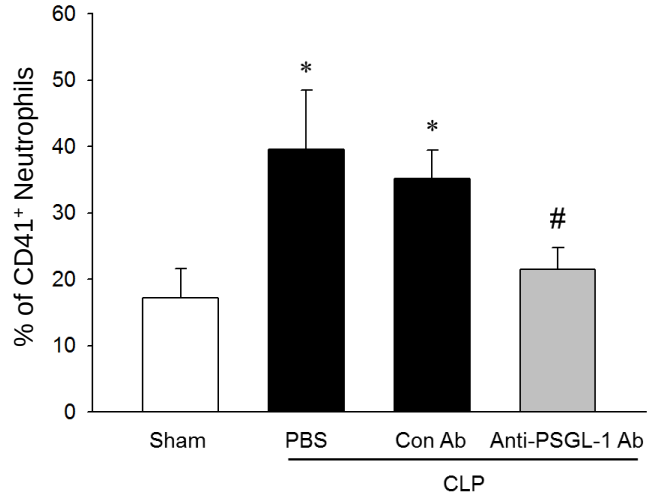

Functional Capillary Density (cm/cm²)
1200
1000
800
600
400
200
0
*
*
#
Sham
PBS
Con Ab
Anti-PSGL-1 Ab
CLP

Fig. 6


Cell Counts (events)
0
10
20
30
40
50
10^0
10^1
10^2
10^3
CLP + Con Ab
CLP + Anti- PSGL-1
Sham
CD41⁺ Neutrophils


% of CD41⁺ Neutrophils
0
10
20
30
40
50
60
*
*
#
Sham
PBS
Con Ab
Anti-PSGL-1 Ab
CLP