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Alterations of adhesion molecule expression and inflammatory mediators in acute lung injury induced by septic and non-septic challenges

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ABSTRACT

The lung is frequently the first failing organ during the sequential development of multiple organ dysfunction under both septic or non-septic conditions. The present study compared polymorphisms of tumor necrosis factor (TNFα), monocyte chemoattractant protein-1 (MCP-1), and adhesion molecule (AM) expression on circulating, recruited, and migrating leukocytes in the development of lung injury after induction of acute pancreatitis (AP) or abdominal sepsis by cecal ligation and puncture (CLP). Pulmonary alveolar barrier and endothelial barrier permeability dysfunction were measured. The expression of AMs (CD11b, CD11b/c, CD31, CD54 and CD62L) on leukocytes isolated from blood, lung tissue, and bronchoalveolar space were measured by flowcytometry. Plasma exudation to the interstitial tissue and the bronchoalveolar space significantly increased 1 and 3 hours after induction of pancreatitis and to the bronchoalveolar space from 6 hours after sepsis. Bronchoalveolar levels of MCP-1 significantly increased earlier than plasma exudation to the alveoli in both pancreatitis and sepsis. Alterations in expression of adhesion molecules on bronchoalveolar lavage (BAL) leukocytes can represent a marker reflecting leukocyte activation in the lung tissue, since both BAL and lung tissue leukocytes showed similar patterns of changes. Expression of adhesion molecules on circulating leukocytes increased 1 hour after induction of pancreatitis. Activating phenotypes of circulating, lung tissue and bronchoalveolar leukocytes may thus be responsible for the development and severity of secondary lung injury.

Key words: Acute pancreatitis, sepsis, acute lung injury, TNFα, MCP-1, PECAM-1, ICAM-1, L-selectin, Mac-1, and C3bi receptor.

INTRODUCTION

Acute lung injury (ALI) and its more severe form, the acute respiratory distress syndrome (ARDS), are syndromes of acute respiratory failure that result from acute pulmonary edema and inflammation. The incidence of ALI/ARDS is continually increasing in the intensive care unit to about 30-35% of the respective 28-day mortality (1). It is clinically characterized by dyspnea, profound hypoxemia, decreased lung compliance, and diffuse bilateral infiltrates on chest radiography and experimentally by tissue edema, increased vascular permeability, and pulmonary dysfunction, and relates to the prognosis. ALI/ARDS has been suggested to be an early and important disorder during the development of the multiple organ dysfunction syndrome (MODS). The development of ALI/ARDS is associated with several clinical disorders, including intra-pulmonary injury from pneumonia and aspiration and extra-pulmonary injury from trauma, sepsis, shock, and acute pancreatitis. Pancreatitis-associated lung injury contributes to mortality in more than 50% in patients with severe AP (2,3) and the incidence of sepsis-associated ARDS was about 20-60%, out of which more than 40-50% died (4,5), although the role of ARDS in development of multiple organ dysfunction syndrome remains unclear.

Experimental studies have demonstrated that the combination of AP and abdominal sepsis or bacterial products aggravated the development, course and outcome of multiple organ dysfunction and increased endothelial barrier dysfunction by altering intercellular signalling (6,7). The pulmonary endothelial cell barrier showed different resistance against the primarily septic (abdominal sepsis) and non-septic challenges (AP) (8). In order to map inflammatory phenotypes in the severity and duration of lung injury induced by primarily extrapulmonary challenges, the present study aimed at comparing early dynamic alterations of inflammatory

mediators and expressions of adhesion molecules on leukocytes from various locations in the development of lung injury in association with extrapulmonary septic- and non-septic challenges. Expression of platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31), intracellular adhesion molecule-1 (ICAM-1, CD54), L-selectin (CD62L), Mac-1 (CD11b), and C3bi receptor (CD11b/c) on circulating, recruited, and migrating leukocytes was measured 1, 3, 6, and 9 hours after the induction of abdominal sepsis or acute pancreatitis in rats. Levels of tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and interleukin-6 (IL-6) in the circulation, lung tissue and bronchoalveolar lavage (BAL) fluid were also assessed in the early phase after challenge.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats, weighing about 250 g, were fed standard rat chow (R_3, Astra-Ewos, Södertälje, Sweden) and water ad libitum. The rats were allowed to acclimatize to our laboratory conditions for 4-6 days and were subjected to a regime of 12 hours day/night cycle living in mesh stainless- steel cages (3 rats/cage) at constant temperature (22^oC). The protocol was approved by the Animal Ethics Committee at Lund University. All animals were handled in accordance with the guidelines set forth by the Swedish Physiological Society.

Induction of AP and abdominal sepsis. AP was induced by the intraductal administration of 0.2 ml of 5% sodium taurodeoxycholate, by use of an infusion pump at a speed of 0.04 ml/min, following clamping of the proximal end of the common bile duct and cannulating the biliary-pancreatic duct by a thin polyethylene catheter (0.66mm OD, Portex Ltd., Hythe, Kent, UK), after sterilizing the sodium taurodeoxycholate solution at 100^oC for 20 min. Abdominal sepsis, in the following text mentioned as sepsis, was induced by cecal ligation and puncture(CLP). The cecum was gently filled with content from the ascending colon, ligated with 3-0 silk and punctured through the cecal walls twice with an 18-gauge needle. Sham operation (controls) included laparotomy and separation of the common bile duct or cecum similar to what was performed in the experimental group, but without bile injection or ligation and puncture of the cecum.

Lung injury and endothelial barrier dysfunction. Pulmonary alveolar barrier permeability was assessed by measuring the passage of ^{125}I-labeled human serum albumin (HSA, Institute for engergiteknikk, Kjeller, Norway) from blood to the alveolar space as a marker of plasma

exudation. 0.5 ml of ^{125}I-HSA (1.8 X 10^6 cpm) was injected into the femoral vein catheter (0.75mm, Portex, Kent, England). After one-hour equilibration, the animals were sacrificed by an over-dose of anesthesia. The trachea was exposed, opened and cannulated with the catheter connected with the BAL system. The system includes a silicon tube connected with a 50 ml syringe containing 20 ml of PBS with 1% fetal bovine serum (FBS) and another channel for exclusion of lavage fluid from the lungs. The bronchoalveolar space was lavaged by opening the liquid entry control under the pressure of 3 cm H_2O for 2 min, after which the lavage fluid was excluded for another minute through the tubes, which were controlled by a switch. The lavage was repeated twice, from which the BAL fluid was kept on ice, centrifuged at X 500g, and separated into supernatant and cells. Levels of ^{125}I in supernatant and blood samples were measured in a γ-counter (1272 Clinigamma, LKB, Wallac OY, Finland) after that the samples were weighed for determining wet tissue weight. Leakage of plasma from the circulation to the pulmonary interstitium was indicated by the HSA influx (8), which was calculated by the ratio of CPM per gram lung tissue and CPM per gram plasma. Leakage of plasma from the circulation to the alveoli was indicated by levels of CPM in BAL supernatant.

Cell isolation. Blood and BAL fluids were collected into tubes containing EDTA, after which centrifugation (3000 rpm, 4^{o}C) for 5-10 min was performed to separate the leukocytes. The cells were then washed with RPMI 1640 containing 10% FBS after that contaminated red blood cells were lysed with hypotonic solution. Tissue leukocytes were isolated from the lungs as described previously (9-11). The trachea was connected to a ventilator (Model II, Bosite Biological Research Apparatus, Comerio-Vavese, Italy) at a volume of 0.4 L/min at 85 breaths/min. The pulmonary artery was cannulated with a catheter (Medical grade tubing, Dow Corning, MI, USA) and perfused with Hank's solution (37^{o}C, pH 7.4) at a speed of 50 ml/min

for 10 min. The lung tissue was sliced into small pieces, incubated with Hank′s balanced salt solution containing 0.01% DNase I and 20 u/ml collagenase D with specific activity >0.47 u/mg (Roche Diagnostics Scandinavia AB, Bromma, Sweden) at 37^{o}C for 3 hours, and washed at centrifugation (1500 rpm, 4^{o}C) thrice for 5 min after filtering through four layers of gauze.

Flowcytometry. Mouse monoclonal antibodies against rat CD11b (WT.5), CD11b/c (OX-42), CD31 (TLD-3A12), CD54 (1A29) and CD62L (HRL1) were purchased from Pharmingen (San Diego, CA, USA). The isolated cells from blood, BAL and lung tissue were stained with FITC (CD11b and CD11b/c) and/or phycoerythrin (CD31, CD54 and CD62L)-conjugated antibodies against cell surface molecules. The cells were washed twice, suspended in 0.5% PBS-BSA, and run on a FACScan (Becton Dickinson, San Diego, CA) with the Cell Quest® analysis program. 10,000 to 20,000 cells were analyzed from each sample. Increased expression of CD11b^{+}, CD54^{+}, CD62L^{+}, CD11b/c^{+}, CD31^{+} on isolated leukocytes was demonstrated as percentage above control levels, calculated by the following formulation: Increased expression (% above controls)= (positive AM percentage from disease groups - mean value of positive AM percentage from control groups)/ mean value of positive AM percentage from control group X 100.

Assay of inflammatory mediators: Levels of TNF-α, MCP-1 and IL-6 in the plasma, lung tissue and BAL fuild were determined by ELISA. Antibodies specific for rat TNF-α, MCP-1 and IL-6 (Pharmingen, San Diego, CA, USA) were coated onto the wells of the microtiter strips (NUNC, Copenhagen, Denmark) and the samples including standards of known rat TNF-α, MCP-1 and IL-6 were pipetted into the wells, incubated and washed. Intensity of the color was determined at 405 nm.

Experimental design: The rats were randomly allocated into three groups: 1). Sham-operated animals (n=72), 2). Animals with AP (n=72), and 3). Animals with abdominal sepsis (CLP; n=72). The animals were terminated one, three, six and nine hours after sham operation or induction of AP and sepsis (CLP) (n=18/group/time point) by an overdose of anesthesia. In the first experiment, leukocytes were separated from blood, lungs and BAL fluid for measuring the AM expression (six animals from each group per time point). In the second experiment, six animals from each group and time point studied received an intravenous injection of radiolabeled HSA to assess plasma exudation as an indicator of pulmonary endothelial barrier dysfunction. In order to avoid radioisotopic contamination, another six animals from each group in the third experiment were perfused for measuring levels of MPO, TNFα, IL-6, and MCP-1 in the lungs, plasma, and BAL fluid following the sample harvest.

Statistics: The data were analyzed using unpaired Student's *t*-test or a nonparametric test (Mann-Whitney U test) where appropriate. Values are described as means ± SEM. A probability of <0.05 was considered as significant.

RESULTS

Levels of HSA influx into pulmonary interstitium were significantly increased from 1 hour onward after induction of AP ($p<0.05$ vs controls; Fig. 1A). Radiolabeled HSA levels in BAL fluid significantly increased from 3 hours after induction of AP and from 6 hours after sepsis induction($p<0.05$ or 0.01 vs controls, respectively; Fig. 1B). Pulmonary plasma exudation in animals with AP at 3 hours was significantly higher than in sepsis ($p<0.01$).

Plasma levels of TNFα were significantly increased from 3 hours after induction of abdominal sepsis and at 3 hours after AP, as compared with controls ($p<0.05$ or 0.01, respectively, Fig. 2A). Plasma levels of TNFα in septic animals were significantly higher than in AP animals at 6 and 9 hours after induction ($p<0.05$ and 0.01, respectively). Levels of TNFα in lung tissue from AP animals were significantly higher 6 and 9 hours after induction ($p<0.05$ and 0.01 vs controls, respectively, Fig. 2B). Levels of TNFα in BAL fluid were significantly higher in animals 3 and 6 hours after the induction of AP or sepsis ($p<0.05$ or 0.01, respectively, Fig. 2C). AP animals had significantly higher levels of TNFα in the lung tissue than septic animals at 6 hours ($P<0.01$ vs septic group; Fig. 2B), while septic animals had significantly higher plasma levels at 6 and 9 hours (Fig. 2A).

Plasma levels of MCP-1 were significantly higher in AP animals at 3 and 6 hours and in sepsis animals at 6 and 9 hours after the induction, as compared to controls (Fig. 3A, $p<0.05$ or less). MCP-1 levels in pulmonary tissue significantly increased 6 ($p<0.05$) and 9 hours ($p<0.01$) after induction of AP or sepsis as compared to controls (Fig. 3B). Septic animals had significantly higher levels of MCP-1 than AP animals at 9 hours ($p<0.01$). A significant increase in BAL levels of MCP-1 was noted from 1 to 6 hours after AP induction and from 3 to 9 hours after

abdominal sepsis, as compared to controls ($p<0.05$ or 0.01, respectively; Fig. 3C). BAL levels of MCP-1 in animals with sepsis were significantly higher than in AP at 6 hours ($p<0.05$).

The percentage of CD11b expression on circulating leukocytes from blood was significantly increased 1 to 6 hours after induction pancreatitis and 3 and 9 hours after sepsis induction as compared to controls (Fig. 4A). Animals with sepsis demonstrated a significantly higher expression of CD11b on circulating leukocytes than those with pancreatitis at 3 and 9 hours, while AP animals had a higher expression at 6 hours. CD11b expression in tissue leukocytes significantly increased at 1, 3 and 6 hours after induction of pancreatitis, as compared to animals with sham operation or abdominal sepsis (Fig. 4B, $p<0.05$ or less). A significant increase in CD11b expression on BAL leukocytes was seen from 1 hour and on after induction of pancreatitis or abdominal sepsis ($p<0.05$ or less, Fig. 4C). CD11b expression on BAL leukocytes from AP rats was significantly higher than in septic rats at 3 hours.

Fig. 5A demonstrates that CD11b/c expression on circulating leukocytes from pancreatitis animals significantly increased at 1, 3 and 6 hours as compared to sham operated animals, and at 6 hours as compared to septic rats ($p<0.05$ or less). Septic animals had significantly higher CD11b/c expression on circulating leukocytes than sham operated animals at 3 and 9 hours, and than AP animals at 9hours. CD11b/c expression on tissue leukocytes significantly increased from 1 hour after induction of AP or abdominal sepsis, as compared to sham operation (Fig. 5B, $p<0.05$ or less vs sham operation). At 1 hour, CD11b/c expression on circulating leukocytes in AP was significantly higher than in sepsis ($p<0.01$). On BAL leukocytes, CD11b/c expression was significantly increased 3 and 6 hours after induction of acute pancreatitis or abdominal sepsis, as compared to sham operation (Fig. 5C, $p<0.05$ or

less). There was a significant difference in CD11b/c expression on BAL leukocytes comparing pancreatitis and sepsis.

CD 31 expression on circulating leukocytes was significantly higher in pancreatitis animals at 1 hour as compared to septic or sham operated rats, ($p<0.05$, Fig. 6A), while significantly lower in septic rats at 1-6 hours and in pancreatitis animals at 6 and 9 hours ($p<0.05$ vs sham operation). CD31 expression on tissue leukocytes from pancreatitis or sepsis animals was higher than following sham operation at 1 and 3 hours (Fig. 6B), while CD31 expression on BAL leukocytes significantly increased up to 300% above controls at 1 hour (Fig. 6C). At 6 hours, CD31 expression on BAL leukocytes was significantly lower in sepsis, but higher in pancreatitis (Fig. 6C).

CD54 expression on circulating leukocytes was significantly lower in sepsis at 1 hour as compared to controls and AP, and decreased in AP from 3 hours after the induction compared to controls and sepsis animals (Fig. 7A, $p<0.05$ or less, respectively). Animals with sepsis had a significantly higher CD54 expression on tissue leukocytes at 1 hour, as compared to sham operation and AP (Fig. 7B). CD54 expression on BAL leukocytes was significantly higher 1 hour after AP and 1 and 6 hours after pancreatitis (Fig. 7C, $p<0.05$ or less vs sham operation). Animals with pancreatitis had lower CD 54 expression on BAL leukocytes at 1 hour and higher at 6 hours, as compared to sepsis.

Animals with pancreatitis had a significant increase in CD62L expression on circulating leukocytes at 1 hour followed by a significant decrease, also noted at 6 and 9 hours after sepsis induction (Fig. 8A, $p<0.05$vs sham operation). Expression of CD62L on tissue leukocytes

significantly increased 1 and 6 hours after pancreatitis and 1 hour after sepsis (Fig. 8B), while the expression in BAL leukocytes was significantly higher 1 hour after induction of sepsis or pancreatitis, followed by a decrease in septic animals at 6 hours (Fig. 8C).

DISCUSSION

The present study demonstrates that a significant increase in HSA leakage from the circulation to the pulmonary interstitial space occurred one hour after induction of AP, followed by an increase in plasma exudation to the bronchoalveolar space at 6 hours, as compared to animals with abdominal sepsis. Increased leakage of albumin may result in increased oncotic buffering by allowing greater access of albumin to the interstitial tissue volume, leading to dilution and decreased interstitial colloid oncotic pressure and an increase of the oncotic gradients (11). Potentially, increased interstitial volume and pressure resulting from pulmonary endothelial barrier dysfunction may increase the resistance against further leakage of injected radiolabeled albumin. Increased plasma exudation from the circulation to the bronchoalveolar space 6 hours after pancreatitis indicates the occurrence of endothelial barrier dysfunction, increase of interstitial volume and pressure, and compromise of alveolar epithelial integrity.

Neutrophil infiltration into the lungs has been reported to significantly increase from 3 hours and on after induction of both pancreatitis and sepsis (12). Comparing pancreatitis and sepsis, neutrophil infiltration into the lungs depicts two pathophysiological characteristics: 1). An increase occurring by time after challenge and 2). A more pronounced neutrophil infiltration in pancreatitis than in sepsis, demonstrating a more rapid course of disease in pancreatitis animals. Although neutrophils may play an important role in the development of pancreatitis-associated lung injury, neutrophil depletion was found to only partially reduce the severity of pancreatitis-associated lung injury (10), indicating that other inflammatory cells or products may contribute to pancreatitis-associated lung injury. Pulmonary macrophages are also involved in pancreatitis-induced endothelial barrier dysfunction, type-II pneumocyte compromise, and tissue injury by increasing cell number, the release of cytokines, and

affecting their biological function (e.g. killing capacity) (13,14). In the present study, expression of CD11b or CD11b/c on leukocytes from blood, lung tissue and bronchoalveolar lavage increased after induction of both pancreatitis and sepsis. It seems that expression of CD11b or CD11b/c on circulating leukocytes correlates with leukocyte infiltration in lung tissue as well as plasma exudation. It is possible that these markers could be used as indicators for the severity of pancreatitis-associated lung injury, in similarity with the consideration that CD11b expression on both monocytes and neutrophils has been suggested to relate to the clinical severity of pancreatic injury (15). Expression of CD31, CD54, or CD62L on circulating leukocytes decreased from 3 hours and on after induction of pancreatitis and sepsis, with a more pronounced expression in pancreatitis animals and the expression of CD62L (selectin) being most increased, followed by CD31 (PECAM-1) and CD54 (ICAM-1). This may be due to that these adhesion molecule-positive cells have migrated into the lungs and other organs or due to a counter-regulatory mechanism that impede extravasation of leukocytes as reported in patients with the systemic inflammatory response syndrome (16). Expression of CD31 and CD54 on lung tissue leukocytes increased earlier than CD62L. Expression of CD11b, CD11b/c, CD31, CD54, or CD62L on leukocytes from BAL indicates the initiation of cellular activation.

Inflammatory mediators produced from inflammatory cells may play an important role in development of extrapancreatic organ injury and dysfunction in pancreatitis, although its mechanisms remain unclear. TNFα and MCP-1 are the early and critical cytokine and chemokine, respectively, in immune responses. It is conflicting whether TNFα levels in the circulation correlate with disease severity or mortality in patients with acute pancreatitis (17,18), although the number of TNFα producing leukocytes in pancreatic tissue was

significantly higher than in the circulation (19) and circulating monocytes have been supposed to be responsible for the increase in TNFα during the development of pancreatitis (18). In order to investigate a potential correlation of TNFα levels with pancreatitis-associated lung injury, the present study measured TNFα levels in the circulation, lung tissue and BAL fluid. The results indicate a polymorphism of TNFα production among these locations, the severity of pulmonary endothelial dysfunction induced by either sepsis or pancreatitis. In contrast, plasma levels of MCP-1 increased in parallel with plasma exudation from the circulation to the alveolar space and BAL levels of MCP-1 with plasma exudation from the circulation to the interstitial tissue. In both sepsis- or pancreatitis-induced lung injury, BAL levels of MCP-1 increased earlier than the occurrence of plasma exudation to the bronchoalveolar space. This indicates that BAL MCP-1 may be a biomarker to predict the severity of secondary lung injury. This supports to the clinical finding that both local (intraperitoneal) and systemic levels of MCP-1 increased early in the course of severe acute pancreatitis in humans (20). Furthermore, the increased levels of systemic MCP-1 have been found to be the closest correlation with the development of pancreatic infections and extrapancreatic organ dysfunction (20). We also noted that levels of MCP-1 increased in the BAL fluid earlier than in the circulation, indicating that local cells are involved in the production of MCP-1 for chemoattracted and activated leukocytes to the areas of injury. For example, mast cells that are normally allocated around the vessel have been considered to be one of the contributors for leukocyte activation and MCP-1 production as well as the sequential development of pancreatitis-associated multiple organ dysfunction (21). The severity of inflammatory responses after induction of abdominal sepsis seems to increase by time, since levels of TNFα and MCP-1 or expressions of CD11b and CD11b/c increased in either the circulation or local tissue/BAL fluid at the late phase of the experiment (6 or 9 hours). In the present study, overexpression of CD11b and CD11b/c on

circulating and tissue leukocytes was noted in a similar pattern to plasma exudation into lung interstitium in rats with acute pancreactitis. Both CD11b and CD11b/c were overexpressed in BAL leukocytes earlier than the occuurence of plasma exudation from the lung tissue into the alveolar space, which can be potential candidates indicating the development of the secondary lung injury.

In conclusion, the present study demonstrates that plasma exudation to the interstitial tissue and bronchoalveolar space in acute pancreatitis occurred earlier and more pronounced than in abdominal sepsis. Alterations in expression of adhesion molecules on BAL leukocytes can be a marker reflecting leukocyte activation in the lung tissue, since both BAL and lung tissue leukocytes showed similar patterns of changes. Expression of adhesion molecules on circulating leukocytes increased 1 hour after induction of pancreatitis. BAL levels of MCP-1 may be an early mediator involved in the initiation of pancreatitis-associated lung injury.

REFERENCES

1. Bersten AD, Edibam C, Hunt T, Moran J. Incidence and mortality of acute lung injury and the acute respiratory distress syndrome in three Australian States. Am J Respir Cril Care Med 2002;165:443-448.

2. Robertsen, C.S., Basran, G.S., and Hardy J.G. 1988. Lung vascular permeability in patients with acute pancreatitis. Pancreas 3:162-165

3. Zhao X, Andersson R, Wang X, Dib M, Wang XD. Acute pancreatitis-associated lung injury: pathophysiological and potential fure therapies. Scand J Gastroenterol 2002;37:1351-8.

4. Eggimann P, Harbarth S, Ricou B, Hugonnet S, Ferriere K, Suter P, Pittet D. Acute respiratory distress syndrome after bacteremic sepsis does not increase mortality. Am J Respir Crit Care Med 2003;167:1210-4.

5. Udobi KF, Childs E, Touijer K. Acute respiratory distress syndrome.Am Fam Physician 2003;67:315-22.

6. Andersson, R., Wang, X.D., and Ihse, I. The influence of abdominal sepsis on acute pancreatitis in rats: A study on mortality, permeability, arterial pressure, and intestinal blood flow. Pancreas 1995;11:365-373.

7. Okabe, A., Hirota, M., Nozawa, F., Shibata, M., Nakano, S., and Ogawa, M. Altered cytokine response in rat acute pancreatitis complicated with endotoxemia. Pancreas 2001;22:32-39.

8. Deng, X.M., Wang, X.D., and Andersson, R. Endothelial barrier resistance in multiple organs after septic and nonseptic challenges in rats. J Appl Physiol 1995;78:2052-2061.

9. Lundberg, A.H., Granger, N., and Russell, J. Temporal correlation of tumor necrosis factor-alpha release, upregulation of pulmonary ICAM-1 and VCAM-1, neutrophil sequestration, and lung injury in diet-induced pancreatitis. J Gastrointestinal. Surg.

2000;4:248-257.

10. Frossard, J.L., Saluja, A., Bhagat, L., Lee, H.S., Bhatia, M., Hofbauer, B., and Steer, M.L. The role of intercellular adhesion molecule 1 and neutrophils in acute pancreatitis and pancreatitis-associated lung injury. Gastroenterology 1999;116:694-701.

11. Parker, J.C., Falgout, H.J., Grimbert, F.A., and Taylor, A.E. The effect of increased vascular pressure on albumin-excluded volume and lung lymph flow in the dog lung. Circ. Res. 1980;47:866-875.

12. Closa, D., Sabater, L., Fernandez-Cruz, L., Prats, N., Gelpi, E., and Rosello-Catafau, J. Activation of alveolar macrophages in lung injury associated with experimental acute pancreatitis is mediated by the liver. Ann. Surg. 1999;229:230-236.

13. Wang, X.D., Börjesson, A., Sun, Z.W., Wallén, R., Deng, X.M., Zhang, H.Y., Hallberg, E., and Andersson, R. The association of type II pneumocytes and endothelial permeability with the pulmonary custocyte system in experimental acute pancreatitis. Eur. J. Clin. Invest. 1998;28:778-785.

14. Wang, X.D., Andersson, R., Soltesz, V., Leveau, P., and Ihse, I. Gut origin sepsis, macrophage function, and oxygen extraction associated with acute pancreatitis in the rat. World. J. Surg. 1996;20:299-308.

15. Mann DV, Kalu P, Foulds S, Edwards R, Glazer G. Neutrophil activation and hyperamylasemia after endoscopic retrograde cholagiopancreatography: potential role for the leukocyte in the pathogenesis of acute pancreatitis. Endoscopy 2001;33:488-453.

16. McGill, S.N., Ahmed, N.A., Hu, F., Micheln, R.P., and Christou, N.V. 1996. Shedding of L-selectin as a mechanism for reduced polymorphonuclear neutrophil exudation in patients with the systemic inflammatory response syndrome. Arch. Surg. 131:1141-1147.

17. Paajanen H, Laato M, Jaakkola M, et al. Serum tumor necrosis factor compared with C

reactive protein in the early assessment of severity of acute pancreatitis. Br J Surg 1995;82:271-273.

18. Brivet FG, Emilie D, Galanaud P. Pro and anti inflammatory cytokines during acute severe pancreatitis: an early and sustained response, although impredictable of death. Crit Care Med 1999;27:749-755.

19. de Dios I, Perez M, de la Mano A, Sevillano S, Orfao A, Ramudo L, Manso MA. Contribution of circulating leukocytes to cytokine production in pancreatic duct obstruction-induced acute pancreatitis in rats. Cytokine 2002;20:295-303.

20. Rau B, Baumgart K, Kruger CM, Schilling M, Beger HG. CC-chemokine activation in acute pancreatitis: enhanced release of monocyte chemosttactant protein-1 in patients with local and systemic complications. Intensive Care Med 2003;29:622-629.

21. Dib M, Zhao X, Wang XD, Andersson R. Role of mast cells in the development pancreatitis-induced multiple organ dysfunction. Br J Surg 2002;89:172-178.

LEGENDS TO FIGURES

Figure 1. Plasma exudation to the interstitial tissue (1A) and to the bronchoalveolar (BAL) space (1B) expressed by the ratio of HSA in lung tisse or BAL fluid and HAS in blood 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. ++ stands for p values less than 0.01, as compared to abdominal sepsis.

Figure 2. Levels of TNFα in plasma (2A), lung tissue (2B) and bronchoalveolar lavage (BAL) fluid (2C) 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. ++ stands for p values less than 0.01, as compared to abdominal sepsis.

Figure 3. Levels of MCP-1 in plasma (3A), lung tissue (3B) and bronchoalveolar lavage (BAL) fluid (3C) 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. + and ++ stand for p values less than 0.05 and 0.01, respectively, as compared to abdominal sepsis.

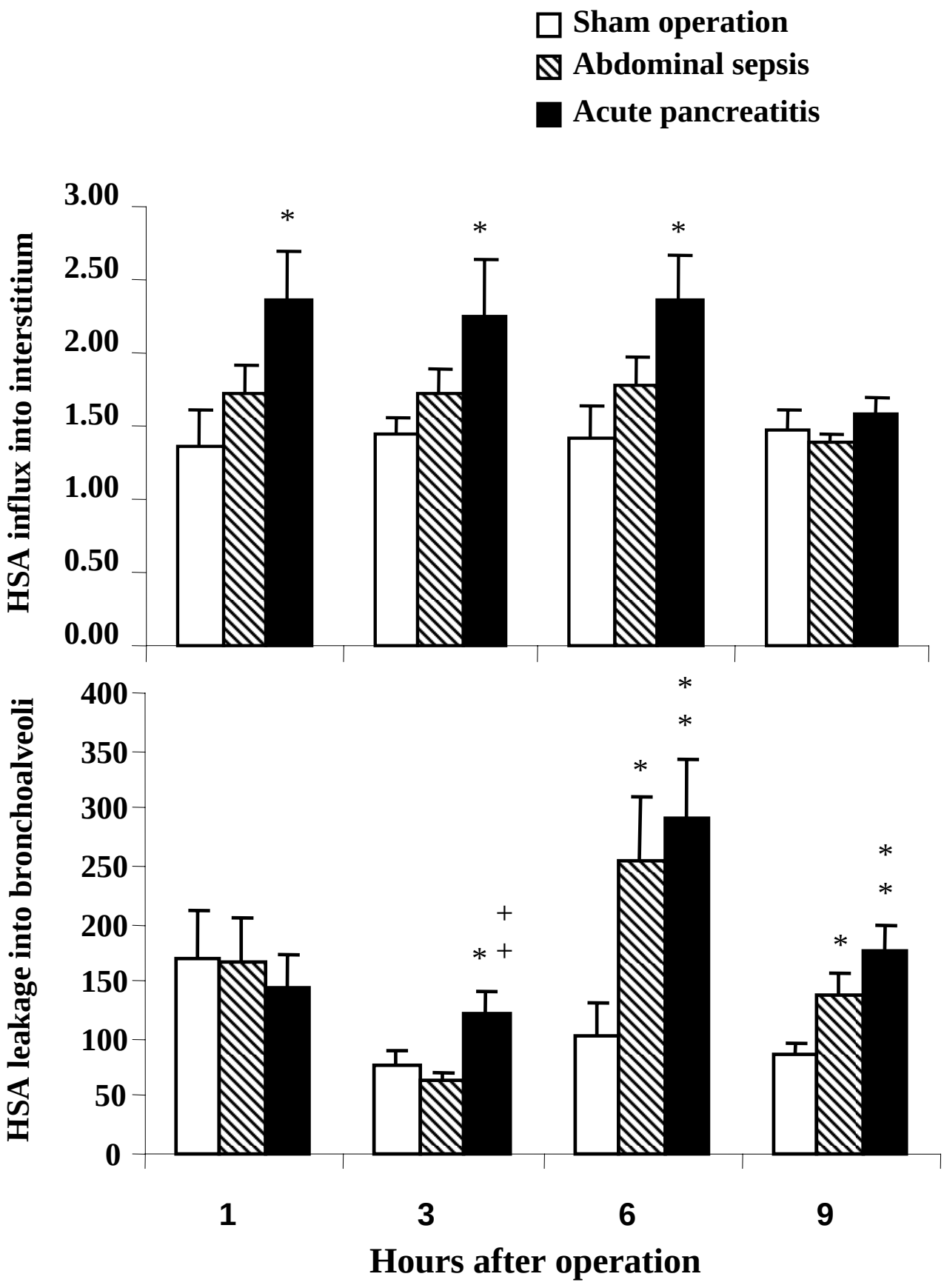
Figure 4. Expression of Mac-1 (CD11b, % above control) on leukocytes isolated from blood (4A), lung tissue (4B) and bronchoalveolar lavage (BAL) fluid (4C) 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. + and ++ stand for p values less than 0.05 and 0.01, respectively, as compared to abdominal sepsis.

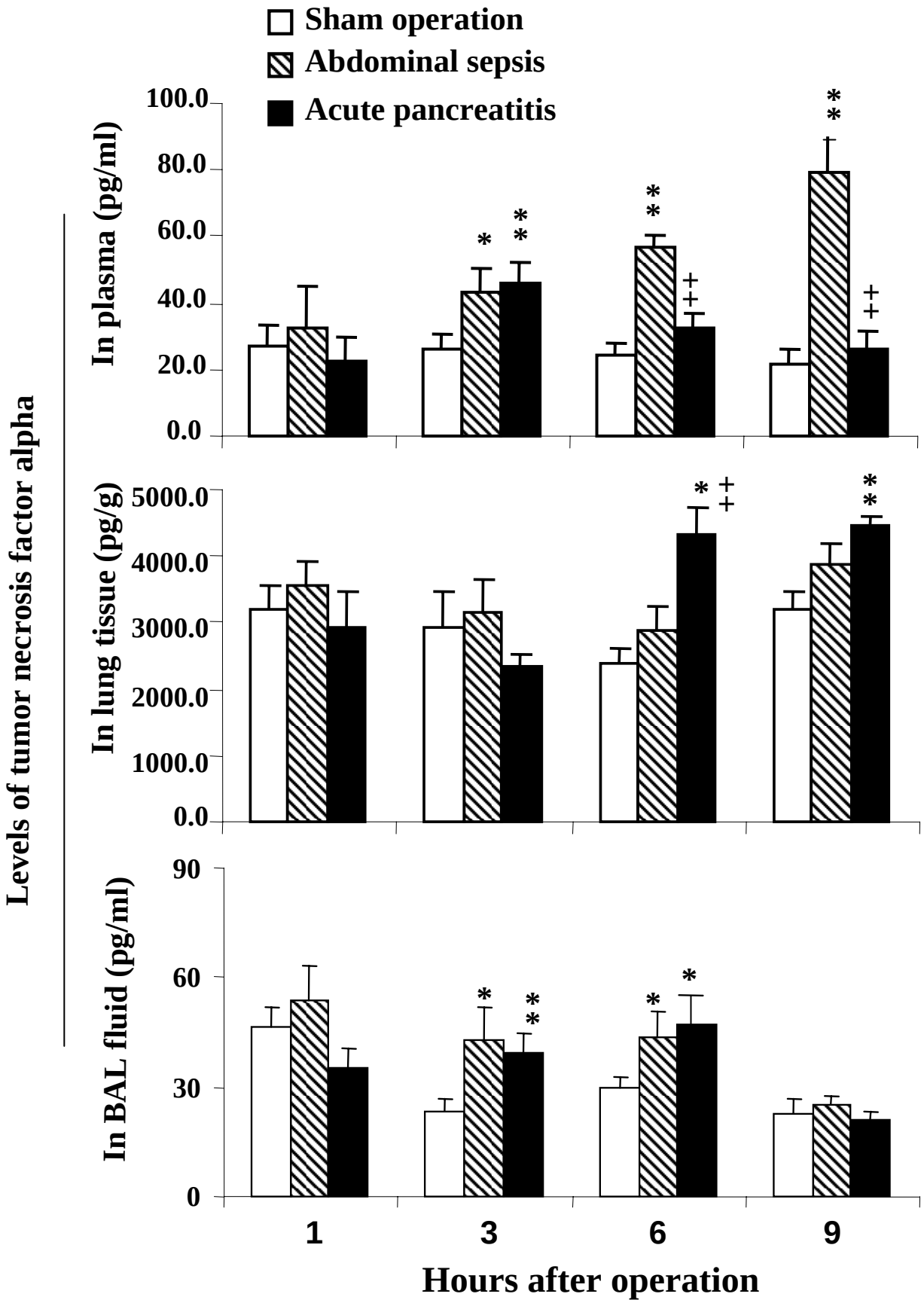
Figure 5. Expression of C3bi receptor (CD11b/c, % above control) on leukocytes isolated from blood (5A), lung tissue (5B) and bronchoalveolar lavage (BAL) fluid (5C) 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. + and ++ stand for p values less than 0.05 and 0.01, respectively, as compared to abdominal sepsis.

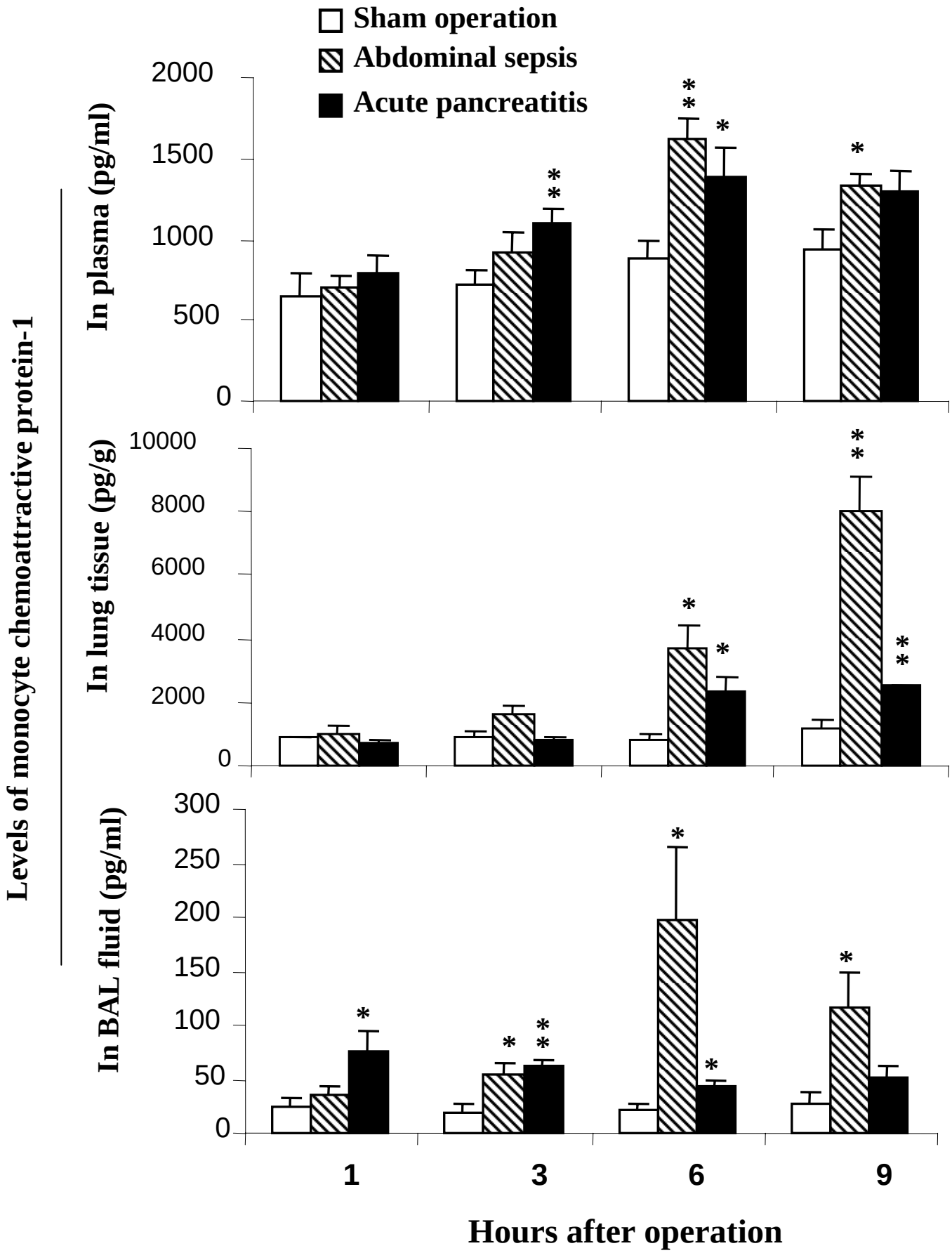
Figure 6. Expression of PECAM-1 (CD31, % above control) on leukocytes isolated from blood (6A), lung tissue (6B) and bronchoalveolar lavage (BAL) fluid (6C) 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. + stands for p values less than 0.05, as compared to abdominal sepsis.

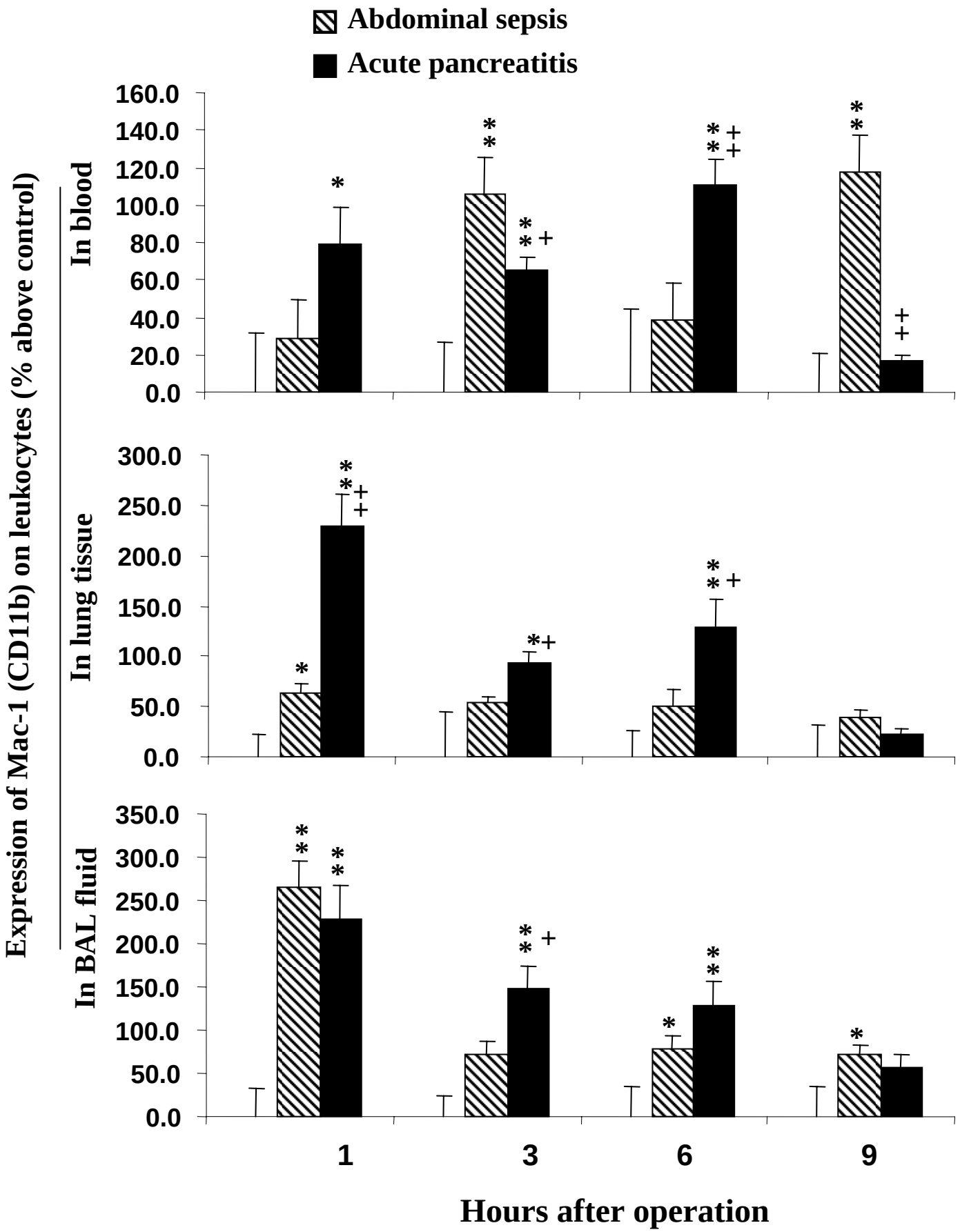
Figure 7. Expression of ICAM-1 (CD54, % above control) on leukocytes isolated from blood (7A), lung tissue (7B) and bronchoalveolar lavage (BAL) fluid (7C) 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. + and ++ stand for p values less than 0.05 and 0.01, respectively, as compared to abdominal sepsis.

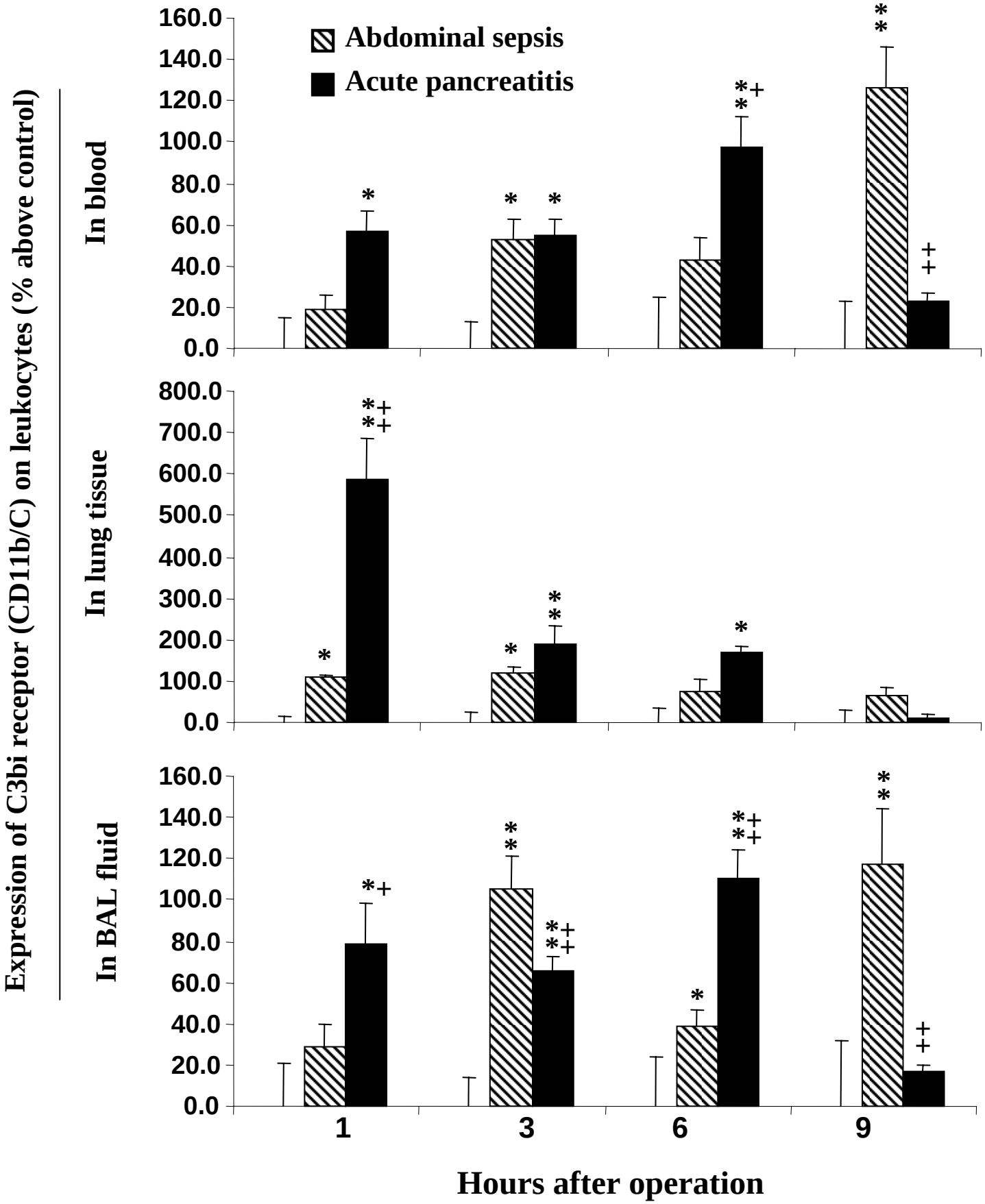
Figure 8. Expression of L-selectin (CD62L, % above control) on leukocytes isolated from blood (8A), lung tissue (8B) and bronchoalveolar lavage (BAL) fluid (8C) 1, 3, 6, and 9 hours after sham operation (controls), induction of acute pancreatitis (AP) and abdominal sepsis. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared to controls. + stands for p values less than 0.05, as compared to abdominal sepsis.

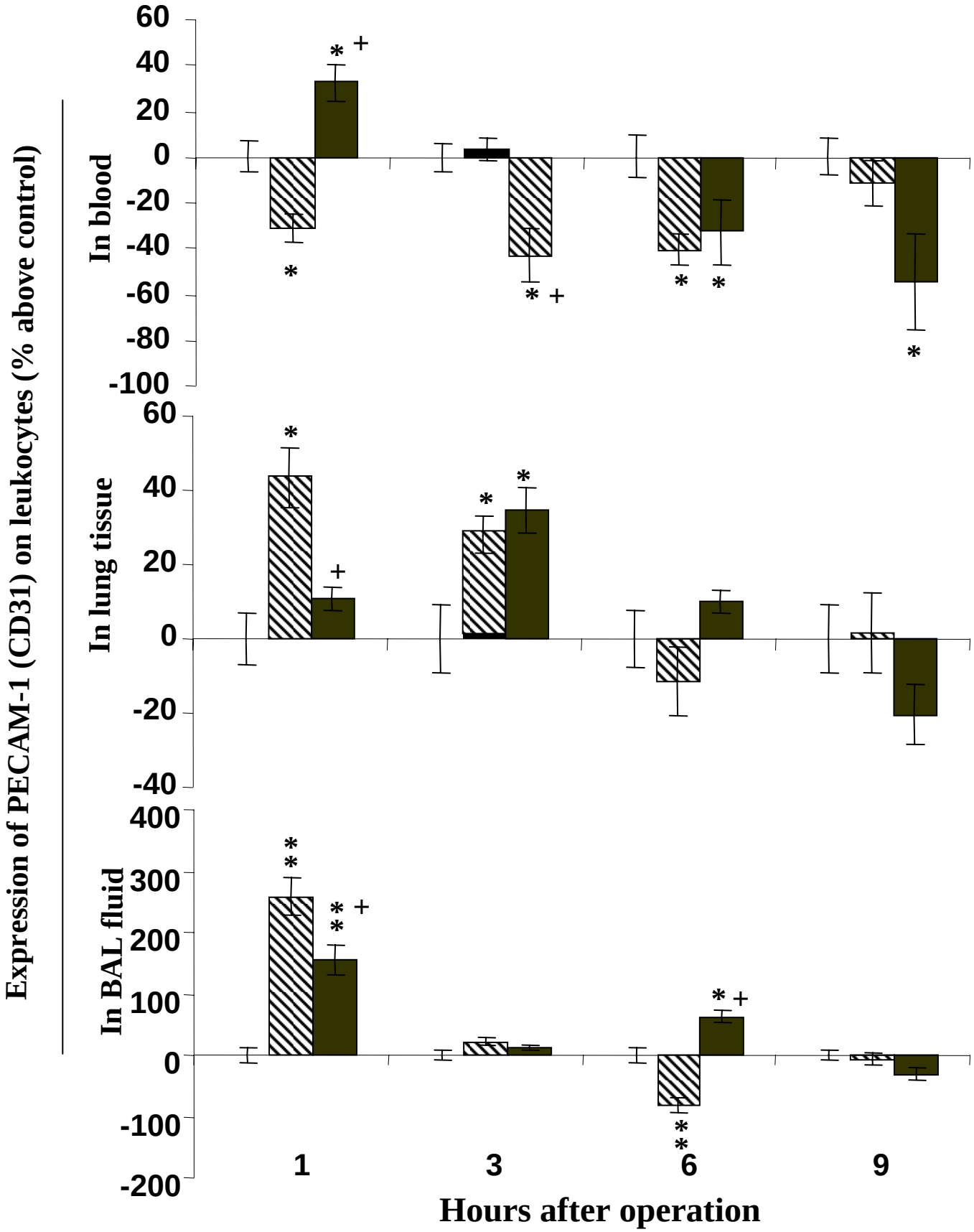

Sham operation
Abdominal sepsis
Acute pancreatitis
HSA influx into interstitium
3.00
2.50
2.00
1.50
1.00
0.50
0.00
HSA leakage into bronchoalveoli
400
350
300
250
200
150
100
50
0
1
3
6
9
Hours after operation

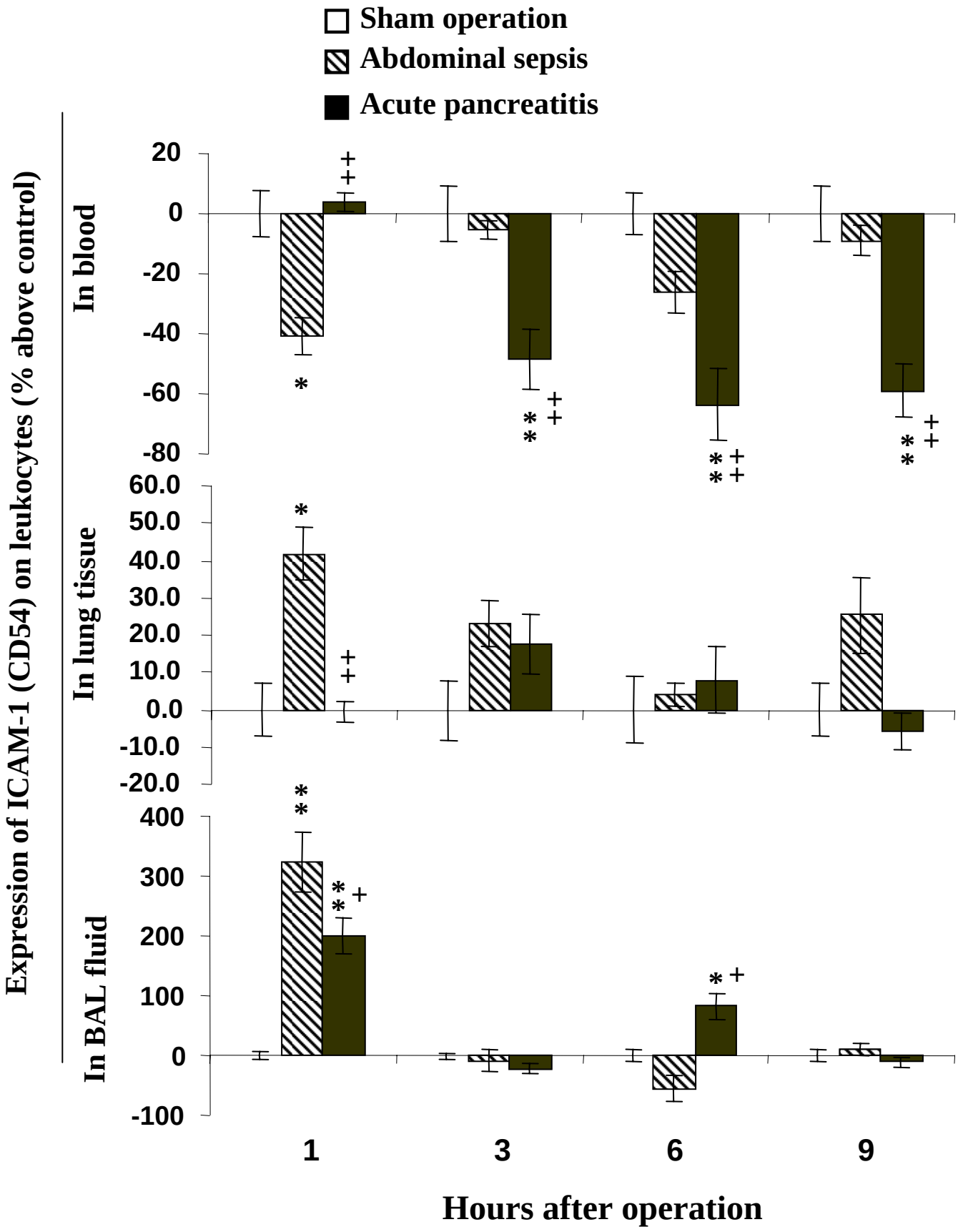

Sham operation
Abdominal sepsis
Acute pancreatitis
Levels of tumor necrosis factor alpha
In plasma (pg/ml)
100.0
80.0
60.0
40.0
20.0
0.0
In lung tissue (pg/g)
5000.0
4000.0
3000.0
2000.0
1000.0
0.0
In BAL fluid (pg/ml)
90
60
30
0
1
3
6
9
Hours after operation

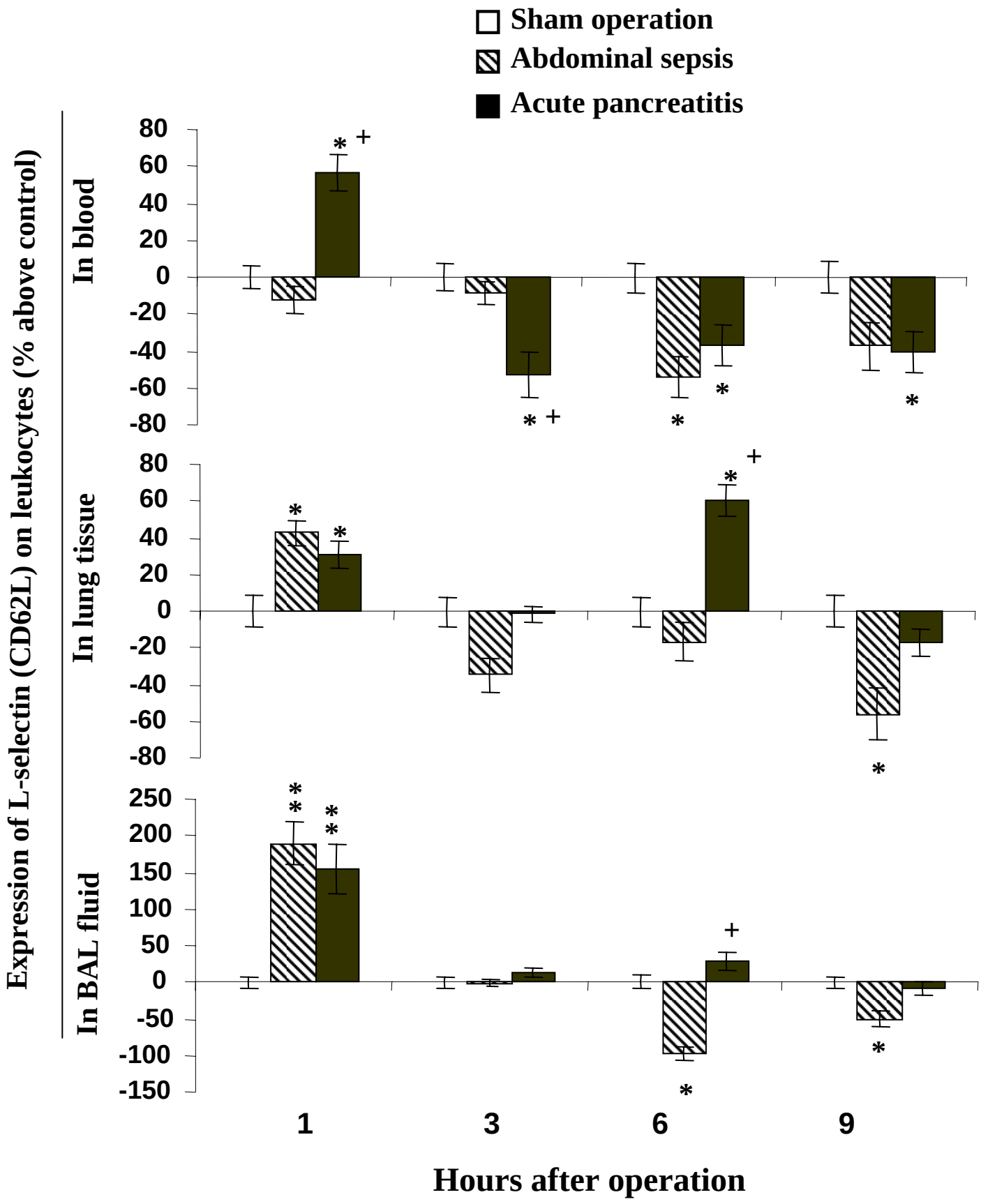

Sham operation
Abdominal sepsis
Acute pancreatitis
Levels of monocyte chemoattractive protein-1
In plasma (pg/ml)
2000
1500
1000
500
0
In lung tissue (pg/g)
10000
8000
6000
4000
2000
0
In BAL fluid (pg/ml)
300
250
200
150
100
50
0
1
3
6
9
Hours after operation


Abdominal sepsis
Acute pancreatitis
Expression of Mac-1 (CD11b) on leukocytes (% above control)
In blood
In lung tissue
In BAL fluid
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
1
3
6
9
Hours after operation


Expression of C3bi receptor (CD11b/C) on leukocytes (% above control)
In blood
In lung tissue
In BAL fluid
Abdominal sepsis
Acute pancreatitis
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
1
3
6
9
Hours after operation


Expression of PECAM-1 (CD31) on leukocytes (% above control)
In blood
In lung tissue
In BAL fluid
60
40
20
0
-20
-40
-60
-80
-100
400
300
200
100
-200
1
3
6
9
Hours after operation


Sham operation
Abdominal sepsis
Acute pancreatitis
Expression of ICAM-1 (CD54) on leukocytes (% above control)
In blood
In lung tissue
In BAL fluid
20
0
-20
-40
-60
-80
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
-20.0
400
300
200
100
0
-100
1
3
6
9
Hours after operation


Sham operation
Abdominal sepsis
Acute pancreatitis
Expression of L-selectin (CD62L) on leukocytes (% above control)
In blood
In lung tissue
In BAL fluid
80
60
40
20
0
-20
-40
-60
-80
250
200
150
100
50
0
-50
-100
-150
1
3
6
9
Hours after operation