TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 816

MEDICA - ODONTOLOGICA

THE ROLE OF ENDOTHELIAL ENZYMES NOS, VAP-1 AND CD73 IN ACUTE LUNG INJURY

by

Jan Kiss

TURUN YLIOPISTO
Turku 2008

From MediCity Research Laboratory, University of Turku and the Department of Surgery, Turku University Central Hospital, Turku, Finland.

Supervised by

Professor Sirpa Jalkanen, MD, PhD
MediCity Research Laboratory
University of Turku
Turku, Finland

Docent Marko Salmi, MD, PhD
MediCity Research Laboratory
University of Turku
Turku, Finland

and

Docent Timo Savunen, MD, PhD
Department of Surgery
Turku University Central Hospital
Turku, Finland

Reviewed by

Docent Karl Lemström
Transplantation Laboratory
University of Helsinki
Department of Cardiothoracic Surgery
Helsinki University Central Hospital
Helsinki, Finland

and

Professor Risto Renkonen
Department of Bacteriology and Immunology
University of Helsinki
Helsinki, Finland

ISBN 978-951-29-3685-4 (PRINT)
ISBN 978-951-29-3686-1 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2008

ABSTRACT

Jan Kiss

The role of endothelial enzymes in acute lung injury

MediCity Research Laboratory, University of Turku and the Department of Surgery, Turku University Central Hospital, Turku, Finland

Acute lung injury (ALI) is a syndrome of acute hypoxemic respiratory failure with bilateral pulmonary infiltrates that is not caused by left atrial hypertension. Since there is no effective treatment available, this frequent clinical syndrome significantly contributes to mortality of both medical and surgical patients. Great majority of the patients with the syndrome suffers from indirect ALI caused by systemic inflammatory response syndrome (SIRS). Sepsis, trauma, major surgery and severe burns, which represent the most common triggers of SIRS, often induce an overwhelming inflammatory reaction leading to dysfunction of several vital organs. Studies of indirect ALI due to SIRS revealed that respiratory dysfunction results from increased permeability of endothelium. Disruption of endothelial barrier allows extravasation of protein-rich liquid and neutrophils to pulmonary parenchyma.

Both under normal conditions and in inflammation, endothelial barrier function is regulated by numerous mechanisms. Endothelial enzymes represent one of the critical control points of vascular permeability and leukocyte trafficking. Some endothelial enzymes prevent disruption of endothelial barrier by production of anti-inflammatory substances. For instance, nitric oxide synthase (NOS) down-regulates leukocyte extravasation in inflammation by generation of nitric oxide. CD73 decreases vascular leakage and neutrophil emigration to inflamed tissues by generation of adenosine. On the other hand, vascular adhesion protein-1 (VAP-1) mediates leukocyte trafficking to the sites of inflammation both by generation of pro-inflammatory substances and by physically acting as an adhesion molecule.

The aims of this study were to define the role of endothelial enzymes NOS, CD73 and VAP-1 in acute lung injury. Our data suggest that increasing substrate availability for NOS reduces both lung edema and neutrophil infiltration and this effect is not enhanced by concomitant administration of antioxidants. CD73 protects from vascular leakage in ALI and its up-regulation by interferon-β represents a novel therapeutic strategy for treatment of this syndrome. Enzymatic activity of VAP-1 mediates neutrophil infiltration in ALI and its inhibition represents an attractive approach to treat ALI.

Keywords: acute lung injury, nitric oxide synthase, vascular adhesion protein-1, CD73.

CONTENTS

ABBREVIATIONS

ALI	acute lung injury
ARDS	acute respiratory distress syndrome
ADP	adenosine diphosphate
AMI	acute mesenteric ischemia
AMP	adenosine monophosphate
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
ELISA	enzyme-linked immunosorbent assay
FITC	fluorescein isothiocyanate
HDMEC	human dermal microvascular endothelial cells
HE	hematoxylin-eosin
HPMEC	human pulmonary microvascular endothelial cells
HUVEC	human umbilical vein endothelial cells
ICAM-1	intercellular adhesion molecule
ICU	intensive care unit
IFN-α	interferon-α
IFN-β	interferon-β
IFN-γ	interferon-γ
IR	ischemia-reperfusion
IRI	ischemia-reperfusion injury
KO	knock-out
mAb	monoclonal antibody
MODS	multiple organ dysfunction syndrome
MPO	myeloperoxidase
NIH	National Institute of Health
NO	nitric oxide
NOS	nitric oxide synthase
PBS	phosphate-buffered solution
SEM	standard error of mean
SIRS	systemic inflammatory response syndrome

SOD	superoxide dismutase
SSAO	semicarbazide-sensitive amine oxidase
TLC	thin-layer chromatography
TNF-α	tumor necrosis factor-α
VAP-1	vascular adhesion protein-1
WT	wild-type

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by roman numerals I-III. Additional unpublished data is also presented.

I Kiss J, Kääpä P, Savunen T. Antioxidants combined with NO donor enhance systemic inflammation in acute lung injury in rats. Scand Cardiovasc J. 2007 Jun;41(3):186-91.

II Kiss J, Yegutkin GG, Koskinen K, Savunen T, Jalkanen S, Salmi M. Interferon-beta protects from acute lung injury via CD73 upregulation. Eur J Immunol. 2007 Dec;37(12):3334-8.

III Kiss J, Jalkanen S, Fülöp F, Savunen T, Salmi M. Ischemia-reperfusion injury is attenuated in VAP-1 deficient mice and by VAP-1 inhibitors. Eur J Immunol. In press..

1. INTRODUCTION

ALI is a frequent clinical syndrome of acute respiratory dysfunction with bilateral pulmonary infiltrates accompanied by hypoxemia. This syndrome is triggered by both pulmonary and nonpulmonary risk factors but is not a consequence of left atrial hypertension (Ware and Matthay, 2000). Despite vast improvements in critical care medicine, mortality rate of the patients suffering from ALI remains as high as 40%. Numerous clinical trials tested with disappointing results treatments, which were reported to be successful in pre-clinical setting. None of the pharmacological interventions improved survival of the patients with ALI. In fact, the only reduction of mortality in this patient group was caused by refined ventilation strategies (Wheeler and Bernard, 2007). The main problem of designing new treatments for acute lung injury seems to be incomplete understanding of the pathophysiology of the disease.

Direct ALI represents pulmonary damage caused by conditions affecting primarily lungs. A pathological process, such as pneumonia or aspiration of gastric content, directly damages lung parenchyma causing respiratory insufficiency. On the other hand, indirect ALI is caused by systemic inflammatory response to a non-pulmonary trigger (Pepe et al., 1982; Fowler et al., 1983; Sloane et al., 1992; Doyle et al., 1995; Hudson et al., 1995; Ware and Matthay, 2000). A primary site of injury, such as non-pulmonary infectious focus, ischemia-reperfusion injury or trauma, triggers a disproportionate inflammation. The response of the immune system is in this case uncontrolled both in terms of intensity and location. Systemic inflammation activates circulating leukocytes, which subsequently extravasate in various organs causing tissue damage (Carden and Granger, 2000; Brown et al., 2006). Lung damage in indirect ALI is caused entirely by inadequate inflammatory response. This overwhelming inflammation results from loss of balance between pro- and anti-inflammatory mechanisms. In order to treat ALI efficiently, we first need to understand the regulation of this balance.

Endothelium forms the innermost layer of the vessels. Under normal conditions, endothelium strictly controls extravasation of macromolecules, liquid and leukocytes. In inflammation, endothelial cells mediate emigration of leukocytes and become leaky to liquid and macromolecules (Stevens et al., 2000). The loss of endothelial barrier function has a central role in the development of ALI (Ware and Matthay, 2000; Wheeler and Bernard, 2007). We decided to study the role of three endothelial enzymes - NOS, CD73 and VAP-1 - in indirect ALI. Besides better understanding of the complicated process of ALI development, we wanted to offer feasible treatment options for the patients suffering from this often fatal disease.

2. REVIEW OF THE LITERATURE

2.1. ACUTE LUNG INJURY

ALI is a common clinical syndrome diagnosed in both surgical and medical patients. The term ALI also includes its more severe form, the acute respiratory distress syndrome (ARDS). Therefore, ALI is used when referring to both conditions. This syndrome is characterized by sudden onset of clinically significant hypoxemia accompanied by diffuse pulmonary infiltrates on chest radiograph. The infiltrates correspond to pulmonary edema resulting from increased pulmonary vascular permeability. This syndrome has been intensely studied for decades because of its high prevalence, unacceptably high mortality rate and no efficient drug therapy (Ware and Matthay, 2000; Wheeler and Bernard, 2007). ALI is a relatively new syndrome, as it was not until 1967 when Ashbaugh and colleagues first described 12 patients with acute respiratory distress, cyanosis refractory to oxygen therapy, decreased lung compliance, and diffuse infiltrates evident on the chest radiograph (Ashbaugh et al., 1967). Ever since, both the name and the diagnostic criteria have been refined (Bernard, 2005).

The vague original definition caused controversies over the natural history of the syndrome, its incidence and the mortality associated with it (Luce, 2005). Therefore, the definition was expanded in 1988 to include quantification of lung function through the use of a scoring system. The system included four points to score the lung injury, namely the level of positive end-expiratory pressure (PEEP), the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO_2/FiO_2), the static lung compliance, and the degree of infiltration evident on chest radiographs (Murray et al., 1988). In addition, the triggering clinical event and the presence or absence of non-pulmonary organ dysfunction was included in the assessment. Interestingly, a recent study showed no difference in mortality between the patients with and without non-pulmonary organ dysfunction (Agarwal et al., 2007). The scoring system proved to be useful in quantification of severity of ALI, but failed to have predictive value for the outcome during the first three days (Doyle et al., 1995). Although higher scores four and seven days after the onset were predictive of a complicated course (Heffner et al., 1995), the clinical usefulness of the scoring system was limited.

A new definition suggested by American–European Consensus Conference Committee in 1994 allows, besides an easy application in the clinical setting, also stratification of the patients according to the severity of lung injury (Bernard et al., 1994). The severity of lung injury is assessed by PaO_2/FiO_2. When $PaO_2/FiO_2<300$, the disorder is called ALI and when $PaO_2/FiO_2<200$, ARDS is diagnosed. Although the simplified definition enables earlier enrolment of patients into clinical trials, it also has several disadvantages. First, oxygenation indices can be readily improved by the use of PEEP. Therefore, the patients who meet the criteria for ARDS prior to ventilation might convert to ALI patients. Similarly, ALI patients might not meet the diagnostic criteria as soon as PEEP is initiated. The second drawback of the definition is that the clinical condition that triggered lung injury is not assessed. Third, the dysfunction of other organs is not taken into account. Finally, clinicians do not recognize accordantly the presence of bilateral infiltrates on chest radiography consistent with the presence of

pulmonary edema (Rubenfeld et al., 1999). Therefore, improvement of the standardization of clinical trials has been achieved by the use of both lung injury scoring system from 1988 and consensus definition from 1994 (Abraham et al., 2000). However, regardless of the improvements, the limitations of randomized controlled trials in ALI have to be acknowledged (Marini, 2006). Many biologic markers and clinical factors, such as the original trigger of acute lung injury or severity of disease, can be used to stratify the patients in different groups. The choice of inclusion criteria and grouping of the patients has a major impact on the outcome of clinical trials (Ware, 2005).

2.1.1. Diagnostic features

The patients suffering from ALI can be identified according to the diagnostic criteria already during the early stages of the disease. Due to the progressive nature of the syndrome, the patients usually experience different stages of the disease with their characteristic features. Sudden respiratory dysfunction refractory to oxygen administration present in the patients with a risk factor is typical for the acute, or exudative, phase of the syndrome. Diffuse bilateral alveolar infiltrates seen on chest radiographs indicate the presence of pulmonary edema (Aberle et al., 1988). Diffuse alveolar damage with infiltrating leukocytes and erythrocytes, hyaline membranes and edema fluid belong to the typical pathological findings (Pratt et al., 1979). Lately, some experts suggested that open-lung biopsy in ALI patients might reveal an unsuspected diagnosis and cause a change in treatment. However, this approach is accompanied by high incidence of complications related to the surgery. These complication possibly outweigh the benefits caused by more appropriate therapy, as there was no survival benefit observed in the biopsied patients (Patel et al., 2004).

The acute phase of ALI might be followed in some cases by a complete resolution. However, in some patients the syndrome progresses to fibroproliferative phase. This phase is characteristic by fibrosing alveolitis with persistent hypoxemia, increased alveolar dead space, and an additional decrease in pulmonary compliance. The proliferating fibroblasts and chronic inflammatory cell infiltrates reduce the pulmonary capillary bed, which results in an increased pulmonary capillary pressure (Pratt et al., 1979). During fibroproliferative phase, linear opacities typical for the presence of evolving fibrosis are found radiographically. Improvements in radiology allow nowadays prediction of prognosis at this stage of the disease (Ichikado et al., 2006). Diffuse interstitial opacities and bullae are the typical findings in computed tomography imaging (Gattinoni et al., 1994). The patients who recover experience improvement in blood gas exchange and lung compliance. In most of the cases, radiographic as well as functional findings return to normal.

2.1.2. Epidemiology

2.1.2.1. Incidence

Due to the use of different definition criteria, numerous triggering conditions and variable clinical manifestation, there has been en extensive dispute regarding the incidence of ALI. National Institute of Health (NIH) Acute Respiratory Distress

Syndrome Network confirmed the original estimate of NIH that the incidence of the syndrome in the US is 75 per 100,000 per year (1977). The incidence of 17.9 per 100,000 for ALI and 13.5 per 100,000 for ARDS were the results of the first epidemiologic study, which used the 1994 consensus definition (Luhr et al., 1999). A more recent study estimates that each year in the United States there are 190,600 cases of ALI, which are associated with 74,500 deaths and 3.6 million hospital days (Rubenfeld et al., 2005).

2.1.2.2. Clinical disorders associated with ALI development

There are several clinical disorders capable of triggering ALI. These disorders are divided according to the mechanism, through which they cause lung damage. The first group of the disorders causes direct injury to the lung and the second group consists of disorders causing lung damage indirectly via systemic inflammation (Figure 1.). Pneumonia and aspiration of gastric contents belong to the most common causes of direct ALI. Pulmonary contusion, fat emboli, near-drowning, inhalational injury and reperfusion pulmonary edema after lung transplantation or pulmonary embolectomy are some of the less common causes. Sepsis and severe trauma with shock and multiple transfusions represent the most common causes of indirect ALI, while cardiopulmonary bypass, drug overdose, acute pancreatitis and transfusions of blood products belong to the less common ones (Pepe et al., 1982; Fowler et al., 1983; Sloane et al., 1992; Doyle et al., 1995; Hudson et al., 1995). Sepsis was shown to be the condition most likely progressing into ALI. As many as 40% of the severe sepsis patients will suffer from indirect lung damage (Pepe et al., 1982; Hudson et al., 1995).

2.1.2.3. Intestinal ischemia-reperfusion as a trigger of ALI

Ischemia occurs when blood supply to an organ becomes compromised. Inadequate perfusion results in deficient oxygen delivery to metabolically active cells and leads to depletion of energy-rich phosphates. Lack of energy slows down active transmembrane ion transport, which causes intracellular ion accumulation with influx of water. Swelling of the cells continues during sufficiently long ischemia to the point of rupture and the cells die of necrosis. Shorter periods of ischemia are characteristic by endothelial cell swelling, stiffening of leukocytes and hemoconcentration, which interfere with microcirculation during the reperfusion causing no-reflow phenomenon (Jerome et al., 1994).

Reperfusion takes place when blood supply to the ischemic organ is restored. Although essential for organ survival, reperfusion significantly enhances the damage caused by ischemia. Reperfusion injury is also called reflow paradox. While capillaries are the site of no-reflow phenomenon, reflow paradox occurs mainly in the post-capillary venules. Leukocyte adhesion to the post-capillary venules results from increased expression of adhesion molecules on both leukocytes and endothelium (Ichikawa et al., 1997). Activated leukocytes damage endothelium by the release of proteases. Due to the disruption of endothelial barrier function, leakage of macromolecules and extravasation of leukocytes extend the reperfusion injury from intravascular space to the interstitium (Kurose et al., 1994). Pro-inflammatory cytokines released by activated immune cells and some resident cells promote

leukocyte accumulation at the site of reperfusion injury and accentuate the damage. Radicals liberated by leukocytes and generated by xanthine oxidase cause direct damage to the cells and close the vicious circle by further increasing expression of adhesion molecules (Granger, 1988; Suzuki et al., 1991).

The inflammatory reaction in the reperfused tissue often reaches sufficient intensity to cause damage in remote organs. The overwhelming reaction of the immune system might lead to dysfunction of several vital organs. This condition is known as multiple organ dysfunction syndrome (MODS) and it represents the main cause of morbidity and mortality in ICU patients (Baue, 2006). Therefore a better understanding of systemic inflammatory response, which is responsible for multiple organ failure, is crucial for targeted treatment. Respiratory dysfunction is present in almost all the patients suffering from multiple organ failure (Guidet et al., 2005).

SIRS is often accompanied by alterations of macro- and microcirculation. Especially splanchnic perfusion was found to be significantly reduced in critical illness due to shunting of blood to the vital organs (Sapirstein et al., 1960; Vatner, 1974; Bulkley et al., 1983; Bailey et al., 2000; Toung et al., 2000). Reduced splanchnic perfusion leads to ischemic damage of intestine and loss of its barrier function (Bounous, 1982). Bacteria and endotoxin translocated through intestinal mucosa to the circulation activate both local and systemic response of the immune system. Therefore, gut was named "motor of MODS" (Swank and Deitch, 1996).

Intestinal ischemia occurs in clinical practice also as a consequence of vascular occlusion. Acute mesenteric ischemia (AMI) represents one of the most dramatic vascular emergencies. This condition is characterized by a sudden occlusion of mesenteric arteries followed by impairment of intestinal blood flow. Although AMI is a relatively infrequent disease, its incidence has been increasing significantly due to longer mean life expectancy. Despite the progress in surgical and intensive care, the in-hospital mortality has remained as high as 70% during the last decades. Besides incomplete understanding of the events triggered by AMI, the major reason of the extremely high mortality rate associated with AMI is the difficulty to recognize this condition. A non-specific clinical presentation and laboratory findings often delay diagnosis to the point when the intestinal necrosis has developed. Bacteria and endotoxin translocated from the damaged intestine trigger systemic inflammatory response syndrome, which often culminates in MODS and death (Yasuhara, 2005). In experimental studies, intestinal ischemia induced by vascular occlusion with a clamp has become an established model of systemic inflammation causing ALI and MODS.

2.1.2.4. ALI in cardiovascular surgery

Cardiac and major vascular surgery is frequently associated with a certain degree of systemic inflammation. Surgical trauma, ischemia-reperfusion injury, release of endotoxin from under-perfused intestine, contact of the patient's blood with the artificial surface of cardiopulmonary bypass (CPB) circuit and transfusion are some of the most important triggers of the inflammatory response (Tonz et al., 1995; Raijmakers et al., 1997; Czerny et al., 2000; Silliman et al., 2005). SIRS significantly contributes to the post-operative complications in cardiovascular surgery, such as dysfunction of the heart, lungs, liver, kidneys, brain, or in the worst case MODS.

ALI belongs to the most common post-operative complication in cardiovascular surgery. The lungs suffer in addition to the systemic inflammatory reaction also from ischemic injury (Schlensak et al., 2000). During CPB, there is no blood flow in the pulmonary artery and the flow in the bronchial arteries is reduced. The importance of this finding is underlined by the fact that lung ischemia and subsequent inflammation can be partially prevented by perfusion of the pulmonary artery with cold blood during CPB (Schlensak et al., 2001; Schlensak et al., 2002).

Results of some studies suggest that the incidence of ALI in the patients undergoing cardiac surgery is as high as 60% (Verheij et al., 2006). Respiratory dysfunction in most of the cases is only temporary and corrects rapidly with the support of artificial ventilation. However, about 20% of the patients undergoing cardiac surgery with the use of CPB require ventilation for more than 48 hours after the operation (Hammermeister et al., 1990). Prolonged ICU stay with artificial ventilation is associated besides increased complication rate also with higher mortality. In fact, mortality of the patients with severe post-operatory respiratory dysfunction in cardiac surgery has been reported to be over 50% (Milot et al., 2001).

2.1.2.5. Outcomes

Mortality rate associated with ALI is about 40% (Fowler et al., 1983; Bell et al., 1983; Montgomery et al., 1985; Sloane et al., 1992; Suchyta et al., 1992; Doyle et al., 1995; Milberg et al., 1995; Zilberberg and Epstein, 1998). The deaths are caused in the most cases by MODS. The reduction of mortality rates caused by the recent improvements in the ventilation strategies shows that a part of the deaths is a direct consequence of lung dysfunction. Although the mortality of patients suffering from ALI has according to some reports a tendency to decrease, it is compensated by the increasing incidence of the syndrome. Chronic liver disease, nonpulmonary organ dysfunction, sepsis, and advanced age are some of the recognized risk factors of death in ALI patients. The severity of hypoxemia, however, has not been shown to be associated with increased mortality (Doyle et al., 1995; Zilberg and Epstein, 1998; Luhr et al., 1999). Critical patients suffering from severe sepsis, the most common cause of ALI and MODS, occupy according to a recent study 40% of beds in ICU and their mortality remains as high as 70% (Guidet et al., 2005). One of the most important components of MODS is ALI. ALI is present in about 90% of MODS patients and represents one of the independent risk factors for death (Stapleton et al., 2005).

Regardless of the severity of the initial lung injury, lung function returns to normal within six to twelve months after the diagnosis in most of the survivors (McHugh et al., 1994). Lung function abnormalities, which persist after the resolution of the syndrome, include gas-exchange deficit with exercise and residual impairment of pulmonary mechanics. These anomalies were believed not cause any symptoms (Elliott et al., 1987; Ghio et al., 1989). The earlier findings were confronted by the results of recent studies, which show that the survivors of ALI tend to have certain degree of functional impairment, diminished health-related quality of life and represent an additional cost to healthcare (Hopkins et al., 2005; Cheung et al., 2006).

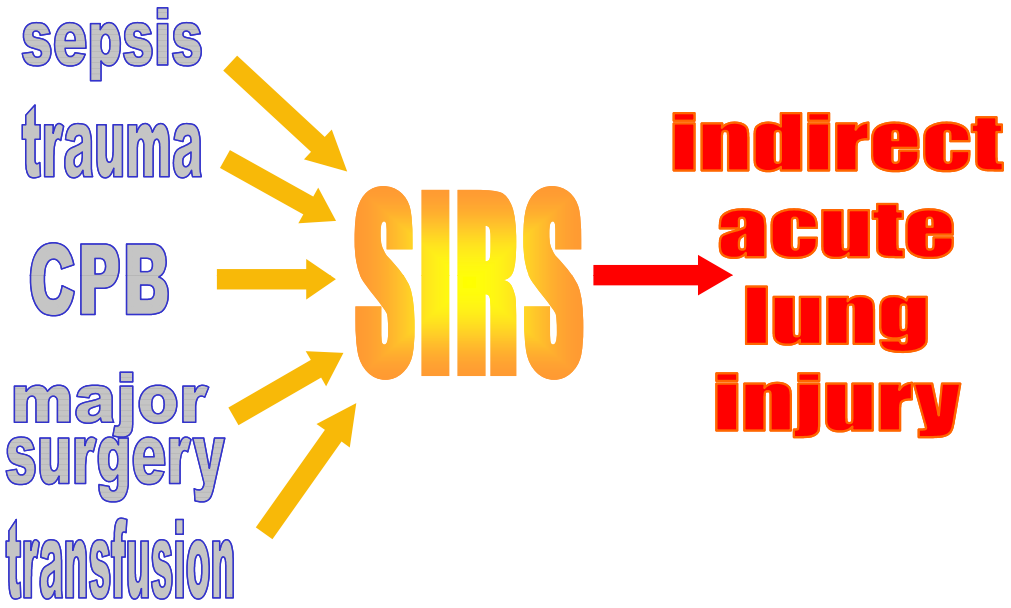

Figure 1. Indirect ALI.
The scheme summarizes the development of indirect ALI in clinical practice. Non-pulmonary disorders trigger SIRS, which results in disruption of alveolo-capillary barrier and subsequent development of indirect ALI.

Especially the patients who experience severe disease and are ventilated during extended periods are at higher risk of having residual impairment (Suchyta et al., 1991; McHugh et al., 1994).

2.1.3. Pathogenesis

2.1.3.1. Endothelial and epithelial injury

Disruption of the alveolar-capillary barrier is a well-established mechanism responsible for the influx of protein-rich edema fluid into the air spaces during the initial phase of ALI (Pugin et al., 1999). The alveolar-capillary barrier consists of microvascular endothelium and alveolar epithelium. Injury of each of the two components of the barrier is responsible for the development of pulmonary edema.
Endothelium covers the luminal surface of the vasculature and is therefore exposed to the substances found in the circulation. During ALI, endothelial cells are being injured by pro-inflammatory cytokines, activated immune cells and reactive species. Endothelial damage contributes to leukocyte recruitment, coagulation disorders and vascular leakage (Figure 2.).

Two cell types comprise the alveolar epithelium under normal circumstances. Up to 90 percent of the alveolar surface is covered by flat type I cells. The remaining area is covered by cuboidal type II cells. The former are very prone to injury, while the latter are fairly resistant, which allows them to differentiate to type I cells after injury. The loss of epithelial integrity contributes importantly to edema formation for several reasons. Epithelial barrier represents the less permeable component of the alveolar-capillary barrier (Wiener-Kronish et al., 1991). Injury to type II cells impairs removal of fluid from alveolar space and surfactant production, which interferes with the normal function of the lungs (Modelska et al., 1999; Sznajder, 1999; Greene et al., 1999). Severe epithelial injury also predisposes to fibrosis during the later phases of ALI (Bitterman, 1992). Moreover, the lack of epithelial barrier allows bacteria present in the patients with pneumonia to enter the circulation and cause sepsis (Kurahashi et al., 1999).

2.1.3.2. Neutrophil-dependent injury

ALI is accompanied by neutrophil sequestration in the lungs (Figure 2.). Most of the animal models show clear neutrophil-dependency of the lung damage (Prescott et al., 1999). In clinical practice, neutrophils were found in the lung biopsies, pulmonary edema fluid and bronchoalveolar lavage fluid of the patients suffering from ALI (Bachofen and Weibel, 1974; Bachofen and Weibel, 1977; Pittet et al., 1997). Upon activation, neutrophils can release oxidants, proteases and proinflammatory molecules. However, it remains unclear whether neutrophil sequestration is a result or a cause of the lung damage. Both animal and clinical studies with neutropenic subjects showed that lung injury can develop also in the absence of polymorphonuclear cells (Laufe et al., 1986).

2.1.3.3. Other pro-inflammatory mechanisms

Pro-inflammatory cytokines have a crucial role in the initiation and evolution of ALI. In the case of direct ALI, the cytokines are mainly produced locally by the infiltrating immune cells, lung epithelial cells and fibroblasts. Extrapulmonary sources of proinflammatory cytokines contribute to lung damage in indirect ALI. However, the extent of inflammatory response depends on the balance between pro- and anti-inflammatory mediators.

Although in many cases life-saving, artificial ventilation represents another mechanism enhancing inflammatory reaction and causing lung damage. Ventilation-induced lung injury has two major components. The first part is the inhalation of high-fraction oxygen, which is toxic for the lung parenchyma (Pratt et al., 1979). Second, high pressures and volumes cause increased vascular permeability inducing edema formation (Webb and Tierney, 1974; Pratt et al., 1979; Parker et al., 1984; Dreyfuss et al., 1988; Corbridge et al., 1990). Moreover, alveolar overdistention accompanied by the repeated collapse and reopening of alveoli causes production of pro-inflammatory mediators in the lungs (Slutsky and Tremblay, 1998). Therefore, development of optimal ventilation strategies is absolutely necessary to reduce additional lung damage.

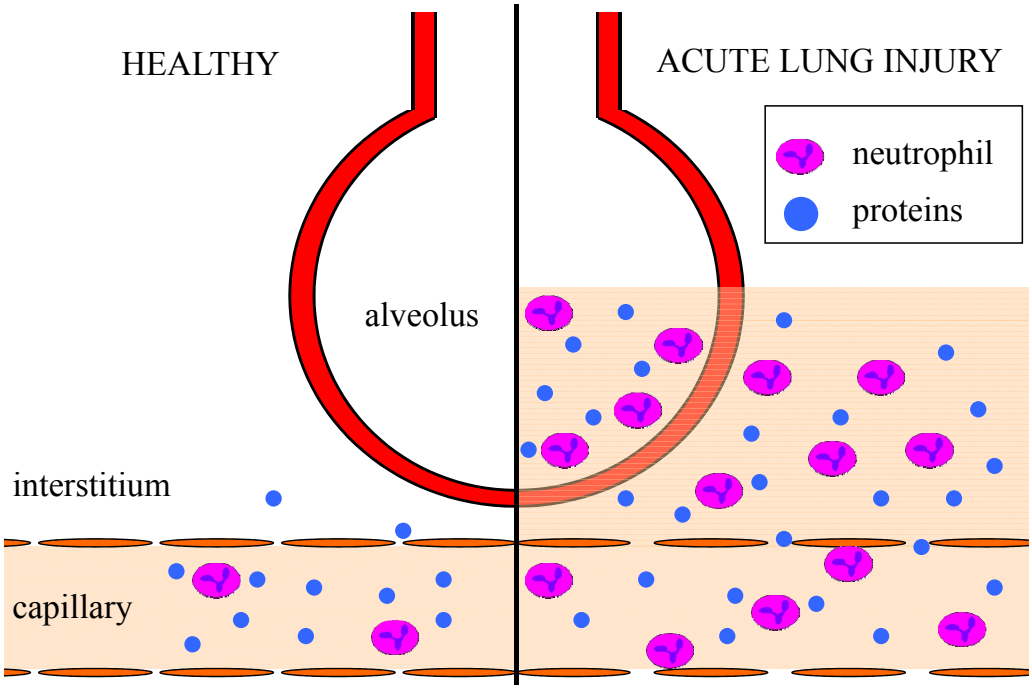

Figure 2. Pathogenesis of ALI.
The figure illustrates the hallmarks of acute lung injury. Compromised endothelial barrier function allows leakage of protein-rich liquid, which is followed by leukocyte extravasation to the interstitium and alveolar spaces.

2.1.4. Treatment

A reduction in mortality rate associated with ALI is mostly a consequence of improvement in supportive care (Milberg et al., 1995; Abel et al., 1998). Clinical trials of drug treatment for ALI, such as corticosteroids and vasodilators, did not improve mortality rates and in some cases they even increased risk of death (Sprung et al., 1984; Matthay et al., 1998; Steinberg et al., 2006; Marini, 2006). Early and more effective treatment of infections represents one of the major advances in supportive care, since uncontrolled infections in critical illness are often fatal (Montgomery et al., 1985). Fluid restriction belongs to the standard therapeutical interventions in the patients with lung edema. However, this approach carries a risk of compromising non-pulmonary organ perfusion. Experimental studies showed that decrease of left atrial pressure was accompanied with decrease of lung edema (Bachofen and Weibel, 1977; Prewitt et al., 1981). A recent clinical study showed that fluid restriction in the patients with ALI improved lung function and shortened the duration of mechanical ventilation and intensive care without increasing nonpulmonary organ failures (Wiedemann et al., 2006).

Improved ventilation strategies are especially important for the patients suffering from respiratory dysfunction. Low tidal volume has been shown to significantly

decrease mortality due to reduced ventilator-induced lung injury (2000). Positive end-expiratory pressure improves oxygenation and allows use of lower fractions of oxygen (Petty and Ashbaugh, 1971; Falke et al., 1972). However, the ventilation strategy in ALI patients still needs to be optimized.

All the abovementioned refinements in supportive care have had significant impact on the mortality rate in the patients with ALI or ARDS, especially in the setting of multiple organ failure. However, the mortality still remains unacceptably high and the incidence of multiple organ failure has increased, which compensates for the improvements in critical care. What more, no effective pharmacological treatment for ALI is available.

2.2. NOVEL THERAPEUTIC STRATEGIES IN THE TREATMENT OF ALI

2.2.1. Nitric oxide

Nitric oxide (NO) is a gas without color and odor. It is relatively water insoluble and does not react with the majority of biologic molecules. However, NO has an unpaired electron. This property allows it to react with other free radicals, some amino acids, and transition metal ions (McCleverty, 2004). Thiols, nitrite, and proteins containing transition metals stabilize nitric oxide in biologic solutions by forming complexes (Stamler et al., 1992).

NO was not given much importance until it was shown to be identical with endothelium-derived relaxing factor, which is an important molecule in the regulation of vascular tone (Palmer et al., 1987). Endogenously, NO generation is governed by nitric oxicle synthase (NOS). This enzyme exists in three isoforms - neural, inducible, and endothelial. Each of these isoforms generates nitric oxide from the semiessential amino acid L-arginine.

2.2.1.1. Positive effects of NO

NO has several protective effects in inflamed lungs, which made it one of the candidates in treatment of ALI. The effect of NO in pulmonary circulation has been profoundly studied. Its inhalation causes a decrease of pulmonary artery pressure and pulmonary vascular resistance by vasodilatation of vasculature in the lungs (Pepke-Zaba et al., 1991; Frostell et al., 1991; Frostell et al., 1993). Since hemoglobin immediately inactivates NO, its effect is local and does not cause systemic hypotension (Rimar and Gillis, 1993). What more, increase of pulmonary blood flow induced by NO is selective for the ventilated areas. Thus, NO improves ventilation-perfusion mismatch and improves oxygenation, which is in contrast with other vasodilators (Rossaint et al., 1993).

Besides its effects on the regulation of vascular tone, NO improves function of the lungs suffering from ALI in many other ways. One of its crucial effects is reduction of neutrophil-mediated damage in acute inflammation. Both the respiratory burst and neutrophil-derived oxidative stress are diminished by NO (Gessler et al., 1996). In vitro studies showed that NO reduces leukocyte adhesion to endothelium (Kubes et al.,

1991). Similar effect was also observed in animal models of ALI, in which NO decreased the sequestration of neutrophils both in the pulmonary vessels and in the alveoli (Sato et al., 1999). NO also modulates platelet function. It was shown to inhibit adhesion of platelets to endothelium and their subsequent aggregation (Moncada et al., 1991). Another finding relevant for direct ALI caused by infection is that endogenously produced NO is capable of killing pathogens (Liew et al., 1990). In addition, production of surfactant was shown to be increased by NO, which adds to its protective properties (Stuart et al., 2003).

2.2.1.2. Negative effects of NO

Toxic effects of NO stem mainly from its reactivity with free radicals. It has been shown in different models that nitrogen reactive species derived from NO cause certain degree of endothelial damage (Heiss et al., 1994; Kristof et al., 1998). However, NO alone is not sufficient to significantly increase generation of nitrogen reactive species. The process requires also high concentration of oxygen radicals (Weinberger et al., 2001). This condition might be met in the patients ventilated with high fraction of oxygen.

2.2.1.3. Use of NO for treatment of ALI in clinical trials

Vasodilatatory and anti-inflammatory effects of NO make it an attractive treatment option for the patients with ALI. 63% of European intensive care specialists surveyed in 1997 and 39% of Canadian intensive care specialists surveyed in 2004 have used NO to treat ALI (Beloucif and Payen, 1998; Meade et al., 2004). Interestingly, none of the 12 randomised trials of inhaled NO in patients with ALI showed mortality benefit (Adhikari et al., 2007). Although administration of NO was associated with limited improvement in oxygenation, the patients had an increased risk of developing renal dysfunction.

2.2.2. Antioxidants

Reactive oxygen species are being continuously generated under physiologic conditions as a result of oxygen metabolism (Frei, 1994). Small quantities of oxidants produced during cellular respiration and localized inflammatory reaction are effectively inactivated by scavenger systems. Endogenous antioxidants can be divided into two groups. Superoxide dismutase, catalase, and glutathione peroxidase belong to enzymatic antioxidants. Glutathione, vitamin E, vitamin C, β-carotene, and heme-binding proteins including ceruloplasmin, transferrin, haptoglobin, and albumin represent non-enzymatic scavengers. Under circumstances which induce a massive increase in oxidants production, antioxidant defence might be insufficient and the tissues suffer from oxidative stress. The effects of oxidative stress range from induction of expression of adhesion molecules through damage of proteins, lipids and DNA to cell death (Halliwell, 1994; Marnett et al., 2003; de Nigris et al., 2003; Szabo, 2003; Virag et al., 2003).

2.2.2.1. Oxidative stress in critical illness

Critical illness is accompanied by substantial increase in generation of oxidants (Gutteridge and Mitchell, 1999). One major source of reactive oxygen species are the activated phagocytes. Respiratory burst of neutrophils, monocytes, macrophages and eosinophils consisting of rapid release of oxygen species significantly increases oxidative stress (Lamb et al., 1999). Xantine dehydrogenase represents additional source of reactive oxygen species (Takeyama et al., 1996). Finally, inducible NOS generates in critical illness increased amounts of NO, which together with reactive oxygen species forms reactive nitrogen species and causes an additional oxidative stress (Rudkowski et al., 2004; Peng et al., 2005).

2.2.2.2. Oxidative stress in ALI

Disruption of oxidant-antioxidant balance has been proposed as one of the mechanisms behind the development of ALI. It has been shown that the patients suffering from ALI have increased levels of reactive species and decreased levels of antioxidants (Zhang et al., 2000; Lang et al., 2002). Increased concentrations of hydrogen peroxide accompanied by peroxidation of membrane phospholipids have been shown in several clinical studies (Baldwin et al., 1986; Sznajder et al., 1989). Concurrently, measurement of antioxidant concentration in the lungs of ALI patients showed diminished quantities of urate, glutathione, ascorbate, α-tocopherol, β-carotene and selenium (Richard et al., 1990; Quinlan et al., 1996; Metnitz et al., 1999).

Disturbed balance between oxidants and antioxidants seems to play an important role especially in the patients with ALI due to SIRS. When these patients suffer from more prominent oxidative stress, they will more likely progress to multiple organ failure and eventually die (Cowley et al., 1996; Motoyama et al., 2003). Although there has been much debate whether this observation is a cause or a consequence of critical illness, antioxidant supplementation might be an alternative therapeutic approach.

2.2.2.3. Use of antioxidants for treatment of ALI in clinical trials

Given that ALI patients suffer from increased production of oxidants and depletion of antioxidants, treatment with antioxidants might restore the lost balance. N-acetylcysteine and procysteine were shown to replete intracellular antioxidant glutathione. After encouraging results from animal experiments (Bernard et al., 1984), these antioxidants were tested in several clinical trials. Although some of the studies showed improved systemic oxygenation and reduced need for ventilatory support, none of the trials showed mortality benefit (Cepkova and Matthay, 2006).

2.2.3. CD73

Purines are powerful signalling molecules both in normal conditions and in inflammation. CD73 (ecto-5'-nucleotidase, 5'NT) is an endothelial-surface expressed glycosyl phosphatidylinositol-linked, membrane-bound ectoenzyme (Zimmermann, 1992). It has been shown that CD73 exists as an endothelium-bound and as a soluble enzyme detectable in circulation. The soluble enzyme is released from endothelial surface upon shear stress (Yegutkin et al., 2000). CD73 controls the balance between

pro- and anti-inflammatory purines (Hunsucker et al., 2005). Adenosine 5'-triphosphate (ATP) is continuously released into extracellular space and this release increases significantly during inflammation. ATP is converted into adenosine 5'-diphosphate (ADP) and further to adenosine 5'-monophosphate (AMP) by CD39 (ecto-apyrase, NTPDase). CD73 catalyzes dephosphorylation of AMP to adenosine (Figure 3.). While ATP and ADP have pro-inflammatory and pro-thrombotic effects, adenosine is a potent anti-inflammatory molecule (Di Virgilio et al., 2001). Adenosine generated by endothelial CD73 binds to G-protein coupled adenosine receptors. There are four different types of adenosine receptors: A_1, A_{2A}, A_{2B} and A_3 (Linden, 2001). These receptors are expressed on different cells and function via distinct intracellular signaling. Taken together, CD73 regulates many physiological responses by extracellular generation of adenosine and subsequent activation of adenosine receptors.

2.2.3.1. The role of CD73 in normal conditions

The knowledge from the earlier studies suggesting that platelet function is regulated by purinergic signaling was expanded using CD73 deficient animals (Koszalka et al., 2004). Although intrinsic platelet function of CD73 knock-out mice studied ex vivo seems to be identical with that of wild-type animals, in vivo studies revealed several differences. Platelet cyclic adenosine monophosphate (cAMP) levels are lower in CD73 deficient mice due to lower levels of plasma adenosine and subsequent decreased activation of adenosine receptors on platelets. This platelet abnormality observed in CD73 knock-out mice is associated with reduction of bleeding time after tail tip resection and vessel occlusion induced by free radical injury (Koszalka et al., 2004).

Adenosine represents one of the crucial regulators of glomerular filtration by mediating message between macula densa and underlying smooth muscle cells. A study in CD73 deficient animals showed that under normal conditions, there is no difference in renal function between these animals and their wild-type controls. However, CD73 knock-out mice challenged with increased tubular perfusion flow had significantly lower superficial nephron glomerular filtration rates. Moreover, CD73 deficient animals had almost no residual feedback response during prolonged perfusion of the loop of Henle (Castrop et al., 2004).

Epithelial cells in the lungs and intestine actively transport water and ions in order to maintain the epithelial surface hydrated. Adenosine was shown to activate electrogenic chloride transport and fluid secretion (Gamba, 2005). Measurements in the abovementioned mucosal organs revealed high CD73 activity (Thompson et al., 2004). However, a direct proof of the importance of CD73 activity in ion transport and hydration of mucosal surfaces of the lungs and intestine is still missing.

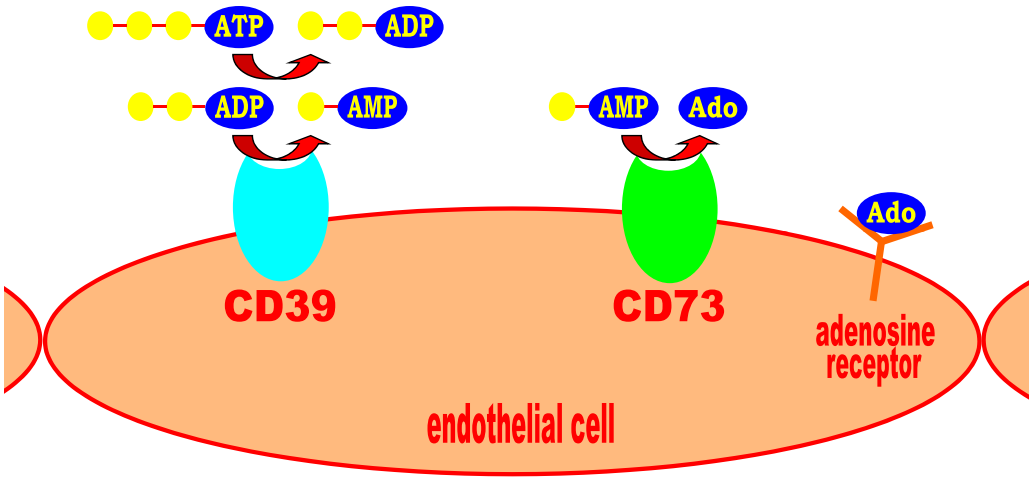

Figure 3. Adenosine generation on endothelial surface
The figure shows extracellular adenosine production. Extracellular ATP and ADP are hydrolyzed by CD39 to AMP. CD73 hydrolyzes AMP generating adenosine, which binds to G-protein coupled adenosine receptors.

CD73 regulates different types of tissue barriers. For instance, intestinal barrier function is CD73-dependent. Specific inhibitor of CD73 administered orally increased intestinal permeability (Synnestvedt et al., 2002). However, the importance of CD73 in the regulation of endothelial barrier function seems to have far more physiological importance. The first mention of the role of CD73 in the regulation of endothelial permeability was suggested almost 10 years ago. Neutrophil-derived AMP was shown to be converted by CD73 to adenosine, which subsequently decreased paracellular permeability. Inhibition of CD73 by enzyme inhibitor or monoclonal antibody resulted in 85% increase of endothelial permeability (Lennon et al., 1998). This concept was later refined by identification of neutrophils as the source of ATP, which is converted to AMP by CD39 (Eltzschig et al., 2004). Thus, adenosine production on endothelial surface is a result of the coordinated phosphohydrolysis of purine nucleotides by CD39 and CD73 (Eltzschig et al., 2003). The original in vitro findings have been confirmed by studies in CD73-deficient animals (Eltzschig et al., 2004; Thompson et al., 2004).

2.2.3.2. The role of CD73 in disease

Studies in human volunteers and in animals performed already two decades ago demonstrated that hypoxia induces increase of endogenous adenosine production

(Gnaiger, 2001; O'Farrell, 2001). One of the possible explanations of amplified need of adenosine is the vasodilatory property of adenosine. Activation of A_1 adenosine receptors results in an increase of blood flow to hypoxic tissue (Bryan and Marshall, 1999). Another reason for increased adenosine production in hypoxia is the need of limitation of inflammatory response.

Augmented adenosine production in hypoxia is attributable to CD73 activity (Kobayashi et al., 2000; Synnestvedt et al., 2002; Eltzschig et al., 2003; Ledoux et al., 2003; Thompson et al., 2004). There are two known mechanisms of CD73 up-regulation. First, hypoxia causes transcriptional induction of CD73 via hypoxia-inducible factor-1 (Synnestvedt et al., 2002). Increased adenosine production by CD73 activates A_{2A} or A_{2B} receptors and thus increases intracellular cAMP. Since CD73 gene promoter contains cAMP responsive element, the product of CD73-catalyzed reaction probably adds to transcriptional up-regulation of CD73 (Hansen et al., 1995).

Hypoxia causes increase in vascular permeability with subsequent extravasation of protein-rich liquid and neutrophils. Vascular leakage induced by hypoxia is emphasized upon inhibition of CD73 and in CD73 deficient animals. Although vascular leakage after hypoxia was present also in the heart, intestine and kidneys of CD73 deficient mice, the most significant changes were observed in the lungs (Thompson et al., 2004; Eltzschig et al., 2004).

Adenosine has been long known to exert protective effect in ischemic myocardium via binding to adenosine receptors on several cell types, including cardiomyocytes, endothelium and immune cells (Eltzschig et al., 2003). Moreover, adenosine can induce tolerance to ischemia in myocardium by a mechanism known as preconditioning (de Jong et al., 2000; Headrick et al., 2003). CD73 is a major source of extracellular adenosine production in the heart and was shown to have an important role in ischemic preconditioning (Koszalka et al., 2004; Eckle et al., 2007b). Adenosine reduces ischemia-reperfusion injury via activation of A_{2A} receptors on inflammatory cells (Linden, 2001).

In inflammation, activated neutrophils extravasate from circulation to the tissues. These cells are believed to play a major role in the development of inflammation-induced injury consisting of cell death and tissue edema. Adenosine reduces activation of neutrophils and thus prevents its potentially deleterious effects in the tissues (Cronstein et al., 1983). This effect is exerted via activation of adenosine receptors on neutrophils. Similar effects have been observed when the receptors were activated by agonists, which are being intensively studied for their therapeutic potential (Rosengren et al., 1991; Mubagwa and Flameng, 2001; McCallion et al., 2004). The importance of endogenous adenosine generation in inflammation was recognized only recently using gene-targeted animals (Koszalka et al., 2004; Eltzschig et al., 2004; Thompson et al., 2004; Grenz et al., 2007; Eckle et al., 2007a; Eckle et al., 2007b; Hart et al., 2008).

Inflammation is accompanied by extracellular release of adenine nucleotides. Endothelial cells, activated neutrophils, platelets and dead cells all contribute to the nucleotide release. ATP, ADP and AMP are readily metabolized by CD39 and CD73 into adenosine. This mechanism abolishes excessive accumulation of neutrophils in the inflamed tissues and prevents excessive tissue injury (Thompson et al., 2004; Eltzschig et al., 2004; Guckelberger et al., 2004; Kohler et al., 2007; Grenz et al., 2007). Another

mechanism of CD73-mediated immunosuppression is adenosine generation on the surface of regulatory T cells (Deaglio et al., 2007).

The potential role of CD73 in the response of host to microbial infection has only been recognized recently. Intestinal epithelial cells damaged by bacterial infection release ATP, which is metabolized by CD39 and CD73. Adenosine produced in this reaction increases fluid secretion by epithelium and produces diarrhea (Crane et al., 2002). Viral infection of endothelial cells was shown to increase both expression and activity of CD73 (Kas-Deelen et al., 2001). One of the possible mechanisms behind CD73 induction in viral infection is release on pro-inflammatory cytokines by the infected cells. One of these cytokines, interferon-α (IFN-α), has been shown to induce CD73 activity and adenosine production in vivo (Niemela et al., 2004).

2.2.3.3. Therapeutic potential of CD73

Beneficial effects of adenosine signaling in numerous diseases make it an attractive candidate for therapeutical use. However, administration of adenosine produces severe side effects, such as hypotension and arrhythmias, which limit its use in clinical practice. Adenosine receptor agonists and antagonists represent another set of potential drugs. In this case, specificity and pharmacodynamics have been serious obstacles (Linden, 2001). Manipulation of endogenous adenosine generation is an approach which has a great therapeutic potential. Increased extracellular adenosine production was shown in the studies in which methotrexate and sulfasalazine were used (Morabito et al., 1998). Yet another approach is exogenous administration of CD73, which was used to dampen tissue damage in hypoxia-induced inflammation (Thompson et al., 2004; Eltzschig et al., 2004).

2.2.4. Vascular adhesion protein-1 (VAP-1)

Leukocyte trafficking to the tissues belongs to the most important mechanisms enabling immunosurveilance under normal conditions and immune response in inflammation (von Andrian and Mempel, 2003; Muller, 2003). Both extravasation of lymphocytes to the secondary lymph organs in search for non-self antigens and emigration of activated lymphocytes and granulocytes to inflamed peripheral tissues occurs through multistep adhesion cascade (Springer, 1994). Leukocyte extravasation is mediated by adhesion molecules on leukocytes and endothelial cells. During the first phase of emigration cascade, endothelial selectins and their carbohydrate ligands on leukocyte surface enable tethering and rolling of leukocytes on endothelium. In the next step, chemoattractans and their serpentine receptors mediate shear-resistant adhesion of leukocytes. Finally, firm adhesion and transmigration of leukocytes through endothelium is dependent on leukocyte integrins and endothelial members of immunoglobulin superfamily. The abovementioned traditional adhesion molecules explain only partially the complex process of leukocyte extravasation. The concept is continuously being improved by discovery of additional molecules implicated in leukocyte emigration cascade (Salmi and Jalkanen, 2005; Ley et al., 2007).

Ecto-enzymes represent a relatively new group of non-classical adhesion molecules. These enzymes expressed on endothelial or leukocyte surface have their catalytic domains outside of the cell membrane (Salmi and Jalkanen, 2005). Most of ecto-

enzymes control leukocyte extravasation by their enzymatic activity, but some of them function also physically as adhesion molecules. Nucleotidases, ADP-ribosyl cyclases, ADP-ribosyl transferases, peptidases, proteases and oxidases are some of the ecto-enzymes known to control leukocyte trafficking (Salmi and Jalkanen, 2005). VAP-1 belongs to ecto-oxidases.

2.2.4.1. Characterization of VAP-1

VAP-1 was discovered using an antibody made against purified synovial vessels from arthritis patient (Salmi and Jalkanen, 1992). This antibody reduced binding of peripheral blood lymphocytes to post-capillary high endothelial venules in frozen section adhesion assays and in flow chamber assays. Endothelial expression of VAP-1 is not limited to synovial vessels. VAP-1 was also found to be expressed on sinusoidal cells in the liver, small-caliber venules in numerous organs and high endothelial cells of lymphatic organs. Interestingly, VAP-1 is also expressed on non-endothelial cell types, such as adipocytes and smooth muscle cells (Salmi et al., 1993).

Cloning of VAP-1 demonstrated that it belongs to semicarbazide-sensitive mono-amine oxidases (SSAO) (Smith et al., 1998). SSAO catalyze oxidative deamination of primary amines producing the corresponding aldehyde, hydrogen peroxide and ammonium (Figure 4.). SSAO contain in their catalytic center copper and topa-quinone, a unique modification of tyrosine residue, as co-factors (Klinman and Mu, 1994; Jalkanen and Salmi, 2001). The reaction catalyzed by SSAO consists of two phases. During the reductive phase, transient Shiff-base is formed between the substrate and topa-quinone, which precedes aldehyde formation. SSAO is reoxidized during the subsequent oxidative phase, which is followed by the release of hydrogen peroxide and ammonium. Carbonyl-reactive substances, such as semi-carbazide and hydroxylamine, are inhibitors of SSAO activity (Klinman and Mu, 1994; Jalkanen and Salmi, 2001).

VAP-1 is stored in endothelial cells under normal conditions intracellularly. Inflammation leads to translocation of VAP-1 from intracellular stores to the luminal surface. In humans, inflammation-induced up-regulation of VAP-1 was shown in synovitis, inflammatory bowel diseases and skin inflammations (Salmi et al., 1993). The animals studies revealed that surface expression of VAP-1 is only present in inflamed tissues (Jaakkola et al., 2000). Hence, translocation of VAP-1 from intracellular stores to luminal surface of vasculature is specific for the sites of inflammation. However, liver and kidney were found to express VAP-1 differently in humans and in mice, which complicates extrapolation of the findings from murine models to the humans (Bono et al., 1999).

Serum SSAO activity was first detected four decades ago (Bergeret et al., 1957). Additional studies revealed that VAP-1 is the major source of SSAO activity in the circulation (Kurkijarvi et al., 2000). Under normal conditions, serum concentration of soluble VAP-1 in humans is 80 ng/ml (Kurkijarvi et al., 1998). This concentration does not change in numerous inflammatory conditions, but it was found to be elevated in diabetes and in certain liver diseases (Kurkijarvi et al., 2000; Salmi et al., 2002).

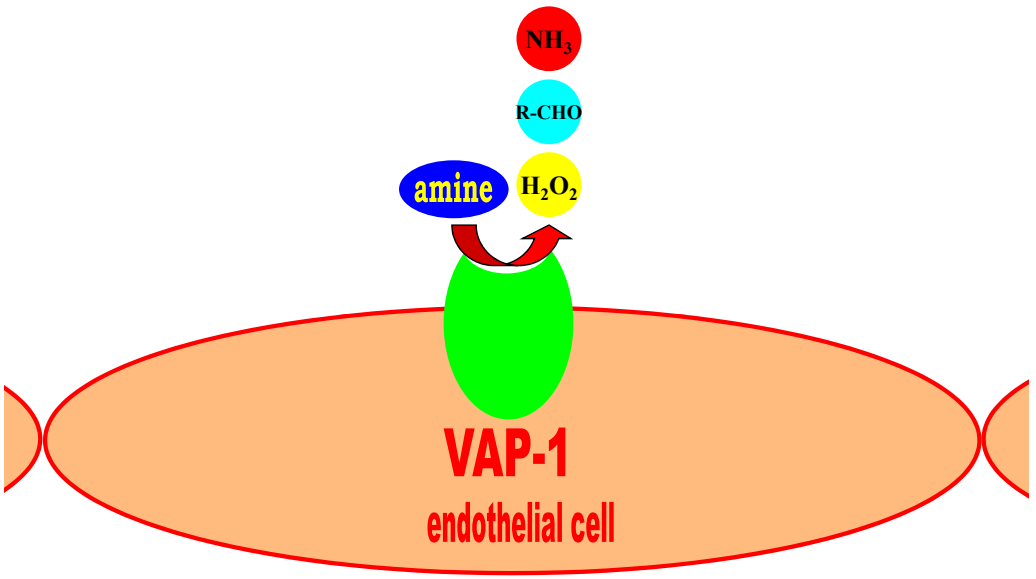

Figure 4. Enzymatic function of VAP-1.
The figure shows the reaction catalyzed by VAP-1, a cell-surface expressed SSAO. VAP-1 catalyzes oxidative deamination of primary amines with NH_3, aldehydes and H_2O_2 as final products.

2.2.4.2. In vitro studies of VAP-1

In vitro inhibition of VAP-1 adhesive activity by anti-VAP-1 monoclonal antibodies diminished leukocyte binding to inflamed vasculature in both lymphoid and non-lymphoid organs. The original observations of VAP-1 dependent leukocyte adhesion to frozen section were confirmed in chamber flow assays, which included defined shear stress. Besides firm adhesion, VAP-1 was shown in this study to play a role in rolling and transmigration of leukocytes through VAP-1 positive endothelial monolayer (Figure 5.) (Salmi and Jalkanen, 1992; Yoong et al., 1998; Lalor et al., 2002).Inhibition of enzymatic activity of VAP-1 by small molecular inhibitors produced in vitro similar reduction of leukocyte-endothelial interaction as use of anti-VAP-1 monoclonal antibodies (Salmi et al., 2001; Lalor et al., 2002; Koskinen et al., 2004). SSAO inhibitors interfered with all three steps of leukocyte extravasation. The importance of VAP-1 enzymatic activity for leukocyte emigration was confirmed using enzymatically inactive mutants (Koskinen et al., 2004). Anti-VAP-1 monoclonal antibodies have no effect on SSAO enzymatic activity and SSAO inhibitors do not alter the expression of antibody-defined epitopes of VAP-1 (Salmi et al., 2001; Koskinen et al., 2004; Bonder et al., 2005). Interestingly, blocking of VAP-1 with antibodies has no additive effect to

SSAO inhibition in leukocyte-endothelial interaction. Thus, VAP-1-mediated leukocyte adhesion to endothelium is believed to have two phases. Leukocyte binds during the first phase to the adhesive epitope recognized by antibodies. During the second phase, enzymatic reaction produces a covalent bond (Salmi and Jalkanen, 2005). Importantly, biologically active end-products of VAP-1 catalyzed reaction induce expression of other adhesion molecules, such as E- and P-selectins, intercellular adhesion molecule-1, and CXCL8 (Jalkanen et al., 2007; Lalor et al., 2007). This way, enzymatic activity of VAP-1 enhances leukocyte binding to endothelium.

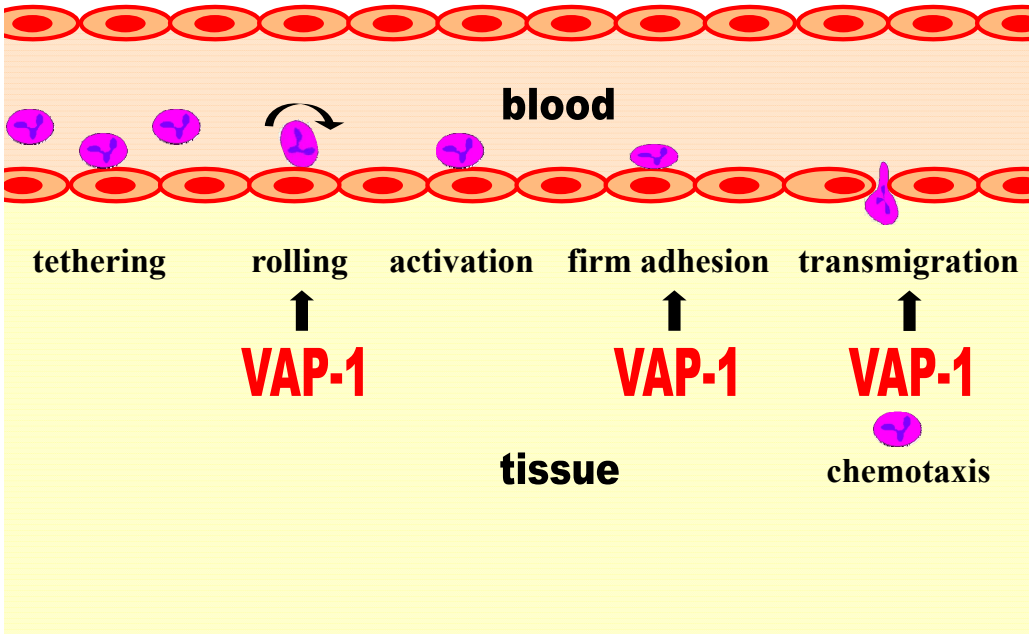

Figure 5. Extravasation cascade and VAP-1.
The figure summarizes the role of VAP-1 in extravasation cascade. VAP-1 is involved in leukocyte rolling, firm adhesion and transmigration from the luminal surface of the endothelium to the tissue.

2.2.4.3. Importance of VAP-1 in vivo

Leukocytes roll faster and the number of firmly adherent and extravasated leukocytes is reduced in inflamed vasculature in vivo after administration of anti-VAP-1 mAb or inhibitors of VAP-1 enzymatic activity. The importance of VAP-1 was first shown in vivo in the studies using anti-VAP antibodies. Inhibition of adhesive function of VAP-1 interfered with leukocyte trafficking in animal models of peritonitis, acute rejection of liver transplant and diabetes (Tohka et al., 2001; Martelius et al., 2004; Bonder et al., 2005; Merinen et al., 2005). Inhibition of VAP-1 enzymatic activity had anti-

inflammatory effect in sepsis, stroke, lung inflammation, colitis, experimental allergic encephalomyelitis, arthritis, skin inflammation and uveitis (Koskinen et al., 2004; Salter-Cid et al., 2005; Marttila-Ichihara et al., 2006; Xu et al., 2006b; Noda et al., 2007; O'Rourke et al., 2007a; O'Rourke et al., 2007b).

Generation of VAP-1 deficient animals confirmed the observations from wild-type mice treated with anti-VAP-1 antibodies and SSAO inhibitors. Gene targeting of VAP-1 resulted in an increase of leukocyte rolling velocity and a decrease of the number of firmly adherent and extravasated leukocytes in inflamed vasculature in vivo (Stolen et al., 2005). Peritonitis and synovitis in VAP-1 deficient mice were accompanied by significantly reduced leukocyte trafficking (Stolen et al., 2005; Marttila-Ichihara et al., 2006). Lymphocyte response after oral immunization was also compromised in the absence of VAP-1 (Koskinen et al., 2007).

Non-enzymatic glycation of proteins represents one of the mechanisms behind development of vascular diseases, such as atherosclerosis. Studies in mice over-expressing VAP-1 on endothelium revealed that VAP-1 enzymatic activity leads to increased generation of advanced glycation end-products, provided there is sufficient substrate for SSAO activity (Stolen et al., 2004). Moreover, these mice develop glucose intolerance and glomerulosclerosis later in life. Products of SSAO activity may contribute to the development of vascular complications. Aldehydes generated by VAP-1 enzymatic activity together with increased plasma glucose levels accelerate non-enzymatic protein glycation (Yu and Zuo, 1997) Hydrogen peroxide, another product of SSAO activity, adds to vascular damage by oxidation of proteins. In humans, plasma concentration of soluble VAP-1 seems to be inversely proportionate to insulin concentration. VAP-1 most likely contributes to the development of vasculopathies also by mediating infiltration of inflammatory cells to the vascular lesions, generation of advanced glycation products and production of oxidants (Salmi et al., 2002).

3. AIMS OF THE STUDY

The aims of the present study were to develop new treatment strategies for ALI by

I combining administration of antioxidants and the substrate for NOS

II up-regulating CD73 activity

III inhibiting enzymatic and adhesive function of VAP-1

4. MATERIALS AND METHODS

Materials and methods are described in more detail in the original publications.

Table 1. Characteristics of the cell cultures used for in vitro studies.

Cell type	Description	Source or reference	Used in
HDMEC	human dermal microvascular endothelial cells	freshly isolated in the laboratory	II
HPMEC	human pulmonary microvascular endothelial cells	ScienCell Research Laboratories, UK	II
HUVEC	human umbilical vein endothelial cells	freshly isolated in the laboratory	II

Table 2. Characteristics of the animals used for in vivo studies.
Male rats and sex-matched mice were used. All the animals used were weight- and age-matched.

animal strain	Description	Source or reference	Used in
mouse C57BL/6 WT	wild-type mice	Central Animal Laboratory, Turku University, Finland	II, III
mouse C57BL/6 $CD73^{-/-}$	CD73 deficient mice	Thompson et al, 2004	II
mouse 129S6 WT	wild-type mice	Central Animal Laboratory, Turku University, Finland	III
mouse 129S6 $VAP\text{-}1^{-/-}$	VAP-1 deficient mice	Stolen et at, 2005	III
mouse 129S6 mTIEhVAP-1 TG/$VAP\text{-}1^{-/-}$	mice deficient in mouse VAP-1 over-expressing human VAP-1 on endothelium	Stolen et at, 2004	III
rat sprague-dawley	wild-type rats	Central Animal Laboratory, Turku University, Finland	I

Table 3. Characteristics of the antibodies used for in vitro and in vivo studies.

Antibody	Antigen	Source or reference	Used in
4G4	human CD73	Airas et al, 1993	II
TK8-14	human VAP-1	Kurkijarvi et al, 2004	III
3G6	chicken T cells (neg. control)	Kurkijarvi et al, 2004	II, III
7-88	murine VAP	Merinen et al, 2005	III
7-106	murine VAP-1	Merinen et al, 2005	III
9B5	human CD44 (neg. control)	Jalkanen et al, 1986	III

Table 4. Materials.

Material	Description	Source	Used in
Ventilator			
neonatal ventilator	Minibird	Bird Company, USA	I
Anesthetics			
ketamine	Ketalar	Pfizer, USA	I, II, III
xylazine	Rompun	Orion, Finland	I, II, III
Therapeutic agents			
superoxid dismutase		Sigma, USA	I
catalase		Sigma, USA	I
L-arginine		Braun, Germany	I
recombinant mouse IFN-β		R&D Systems, USA	II
Avonex	recombinant human IFN-β	Biogen Idec, USA	II
BTT2052/ SZE5302	SSAO inhibitor	Marttila-Ichihara et al, 2006	III
Microscopy tools			
light microscope	BX-60	Olympus, Japan	I, II, III
digital camera	ColorView 12	Olympus, Japan	I, II, III
Detection tools			
rat TNF-α detection kit	ELISA	R&D Systems, USA	I
MPO assay kit	ELISA	HyCult, Netherlands	III
EIA reader	Spectra II	Wallac, Finland	I

Material	Description	Source	Used in
Detection tools			
spectrometer	Model 1409	Wallac, Finland	II
protein assay kit	BCA	Pierce, USA	II, III
fluorescence reader	Ultra	Tecan, Switzerland	II, III
fluorescence reader	Infinite	Tecan, Switzerland	II, III
Software			
MultiCalc Advanced	fitting software	PerkinElmer, USA	I
SAS Enterprise guide 3.0	statistical software	SAS Institute, USA	I, II, III
ImageJ	image processing software	freely available	I, II
Other			
glycerophosphate		Sigma, USA	II
AMP		Sigma, USA	II
[2-^{3}H]AMP	18.6 Ci/mmol	Amersham, U.K.	II
TLC sheets	Alugram SIL G/UV$_{254}$	Macherey-Nagel, Germany	II
Multiwell 96 inserts		BD Falcon, USA	II
FITC-conjugated dextran	70 kD	Molecular Probes	II
RPMI		Sigma, USA	II

4.1. ANIMAL INSTRUMENTATION (I, II, III)

The protocols were approved by the Committee on Animal Ethics of Turku University.

4.1.1. Rats (I)

Intramuscular injection of ketamine hydrochloride (110mg/kg of body weight) and xylazine (10 mg/kg of body weight) was used to induce anesthesia in rats. The depth of anesthesia was maintained by further intramuscular injections of ketamine and xylazine mixture. The body temperature of animals throughout the experiments was maintained using a heating lamp. Animals were mechanically ventilated via silicone tube inserted in the trachea with a mixture of room air and 25% O_2 using neonatal ventilator. Maximum inspiration pressure was 20cm of H_2O and the frequency was 30 breaths per minute. Venous catheter placed in the right femoral vein was used for fluid and drug administration. Intestinal ischemia was induced by placing a microvascular clamp on SMA at its aortic origin for 30 minutes. Ischemia phase was followed by 120 minutes

of reperfusion. Sham-operated rats underwent laparotomy and dissection of SMA only. This protocol is an established and reproducible ALI model (Vejchapipat et al., 2006).

4.1.2. Mice (II, III)

Anesthesia in mice was induced by intraperitoneal injection of ketamine hydrochloride (110mg/kg of body weight) and xylazine (10 mg/kg of body weight). The depth of anesthesia was maintained by additional intraperitoneal injections of ketamine and xylazine. The mice spontaneously ventilated room air. Fluid loss was compensated by subcutaneous injections of saline. SMA was dissected via midline laparotomy and occluded by microvascular clamp for 30 min. Sham animals underwent superior mesenteric artery dissection without vascular occlusion. The wound was sutured in one layer. The ischemia phase was followed by the release of the microvascular clamp and wound closure. Mice were sacrificed after 240 minutes and the tissue samples were collected. This protocol has been used previously to induce indirect ALI (Ohara et al., 2001).

4.2. GRADING OF INTESTINAL DAMAGE (I, II, III)

Paraffin-embedded samples of intestine were cut into 4 μm sections and stained with hematoxylin and eosin. Intestinal injury was scored according to original (I) or modified (II, III) Park's grading (Park et al., 1990). The original grading includes all degrees of intestinal IRI: grade 0, normal mucosa; grade 1, subepithelial space at the tips of the villi; grade 2, extended subepithelial space at the tips of the villi; grade 3, massive epithelial lifting down the sides of the villi; grade 4, villi denuded of epithelium; grade 5, loss of the villi; grade 6, injury of intestinal crypt layer; grade 7, necrosis of the entire intestinal mucosa; grade 8, transmural infarction. To simplify the scheme and include only the grades of injury we observed in our model, we modified the grading as follows: grade 0, normal mucosa; grade 1, subepithelial space and/or epithelial lifting in the villi; grade 2, villi denuded of epithelium, and grade 3, loss of the villi. In each slide, 5 randomly chosen fields of view under high magnification were graded.

4.3. MEASUREMENT OF LUNG NEUTROPHIL INFILTRATION (I, III)

To quantify the pulmonary neutrophil sequestration, we measured myeloperoxidase (MPO) concentration in the lungs using two different methods. We measured MPO activity (I) using previously described assay (Grisham et al., 1990). The lungs were excised after euthanasia and mechanically homogenized in a phosphate buffer. The solution was centrifuged twice (1500g at 4 °C for 15 minutes) to separate tissue debris. Hydrogen peroxide and 3.3'.5.5'-tetramethylbenzidine were added to the supernatant. Absorbance was measured by spectrophotometer at 655 nm. The concentration of MPO was calculated from the standard curve. The values were expressed per milligram of protein.

MPO concentration in the lungs was measured by ELISA (III). Animals were euthanized and lungs were perfused with 5 ml of saline through the right ventricle. Lungs were excised and mechanically homogenized in a buffer containing 200 mM NaCl, 5 mM EDTA, 10 mM tris, 10% glycine, 1 mM PMSF and 28 μg/ml aprotinin (pH 7.4). The solution was centrifuged twice (1500g at 4 °C for 15 minutes) to separate tissue debris. The supernatant was then assayed for MPO activity using a commercially available ELISA kit according to manufacturer's instructions. To normalize the values from different experimental groups, average lung MPO concentration in non-treated animals with intestinal IRI and ALI was assigned 100%.

4.4. LUNG TISSUE-AIR RATIO MEASUREMENT (I)

To assess the degree of alveolar wall thickening caused by edema and cellular infiltration, paraffin-embedded samples of lungs were cut into 4 μm sections and stained with hematoxylin and eosin. Images from the sections obtained by digital camera were analyzed by image processing software ImageJ. The program was used according to manufacturers' instructions to calculate tissue-air ratio in lungs.

4.5. LUNG WET-DRY RATIO MEASUREMENT (I)

To assess the lung vascular leakage, we measured lung wet-dry ratio. The lungs were removed and wet weight recorded. Lungs were afterwards placed in the oven at 70°C and dry weight was determined after 24 hours.

4.6. LUNG AIRSPACE HEMORRHAGE MEASUREMENT (I)

Airspace hemorrhage was graded as described previously (Tassiopoulos et al., 1997). Briefly, hematoxylin-eosin-stained lung sections from all animals were analyzed by a single blinded examiner and assigned one of the following grades of the extent of airspace hemorrhage: grade 0 - no changes, grade 1 - focal mild and subtle changes, grade 2 - multifocal mild changes, grade 3 - multifocal prominent changes, grade 4 - extensive prominent changes.

4.7. SERUM TNF-α MEASUREMENT (I)

Rat TNF-α concentration in serum was measured with ELISA according to manufacturer's instructions. Absorbance was read at 450 nm on an EIA reader with fitting statistical software to calculate the results.

4.8. IN VIVO ENDOTHELIAL PERMEABILITY MEASUREMENT (II)

In vivo endothelial permeability was measured by assessment of FITC-conjugated dextran extravasation. Mice were injected intravenously 70 kD FITC-conjugated dextran (25 mg/kg body weight in 0.2 ml sterile saline) 5 minutes prior to euthanasia. Lung tissue samples were collected after killing and snap frozen in liquid nitrogen.

Frozen lung samples were cut into 7 μm sections and observed in fluorescence microscope. Color images of three randomly chosen fields under high magnification obtained by digital camera were analyzed by image processing software ImageJ. The percentage of section area exhibiting fluorescence above an arbitrarily chosen background value was used to determine the extent of vascular leakage.

4.9. IN VITRO ENDOTHELIAL PERMEABILITY MEASUREMENT (II)

HPMEC were grown to confluency in Multiwell 96 inserts (8-μm pore size). The cells were treated 24 hours prior to the experiment with IFN-β (500 U/ml) or left untreated. AMP (50 μM) was added for the last 15 minutes to ensure the presence of CD73 substrate. 70 kD FITC-conjugated dextran (500 μg/ml) was applied to the upper wells. The flux of the labelled dextran to the bottom wells was measured kinetically using a fluorescence reader (Tecan Ultra).

4.10. CD73 ACTIVITY MEASUREMENT (II)

CD73 activity in the lung lysates and on the surface of HPMEC was assayed by thin-layer chromatography (TLC), as described previously (Yegutkin et al., 2001). Briefly, the analyzed samples consisted of lung lysate, RPMI 1640, 5 mmol/l β-glycerophosphate, and AMP with tracer [2–3H]AMP. Incubation times were chosen to ensure the linearity of the reaction with time, so that only up to 10% of the initially introduced substrate would be metabolized during the assay. Aliquots of the mixture were applied to Alugram SIL G/UV254 TLC sheets and separated with isobutanol/isoamyl alcohol/2-ethoxyethanol/ammonia/H_20 (9:6:18:9:15) as solvent. ^{3}H-labeled AMP and its dephosphorylated nucleoside derivatives were visualized in UV light and quantified using a Wallac-1409β spectrometer. CD73 activity was expressed as nmol AMP hydrolyzed by 1 mg protein/h. BCA Protein Assay Kit was used to determine the protein concentration in the lysates.

4.11. CD73 EXPRESSION MEASUREMENT (II)

Expression of CD73 on endothelial surface was studied by immunofluorescence analyses as previously described (Airas et al., 1993). EDTA-trypsin (5 mM) was used to detach HPMEC, which were subsequently incubated with mAb (5 x 10^5 cells per staining, mAb concentration 10 μg/ml). The cells were incubated either with 3G6 (negative control mAb) or 4G4 (anti-CD73 mAb) for 20 min at 4°C and washed twice. Thereafter, the cells were incubated for 20 min at 4°C with 1/100 diluted FITC-conjugated sheep anti-mouse-IgG mAb containing 5% AB serum and washed twice. Finally, the cells were fixed with 1% paraformaldehyde. All incubations and washes were performed with PBS. Flow cytometry was used to detect fluorescence. The mean fluorescence intensity was calculated by subtraction of the fluorescence intensity of the cells stained with negative control mAb from the fluorescence intensity of the cells stained with anti-CD73 mAb.

4.12. SSAO ACTIVITY MEASUREMENT (III)

Lung and abdominal fat samples were collected at the end of reperfusion. Tissue samples were cut into small pieces and lysed in an equal volume of lysis buffer (PBS and 0.2% Triton X-100). Enzymatic activity of VAP-1 was determined by measurement of hydrogen peroxide production using fluoropolarimetric assay, as described previously (Salmi et al., 2001).

4.13. STATISTICAL ANALYSIS (I, II, III)

Statistical analysis was performed using non-parametric one-way ANOVA (Kruskal-Wallis and Mann-Whitney U tests) and repeated measures for ANOVA (cell culture permeability assays). SAS Enterprise guide 3.0 was used to calculate exact p-values. $P<0.05$ was considered statistically significant. The results were expressed as mean±SEM.

5. RESULTS

5.1. INTESTINAL AND LUNG DAMAGE AFTER INTESTINAL ISCHEMIA

Ischemia-reperfusion (IR) of intestine may cause damage to all parts of intestinal wall, provided that the duration of ischemia and reperfusion periods is sufficiently long. In the rats (I), 30 minutes long ischemia and 120 minutes long reperfusion caused a 5.7-fold increase of intestinal damage when compared to the sham-operated animals ($p=0.0001$). In the mice (II, III), average grade of intestinal damage observed in the sham-operated animals was 0.4 (II) and 0.1 (III). Grade of intestinal injury was significantly increased by 30 minutes of intestinal ischemia and 240 minutes of reperfusion to 1.35 (II, $p=0.002$) and 2.1 (III, $p=0.007$).

The potential of intestinal IR to trigger SIRS with subsequent damage to remote organs is well established. Both in the rats (I) and in the mice (II, III), we confirmed the presence of lung injury by histological examination of HE-stained lung sections from the pilot animals. Intestinal IR caused in the rats besides the morphological changes also significant 1.9-fold increase of lung wet-dry ratio when compared to sham-operated animals (I, $p=0.0001$). In the mice, we observed a 5.25-fold increase of lung vascular leakage (II, $p=0.02$) and 2.3-fold increase of lung neutrophil infiltration (III, $p=0.002$).

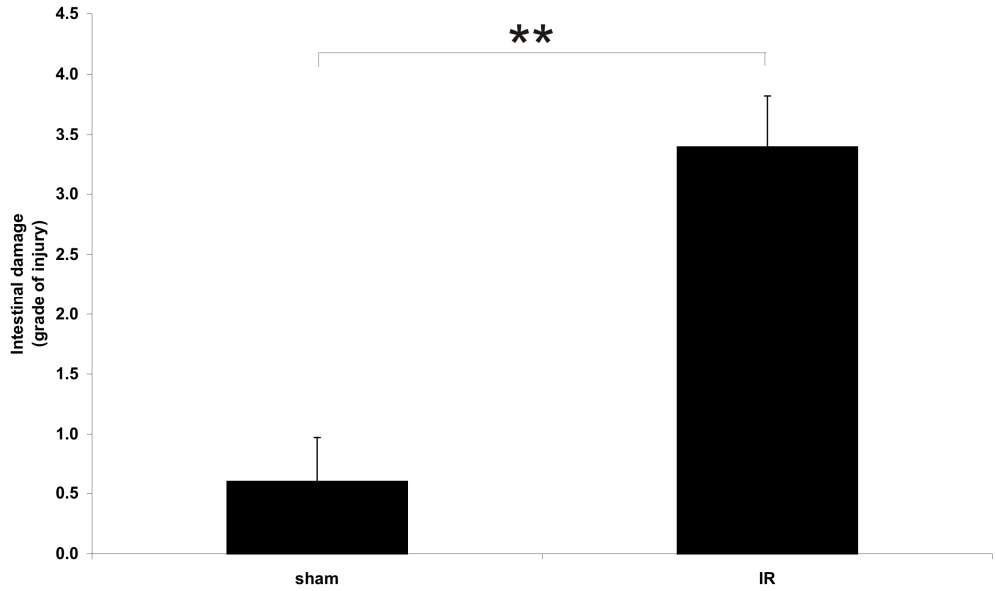

Figure 6. Intestinal IR injury in rats.
Sprague-dawley rats underwent sham operation (n=10) or 30 minutes long intestinal ischemia and 120 minutes long reperfusion (n=10). Intestinal damage was semiquantitatively graded according to the original Park's scheme and expressed as mean±SEM. ** $p<0.01$

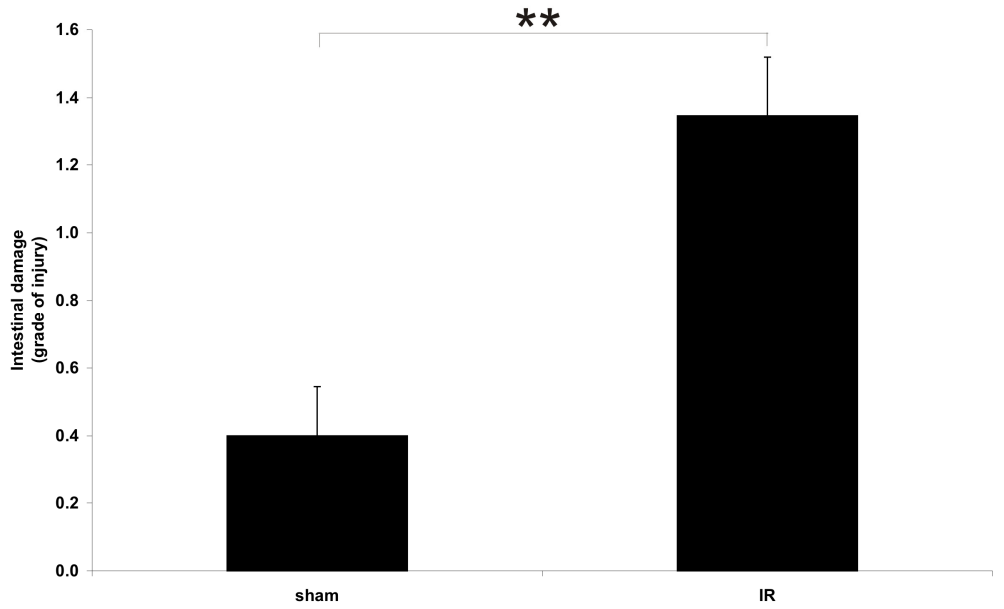

Figure 7. Intestinal IRI in C57BL/6 mice.
C57BL/6 mice underwent sham operation (n=7) or 30 minutes long intestinal ischemia and 240 minutes long reperfusion (n=15). Intestinal damage was semiquantitatively graded according to the modified Park's scheme and expressed as mean±SEM.
** $p<0.01$

5.2. COMBINATION OF NO DONOR AND ANTIOXIDANTS IN ALI

5.2.1. Supplementation of arginine reduces lung damage in ALI

NO has several protective effects in ALI. Depletion of NO in ALI can only be compensated by increased activity of NOS with greater demand for arginine, which is its substrate. In our model, administration of arginine reduced lung wet-dry ratio by 3% ($p=0.03$), MPO concentration by more than 50% (p non-significant), tissue-air ratio by more than 50% ($p<0.001$), and degree of air-space hemorrhage by 60% (p non-significant). In conclusion, increasing availability of NOS substrate reduced lung damage after intestinal IR.

5.2.2. Administration of antioxidants protects from ALI

ALI is accompanied by massive increase of oxygen and nitrogen radical formation in the lungs. We found that antioxidant administration decreased lung wet-dry ratio by 6% ($p=0.03$), MPO concentration by 70% ($p=0.002$), tissue-air ratio by almost 40% ($p=0.001$) and degree of air-space hemorrhage by more than 70% ($p=0.03$). We conclude that supplementation of oxygen radical scavengers attenuated lung injury after intestinal IR.

5.2.3. Combination of arginine and antioxidants abolishes positive effects of each treatment and enhances systemic inflammation

When we compared the animals treated by arginine and antioxidants with non-treated controls, we found that the treatment had no effect on any of the measured parameters of lung damage. However, concomitant administration of arginine and antioxidants lead to highly significant 36-fold increase in plasma TNF-α concentration ($p=0.001$).

In conclusion, NO donor and antioxidants reduced lung damage after intestinal IR when administered alone. Concomitant administration of arginine and antioxidants abolished positive effects of each of these treatments. Morever, the combination treatment enhanced systemic inflammatory response.

5.3. THE ROLE OF CD73 IN ALI

5.3.1. CD73 has a critical role in intestinal IRI and ALI

After sham operation, both CD73 deficient and WT mice had similarly low grade of intestinal damage. IR caused significant increase in damage in both groups. However, the increase in CD73 deficient mice was more than twice as dramatic as in the WT animals. What more, there was a significant difference between the intestinal damage observed in CD73 deficient and WT mice ($p=0.03$).

Quantification of pulmonary extravasation of intravenously administered FITC-labeled dextran showed that the leakage in sham-operated CD73 deficient mice was slightly increased when compared to their WT controls (statistically non-significant difference). Pulmonary vascular leakage increased significantly in both WT and CD73 deficient mice after intestinal IRI. However, ALI in CD73 deficient mice was associated with 80% more pulmonary leakage than their WT littermates ($p=0.03$). This finding underlines the importance of CD73 in development of indirect ALI.

5.3.2. IFN-β administration prevents and treats acute lung injury

Marginal pulmonary leakage observed in WT mice undergoing sham operation was increased over 5-fold in our model of indirect ALI ($p=0.005$). Importantly, IFN-β preventive treatment reduced the leakage in the lungs of WT mice by more than 90% when compared to nontreated littermates ($p=0.0001$). The vascular leakage in IFN-β pre-treated mice was similar to sham-operated animals. The effect of IFN-β pretreatment on the lung damage is not mediated by reduction of primary organ injury, since there was no significant difference in intestinal IRI in the IFN-β treated and non-treated animals. Therefore, IFN-β seems to reduce pulmonary endothelial permeability by acting directly in the lungs.

A single dose of IFN-β administered intravenously at the beginning of reperfusion period promoted vascular barrier function as efficiently as a three-day pre-treatment. FITC-labeled dextran extravasation was reduced by 90±9 % in this group when compared to the controls ($p<0.001$). Therefore, IFN-β diminishes vascular leakage in indirect ALI also when administered after the onset of the disease.

5.3.3. Positive effect of IFN-β administration is CD73-dependent

While lung leakage was completely prevented in WT mice suffering from ALI, CD73 deficient mice did not respond to treatment at all. In non-treated animals, there was only less than two-fold difference in vascular leakage between CD73 deficient and WT mice. After IFN-β treatment the difference became over 16-fold. This evidence strongly points towards CD73 dependency of the protective effect of IFN-β.

5.3.4. IFN-β functions via CD73 up-regulation

In vitro screening for inducers of CD73 on endothelial surface identified IFN-β as a potential candidate. CD73 activity in the lungs of WT mice receiving IFN-β pretreatment for 3 days prior to induction of ALI caused a 2.3-fold increase ($p=0.002$). Importantly, the dose used was the same as in the treatment of multiple sclerosis. Next, we found that expression of CD73 by HPMEC increased more than 2-fold after a 24 h IFN-β treatment. CD73 activity in HPMEC was also augmented ($p=0.049$). CD73 was also inducible on other endothelial cell types such as HUVEC and HDMEC by IFN-β treatment. For instance, on HUVEC both the surface expression (51±14 % increase in specific MFI for CD73, mean±SEM, $n=7$, $p=0.02$) and catalytic activity (94±9 % increase in CD73 activity mean±SEM, $n=3$, $p<0.05$) were induced with 1000 U/ml of IFN-β when compared to non-stimulated cells. IFN-β treatment decreased HPMEC permeability by almost 40% when compared to non-treated control ($p=0.005$). Thus, IFN-β treatment increases CD73 expression and activity and inhibits leakage also in human pulmonary endothelial cells.

5.4. THE ROLE OF VAP-1 IN ALI

5.4.1. VAP-1 expression in WT, gene-modified mice and humans

Expression of VAP-1 on the pulmonary capillaries and larger vessels of WT mice is irregular and weak. Pulmonary vessels in $VAP1^{-/-}$ mice are completely devoid of VAP-1 expression. Several vessels in the lungs of transgenic mice and all the pulmonary vessels in humans are strongly VAP-1 positive.

Intestinal vasculature and smooth muscle layer in WT mice are weakly VAP-1 positive. $VAP\text{-}1^{-/-}$ mice do not express VAP-1 on any structures within intestine. In contrast, transgenic mice have strongly VAP-1-positive intestinal vessels but negative smooth muscle layer. Intestinal vasculature and smooth muscle cells in humans are strongly VAP-1 positive.

5.4.2. VAP-1 is crucial in the development of intestinal IRI and indirect ALI

Intestinal IR resulted in the destruction of intestinal villi and inflammation of the intestinal crypts. Grading of intestinal damage revealed that VAP-1 deficiency was associated with almost 40% reduction of tissue injury ($p=0.02$). Moreover, VAP-1 deficient mice had almost 40% less lung damage when compared to the WT controls ($p=0.02$). These data identify VAP-1 as a crucial molecule in the development of both IRI and ALI by using genetic targeting of the molecule.

5.4.3. Enzymatic activity of murine VAP-1 contributes to the development of intestinal IRI and indirect ALI

Intestinal damage increased more than 18-fold after IR when compared to the sham-operated animals (p=0.007). SSAO inhibitor-treated mice had intestinal damage decreased by almost 80% when compared to the vehicle-treated controls (p=0.03). Administration of anti-VAP-1 mAb had no significant effect on intestinal IRI when compared to non-specific mAb-treated controls. Therefore, enzymatic activity of VAP-1 mediates the development of intestinal IRI in WT mice.

Intestinal IRI caused 130% increase in lung damage when compared to sham-operated animals (p=0.002). Administration of SSAO inhibitor lead to over 30% reduction of lung neutrophil infiltration when compared to the vehicle-treated controls (p=0.03). Use of anti-VAP-1 mAb had no effect on lung damage. Thus, catalytic activity of VAP-1 mediates the development of indirect ALI in WT mice.

5.4.4. IRI and ALI do not induce VAP-1 activity

SSAO activity in the tissues of VAP-1-deficient mice was undetectable, which suggests that VAP-1 is the major source of SSAO activity both in abdominal fat and in the lungs. Neither IRI nor ALI lead to induction of VAP-1 activity in the tissues when compared to sham-operated animals. VAP-1 catalytic activity was decreased by 60% in the abdominal fat and by over 80% in the lungs after administration of SSAO inhibitor. Administration of anti-VAP-1 mAb did not affect VAP-1 enzymatic activity in the tissues. Therefore, neither IRI nor ALI induces the catalytic activity of VAP-1.

5.4.5. VAP-1 mediates intestinal IRI and indirect ALI in humanized mice

Administration of SSAO inhibitor to the humanized VAP-1 mice resulted in almost 50% decrease in intestinal IRI (p=0.01). Administration of anti-VAP-1 mAb did not affect intestinal IRI. In indirect ALI, inhibition of enzymatic activity of human VAP-1 caused 30% decrease in lung damage when compared to the vehicle-treated controls (p=0.05) Interference with the adhesive function of VAP-1 using anti-VAP-1 mAb had no significant effect on the lung injury. In conclusion, enzymatic function of human VAP-1 mediates development of tissue damage in both IRI and ALI.

6. DISCUSSION

Indirect ALI is a consequence of SIRS. In experimental setting, there are numerous approaches to trigger SIRS and thus induce indirect ALI. Intravenous injection of LPS, similarly as cecal ligation and puncture, mimic sepsis-induced ALI (Chatterjee et al., 2007; Wu et al., 2007). Hemorrhage shock either alone or in combination with trauma represents another frequently used model of indirect ALI (Powers et al., 2003; Homma et al., 2005). Since SIRS is accompanied by compromised visceral perfusion, intestinal IRI is a well established and reproducible model of indirect ALI (Ohara et al., 2001). We decided to induce ALI by intestinal IRI, because this model allowed us to investigate the role of endothelial enzymes in both IRI and ALI.

We used two models of intestinal IRI with different lengths of reperfusion. 30 minutes long intestinal ischemia followed by 120 minutes long reperfusion in the rats produced similar morphological changes in the intestine as 30 minutes long ischemia followed by 240 minutes reperfusion. Loss of epithelial layer and loss of entire villi belonged to the most common findings. However, the lung changes after longer reperfusion period were more pronounced. While the increase of lung water content after 120 minutes of reperfusion was less than two-fold, 240 minutes of reperfusion allowed vascular leakage to increase over five-fold. Thus, intestinal IRI resulted in intestinal and lung damage in both cases.

In the first part of our study, we focused on the role of NOS in indirect ALI. Bioavailability of NO is decreased during ALI due to its peroxynitrite-generating reaction with superoxide (Stuart-Smith and Jeremy, 2001). Depletion of NO results in pulmonary vasoconstriction and enhanced adhesion of leukocytes to endothelium. NO donors have protective effects in ALI by increasing availability of NO in the pulmonary circulation (Sheridan et al., 1999; Chu et al., 2005). We challenged this concept in the rat model of intestinal IRI-induced ALI. We chose to administer NO intravenously. Administration of NO via inhalation was not suitable, because our aim was to improve both local changes in the lungs and attenuate the systemic inflammation. Inhaled NO was shown to be inactivated by haemoglobin in the lungs and not to have systemic effects (Rimar and Gillis, 1993).

In our model, increasing availability of NOS substrate improved all the measured parameters. Arginine administration reduced vascular leakage, as reflected by wet-dry ratio. Moreover, tissue-air ratio and intra-alveolar hemorrhage, which are parameters of pulmonary morphological damage, were significantly improved by increasing availability of NO. Interestingly, we observed a strong trend towards reduction of neutrophil infiltration after arginine administration when compared to non-treated controls. However, this 50% decrease was not statistically significant ($p=0.08$), although it most likely has a biological importance. We can conclude that intravenously administered NO donor attenuates lung damage in ALI.

Although pathophysiology of indirect ALI is still incompletely understood, one of the accepted mechanisms is a substantial increase in oxidative stress (Chabot et al., 1998). Granulocytes and endothelial cells in the lungs get activated during excessive inflammatory response. These activated cells are capable of generating large quantities of reactive oxygen species during ALI. In healthy individuals, the radicals are scavenged by endogenous antioxidants, for instance SOD, catalase and glutathione. In

ALI, increased production of reactive oxygen species results in depletion of antioxidants. These conditions facilitate accumulation of oxygen and nitrogen radicals in ALI patients (Lang et al., 2002). Interestingly, high degree of oxidant stress in ICU patients was found in several studies to be associated with decreased survival (Cowley et al., 1996; Motoyama et al., 2003).

Identification of increased oxidant stress in the critically ill patients were followed by numerous experimental studies. Administration of antioxidant significantly reduced pulmonary damage in ALI in different animal models (Frei, 1994). Our findings are in line with the previously published literature. Treatment with antioxidants decreased significantly all the measured parameters of lung damage. Wet-dry ratio was reduced by 6%, tissue-air ratio by 40%, neutrophil infiltration by 70% and intra-alveolar hemorrhage by more than 70%.

The beneficial effects of antioxidant administration in ALI were never confirmed in the clinical trials. Although antioxidants improved in some cases lung physiology of ALI patients, mortality in this patient group was not significantly decreased (Calfee and Matthay, 2007). Naturally, increased oxidant stress contributes only partially to the complex pathophysiology of ALI development. Therefore, a combination of antioxidants with another treatment might have synergic effect. Administration of NO donor was an especially attractive option, because its effects do not overlap with the effects of antioxidants. Intriguingly, we found that only a separate administration of antioxidants and arginine reduced lung damage in ALI. Simultaneous administration of scavengers with NO donor failed to improve any of the parameters of lung injury in our model of indirect ALI. Interestingly, this combination enhanced systemic inflammatory response resulting in an important increase of serum TNF-α. One possible explanation of our findings is that increased availability of NO lead to increased production of nitrogen reactive species and worsened already present oxidants stress. Another possibility is an interaction between the administered compounds resulting in inactivation of both of them. However, this unexpected effect of combining two beneficial treatments requires future studies.

Extravasation of protein-rich fluid with its deposition in pulmonary interstitial and alveolar space is a characteristic feature of ALI. Increased capillary permeability leads to development of pulmonary edema and it compromises alveolar-capillary barrier. Congested lungs perform insufficient gas exchange, which results in arterial hypoxemia refractory to supplementary oxygen therapy. The only treatment of ALI/ARDS that was shown to reduce mortality is based on ventilation strategies, which highlights the importance of alveolar-capillary barrier disruption for the outcome in this frequent syndrome (Ware and Matthay, 2000).

Endothelium is the main control point of macromolecular and fluid extravasation Therefore, the response of capillary endothelium to inflammatory cytokines might be a key moment in ALI development. Intestinal IR is an established model of ALI and is associated with high levels of circulating pro-inflammatory cytokines. Our in-vivo model of ALI induced 25% decrease in CD73 activity in the mouse lungs. CD73 is up-regulated in certain models of inflammation, such as hypoxia and ventilator-induced lung injury, as a part of innate anti-inflammatory response. Increased adenosine production by CD73 prevents uncontrolled inflammation with detrimental effects. Our findings suggest that systemic inflammation down-regulates adenosine production.

This down-regulation participates in the loss of balance between pro- and anti-inflammatory mechanisms, thus favoring development of inflammation-mediated damage.

CD73 has a crucial role in the regulation of endothelial barrier function. The control of endothelial permeability by CD73 is mediated by generation of adenosine, which binds to its receptors expressed on endothelial surface. CD73 has been shown to control pulmonary leakage in normal conditions, hypoxia-induced inflammation and ventilator-induced lung damage (Lennon et al., 1998; Thompson et al., 2004; Eckle et al., 2007). However, most of the critical patients suffering from respiratory dysfunction develop SIRS-induced indirect ALI. Intestinal IRI is a widely used experimental model of indirect ALI. This model has a clinical relevance, since the critical patients have often under-perfused splanchnic area resulting in non-occlusive intestinal ischemia. Occlusion of mesenteric artery by en embolus or a thrombus is often the cause of AMI. Majority of the patients with this diagnosis dies of MODS with ALI as one of its components. In our model, intestinal IRI-induced indirect ALI resulted in a significant increase of pulmonary vascular permeability in both WT and CD73 deficient mice. Importantly, CD73 deficient animals developed more pronounced endothelial barrier dysruption than their WT controls. Sham operated CD73 deficient mice had only mildly increased pulmonary leakage when compared to their WT littermates. Therefore, it can be concluded that the lack of CD73 is compensated by other mechanisms under normal conditions and the endothelial barrier function is maintained. However, after induction of SIRS, CD73-generated adenosine becomes a key player in the control of endothelial permeability in the lungs.

Direct lung injury caused by hypoxia or mechanical ventilation results in significant up-regulation of CD73 (Eckle et al., 2007; Eltzschig et al., 2004; Thompson et al., 2004). Thus, CD73 seems to form part of innate anti-inflammatory response. In contrast, SIRS induced indirect ALI caused 25% decrease in CD73 activity. In this case, pathological down-regulation of endogenous adenosine generation might be one of the mechanisms behind dysruption of endothelial barrier function. Exogenously administered CD73 prevents pulmonary vascular leakage via generation of adenosine, which subsequently activates A_{2B} adenosine receptors (Eckle et al., 2007). However, clinical use of CD73 purified from snake venom is not feasible without extensive clinical trials. On the contrary, IFN-β is a clinically used drug capable of increasing endogenous CD73 activity. IFN-β caused about two-fold induction of CD73 expression and activity on endothelium in the normal conditions. In ALI, IFN-β administration completely prevented the decrease of CD73 activity and led to 130% increase of enzyme activity . This way, even in a model of inflammation which otherwise down-regulates CD73 activity, we were able to induce pharmacologically a strong anti-inflammatory reaction. Increased adenosine availability could thus re-establish the balance between pro- and anti-inflammatory mechanisms attenuating the damage in several organs. To test whether IFN-β effect was CD73-dependent, we treated CD73 deficient animals. Strikingly, IFN-β had no beneficial effect in these animals. Thus, we conclude that IFN-β-mediated protection from vascular leakage in lungs during indirect ALI is CD73 dependent and most likely is mediated via adenosine signalling.

Although IFN-β influences numerous biologic functions, the most prominent effects of this cytokine reside in the modulation of immune system (Theofilopoulos et al.,

2005). IFN-β functions via binding to its receptors expessed on immune as well as non-immune cells, including endothelial cells (Indraccolo et al., 2007). Binding of IFN-β to the receptors changes expression of hundreds of genes and results in modulation of both innate and adaptive immune processes (Der et al., 1998). Administration of IFN-β in multiple sclerosis patients was found to enhance blood-brain barrier function (Stone et al., 1995). In animal models of stroke, IFN-β reduced the size of necrotic area, diminished inflammatory cell infiltration and prevented dysruption of blood-brain barrier (Liu et al., 2002 and Veldhuis et al., 2003). However, the molecular mechanism underlying the enhancement of endothelial barrier function by IFN-β has not been suggested previously. We are the first to identify induction of CD73 as a key control point of IFN-β-mediated reduction of vascular leakage. Interestingly, delayed administration of IFN-β enhanced endothelial barrier function as efficiently as when administered preventively. Therefore, our data shows that acute lung injury can be both prevented and treated by pharmacological induction of CD73. Our findings regarding inducibility of CD73 on several endothelial cell types by IFN-β suggest that this treatment could have similar effects in other syndromes associated with dysruption of endothelial barrier. Up-regulation of CD73 expression and activity on endothelial cells has been previously shown after treatment with IFN-α, which shares the receptor with IFN-β (Niemela et al., 2004). Notably, other tested cytokines did not up-regulate CD73 on endothelium. Therefore, induction of CD73 activity is a novel molecular mechanism behind IFN-β-mediated promotion of endothelial barrier function.

Increased leukocyte trafficking to the tissues suffering from IRI significantly contributes to the tissue damage. Studies performed in the mice deficient in the classical endothelial adhesion molecules have shown that lack of these molecules is associated with decreased tissue damage in IRI (Kakkar and Lefer, 2004). In the model of myocardial IRI, deficiency in P-selectin and E-selectin reduced the tissue damage by 40% (Jones et al., 2000). In the same model, genetic targeting of ICAM-1 resulted in 40% attenuation of tissue damage (Jones et al., 2000). In addition to the traditional adhesion molecules, adhesion cascade is controlled also by ectoenzymes expressed on endothelial surface (Salmi and Jalkanen, 2005). We provide the first genetic evidence that the tissue damage in IRI is reduced in VAP-1 deficiency. Genetic targeting of VAP-1 caused almost 40% decrease of tissue damage in the model of intestinal IRI. Therefore, VAP-1 seems to contribute to the development of IRI to a similar extent as the classical adhesion molecules.

Exaggerated inflammatory response accompanied by leukocyte infiltration mediates tissue damage in indirect ALI. In pre-clinical studies of ALI, neutrophil depletion resulted in improved outcome (Abraham et al., 2000). The critical patients with high neutrophil blood counts experience more pronounced respiratory dysfunction and have poor prognosis (Baughman et al., 1996; Azoulay et al., 2002). Genetic targeting of the classical adhesion molecules in ALI has been shown to be associated with decreased tissue damage (Kamochi et al., 1999). Some ectoenzymes expressed on endothelial surface also control development of ALI (Eckle et al., 2007a). Using the model of intestinal IRI-induced ALI, we found that VAP-1 deficiency diminishes lung damage by almost 40%. Our results are similar to the observations in selectin deficient animals. Lung injury in P-selectin deficient mice was reduced by 30% (Kamochi et al., 1999). Strikingly, deficiency in ICAM-1 decreased lung damage in ALI by 80% (Kamochi et

al., 1999). ICAM-1 is expressed on pulmonary endothelial surface much more than VAP-1 or P-selectin, which is probably responsible for its more important role in the development of ALI.

Recognition of VAP-1 as an important molecule in the development of IRI and indirect ALI led to further studies. In these studies, we separately investigated the contribution of each of the two functional modalities of VAP-1 to the tissue damage in IRI and ALI. Interestingly, inhibition of enzymatic activity of VAP-1 caused 80% reduction of tissue damage in IRI. Almost identical protection from IRI by SSAO inhibition has been shown in the model of stroke, where the tissue damage was diminished by 75% (Xu et al., 2006). Although inhibitors of VAP-1 catalytic activity used in both studies have been screened with panels of kinases and phosphatases, non-specific effects of these compounds cannot be excluded. In fact, the SSAO inhibitor we used in the treatment of IRI has been shown to inhibit together with VAP-1 also other amine oxidases. Genetic targeting is the only way to specifically inactivate the molecule of interest. Hence, superior protection from IRI by SSAO inhibitors might be a consequence of non-specific effects of inhibitors. A different reason for less effective reduction of tissue damage in IRI by genetic targeting might be a compensation for lacking molecule. The acute start of VAP-1 inactivation by SSAO inhibitors does not allow for a similar compensation. Interestingly, diabetic estrogen-treated ovariectomized female rats used in the stroke study were protected from IRI to a similar degree as the mice in our study. These results suggest that SSAO inhibition is a potent anti-inflammatory therapy even the subjects more susceptible to post-ischemic inflammation.

SSAO inhibitors attenuated pulmonary inflammation in indirect ALI by 30%. Although expression of VAP-1 on pulmonary endothelium was much higher in the humanized mice than in the wild-type animals, enzymatic inhibition of both murine and human VAP-1 resulted in a similar reduction of tissue damage. Earlier studies suggested that SSAO inhibition might be beneficial in LPS-induced pulmonary inflammation (Yu et al., 2006). 40% reduction of pulmonary inflammation offered by SSAO inhibitor used in this study might be partially attributed to a different model. The authors induced direct lung injury by instillation of LPS in the trachea, which models pneumonia. In contrast, we induced indirect ALI by intestinal IRI-triggered systemic inflammation. This model is more relevant for clinical practice, as most of the critical patients suffer from indirect ALI caused by systemic inflammation. Interestingly, contribution of VAP-1 to the pulmonary injury seems to be similar regardless of the model.

Use of anti-VAP-1 mAb has been shown to be beneficial in a number of models of inflammation. However, the potential of blocking adhesive function of VAP-1 in IRI and ALI remains unknown. We did not observe any significant effect of anti-adhesive therapy targeted at VAP-1 in the model of intestinal IRI. Neither WT nor humanized mice benefited from administration of anti-VAP-1 mAb in this model. Likewise, pulmonary injury was not diminished by use of anti-VAP-1 mAb in our model of indirect ALI. More potent anti-inflammatory effect of blocking adhesive function of VAP-1 in the earlier studies might stem from a different expression pattern of the molecule in different tissues. Also, VAP-1 has been shown to be inducible by inflammation in several inflammatory conditions. However, we found that IRI and ALI

do not induce VAP-1, which might explain the lack of effect of anti-VAP-1 anti-adhesive therapy in these models.

In both models of acute inflammation, we found enzymatic activity of VAP-1 to be more important than its mAb-inhibitable adhesive function in the development of tissue injury. One of the possible explanations for the more potent anti-inflammatory effects of SSAO inhibitors is their capability to freely diffuse to the tissues and the cells. Thus, inhibitors of VAP-1 enzymatic activity inhibit besides endothelial surface-expressed also intracellularly stored VAP-1. In contrast, anti-VAP-1 mAb only block the molecules expressed on the endothelium. Moreover, anti-VAP-1 mAb only block adhesive function of VAP-1 without interference with its enzymatic activity. SSAO inhibitors affect negatively both functional modalities of VAP-1, as inhibition of VAP-1 enzymatic activity interferes also with the adhesion cascade. Therefore, protective effects of SSAO inhibitors and no significant effects of anti-VAP-1 mAb in the models of IRI and ALI seem rational.

In conclusion, we provide the first genetic evidence of the crucial role of VAP-1 in IRI and in ALI. Our data show that it is the enzymatic activity of VAP-1 rather than its mAb inhibitable adhesive function, which contributes to the tissue injury in these clinically relevant models of acute inflammation. We also show that human VAP-1 in transgenic animals contributes to the development of tissue damage in IRI and ALI similarly to murine VAP-1. A new generation of highly selective SSAO inhibitors suitable for oral administration has become recently available. Future clinical trials will evaluate potential benefits of third-generation SSAO inhibitors in IRI and ALI.

Taken together, we have identified two novel treatment strategies for ALI. IFN-β-induced up-regulation of CD73 enhanced endothelial barrier function both in vitro and in vivo. Inhibition of VAP-1 enzymatic activity by small molecular SSAO inhibitor reduced lung neutrophil infiltration in ALI. Efficacy of both IFN-β and third-generation SSAO inhibitors can be readily tested in clinical trials. Moreover, we have recognized a potentially harmful combination of antioxidants and NO donor. Combining these treatments in indirect ALI augmented systemic inflammation, which could be fatal in critical patients.

7. CONCLUSIONS

ALI is a clinical syndrome of respiratory dysfunction of sudden onset accompanied by bilateral pulmonary infiltrates, which are not caused by left atrial hypertension. Mortality rate of the patients with acute lung injury remains despite the progress in intensive care medicine around 40%. Since neutrophil infiltration and extravasation of protein-rich liquid are the hallmarks of the first phase of acute lung injury, we decided to study endothelial enzymes capable of controlling these events.

First, we hypothesized that availability of NO can be increased more efficiently by administration of both arginine and antioxidants. Surprisingly, the substances had only beneficial effect in ALI when administer separately. Their combination abolished these positive effects and enhanced systemic inflammatory response. Second, we decided to study the role CD73-generated adenosine in indirect ALI. We observed that CD73 controls pulmonary vascular permeability in this model. Besides identifying CD73 as a crucial player in the development of vascular leakage in ALI, we also pharmacologically modulated its activity to reduce lung edema. Treatment with IFN-β prevented dysruption of endothelial barrier via induction of CD73 activity. Most importantly, IFN-β treatment was equally efficient in treatment of pulmonary vascular leakage when the disease had already started. Third, we tested whether VAP-1 mediates lung damage in indirect ALI. We showed that lung neutrophil infiltration is mediated mainly via enzymatic function of VAP-1.

In conclusion, we identified two novel strategies in treatment of ALI and one potentially dangerous combination of drugs beneficial when administered separately. We recommend to avoid combination of arginine and antioxidants in the treatment of ALI. IFN-β should be tested in a clinical trial for treatment of ALI and possibly other conditions accompanied by increased vascular permeability. Finally, we propose inhibition of enzymatic function of VAP-1 as a novel target in treatment of indirect ALI.

8. ACKNOWLEDGEMENTS

This study was carried out at MediCity Research Laboratory and Research Center for Applied and Preventive Cardiovascular Medicine of Turku University, and the Department of Surgery, Turku University Central Hospital, Turku, Finland during the years 2003-2008.

I am deeply indebted to my supervisor professor Sirpa Jalkanen for guidance during the work on my thesis. I am grateful for the opportunity to perform the studies and for the motivating conversations.

My second supervisor docent Marko Salmi is gratefully acknowledged for the invaluable help with designing and conducting this project. I would like to express gratitude for constant supervision and ability to overcome all the practical difficulties.

I owe my sincere gratitude to my third supervisor docent Timo Savunen for making it possible for me to do my doctoral thesis in Turku. I would also like to thank for teaching me the basics of heart surgery and making me feel in Finland at home.

I warmly thank the reviewers professor Risto Renkonen and docent Karl Lemström for encouragement and constructive criticism during the final preparation of this thesis.

I am deeply grateful to Gennady Yegutkin for introducing to me the fascinating world of purinergic signalling and being a good friend. I wish to thank Kaisa Auvinen for help with all the permeability assays, statistics and complex mathematical curve-fitting. I would also like to thank Pirjo Heinilä, Etta-Liisa Väänänen, Riikka Sjöroos, Sari Mäki and Laila Reunanen for invaluable technical help during all my experiments. I am grateful to professor Pekka Kääpä, MD, PhD, for continuous help and encouragement during the work on my first publication. I was fortunate to work with many talented and nice people in Leukocyte Trafficking research group in MediCity. I wish to thank all for creating a friendly work environment.

I am grateful to my "Finnish" friends in Turku for all the moments we shared. The time spent with the "surgery pig team" – Markus Malmberg and Tommi Vähäsilta and "PET pig team" – Ronald Borra, Alexandru Naum, Antti Viljanen and Mikko Järvisalo will never be forgotten. I owe my gratitude to Rafael Frias for all the lunch talks and the opportunity to try what teaching feels like. I would like to thank Ronald Sutter for the climbing and culinary fun and Eero Riissanen for the long dog-walking talks.

This work would not have been possible without the support of my friends from Slovakia. Although nowadays I see Peter Pihorna, Ivana Urbancikova, Milan Huzvar, Gabika Kukova and Vierka Miskovska only occasionally, it always feels like back in the days. I would also like to thank the "Histology crew" lead by professor Mikulas Pomfy for the great time I had during the first years of my research experience.

I owe my warmest love and gratitude to my mother Meli and my sisters Jana and Veronika for the support, guidance and love throughout my life. From the bottom of my heart I thank my love Elina for support and encouragement. I am grateful for each moment we share.

This study was financially supported by the the Finnish Cultural Foundation, CIMO Foundation, the Special Grant for Clinical Research (EVO) of Department of Surgery of Turku University Central Hospital, Gastroenterology Research Foundation, the University of Turku Foundation and the Research Foundation of Instrumentarium.

Freiburg, September 2008

9. REFERENCES

(1977) Conference Report: Mechanisms of Acute Respiratory Failure. *Am Rev Respir Dis* **115**:1071-1078.

(2000) Ventilation With Lower Tidal Volumes As Compared With Traditional Tidal Volumes for Acute Lung Injury and the Acute Respiratory Distress Syndrome. The Acute Respiratory Distress Syndrome Network. *N Engl J Med* **342**:1301-1308.

Abel SJ, Finney SJ, Brett SJ, Keogh BF, Morgan CJ and Evans TW (1998) Reduced Mortality in Association With the Acute Respiratory Distress Syndrome (ARDS). *Thorax* **53**:292-294.

Aberle DR, Wiener-Kronish JP, Webb WR and Matthay MA (1988) Hydrostatic Versus Increased Permeability Pulmonary Edema: Diagnosis Based on Radiographic Criteria in Critically Ill Patients. *Radiology* **168**:73-79.

Abraham E, Carmody A, Shenkar R and Arcaroli J (2000a) Neutrophils As Early Immunologic Effectors in Hemorrhage- or Endotoxemia-Induced Acute Lung Injury. *Am J Physiol Lung Cell Mol Physiol* **279**:L1137-L1145.

Abraham E, Matthay MA, Dinarello CA, Vincent JL, Cohen J, Opal SM, Glauser M, Parsons P, Fisher CJ, Jr. and Repine JE (2000) Consensus Conference Definitions for Sepsis, Septic Shock, Acute Lung Injury, and Acute Respiratory Distress Syndrome: Time for a Reevaluation. *Crit Care Med* **28**:232-235.

Adhikari NK, Burns KE, Friedrich JO, Granton JT, Cook DJ and Meade MO (2007) Effect of Nitric Oxide on Oxygenation and Mortality in Acute Lung Injury: Systematic Review and Meta-Analysis. *BMJ* **334**:779.

Agarwal R, Srinivas R, Nath A and Jindal SK (2007) Is the Mortality Higher in the Pulmonary Versus the Extrapulmonary ARDS? A Meta-Analysis. *Chest*.

Airas L, Salmi M and Jalkanen S (1993) Lymphocyte-Vascular Adhesion Protein-2 Is a Novel 70-KDa Molecule Involved in Lymphocyte Adhesion to Vascular Endothelium. *J Immunol* **151**:4228-4238.

Ashbaugh DG, Bigelow DB, Petty TL and Levine BE (1967) Acute Respiratory Distress in Adults. *Lancet* **2**:319-323.

Azoulay E, Darmon M, Delclaux C, Fieux F, Bornstain C, Moreau D, Attalah H, Le G, Jr. and Schlemmer B (2002) Deterioration of Previous Acute Lung Injury During Neutropenia Recovery. *Crit Care Med* **30**:781-786.

Bachofen M and Weibel ER (1974) Basic Pattern of Tissue Repair in Human Lungs Following Unspecific Injury. *Chest* **65**:Suppl-19S.

Bachofen M and Weibel ER (1977) Alterations of the Gas Exchange Apparatus in Adult Respiratory Insufficiency Associated With Septicemia. *Am Rev Respir Dis* **116**:589-615.

Bailey RW, Brengman ML, Fuh KC, Hamilton SR, Herlong HF and Bulkley GB (2000) Hemodynamic Pathogenesis of Ischemic Hepatic Injury Following Cardiogenic Shock/Resuscitation. *Shock* **14**:451-459.

Baldwin SR, Simon RH, Grum CM, Ketai LH, Boxer LA and Devall LJ (1986) Oxidant Activity in Expired Breath of Patients With Adult Respiratory Distress Syndrome. *Lancet* **1**:11-14.

Baue AE (2006) MOF, MODS, and SIRS: What Is in a Name or an Acronym? *Shock* **26**:438-449.

Baughman RP, Gunther KL, Rashkin MC, Keeton DA and Pattishall EN (1996) Changes in the Inflammatory Response of the Lung During Acute Respiratory Distress Syndrome: Prognostic Indicators. *Am J Respir Crit Care Med* **154**:76-81.

Bell RC, Coalson JJ, Smith JD and Johanson WG, Jr. (1983) Multiple Organ System Failure and Infection in Adult Respiratory Distress Syndrome. *Ann Intern Med* **99**:293-298.

Beloucif S and Payen D (1998) A European Survey of the Use of Inhaled Nitric Oxide in the ICU. Working Group on Inhaled NO in the ICU of the European Society of Intensive Care Medicine. *Intensive Care Med* **24**:864-877.

Bergeret B, Blaschko H and Hawes R (1957) Occurrence of an Amine Oxidase in Horse Serum. *Nature* **180**:1127-1128.

Bernard GR (2005) Acute Respiratory Distress Syndrome: a Historical Perspective. *Am J Respir Crit Care Med* **172**:798-806.

Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, LeGall JR, Morris A and Spragg R (1994) The American-European Consensus Conference on ARDS. Definitions, Mechanisms, Relevant Outcomes, and Clinical

Trial Coordination. *Am J Respir Crit Care Med* **149**:818-824.

Bernard GR, Lucht WD, Niedermeyer ME, Snapper JR, Ogletree ML and Brigham KL (1984) Effect of N-Acetylcysteine on the Pulmonary Response to Endotoxin in the Awake Sheep and Upon in Vitro Granulocyte Function. *J Clin Invest* **73**:1772-1784.

Bitterman PB (1992) Pathogenesis of Fibrosis in Acute Lung Injury. *Am J Med* **92**:39S-43S.

Bonder CS, Norman MU, Swain MG, Zbytnuik LD, Yamanouchi J, Santamaria P, Ajuebor M, Salmi M, Jalkanen S and Kubes P (2005) Rules of Recruitment for Th1 and Th2 Lymphocytes in Inflamed Liver: a Role for Alpha-4 Integrin and Vascular Adhesion Protein-1. *Immunity* **23**:153-163.

Bono P, Jalkanen S and Salmi M (1999) Mouse Vascular Adhesion Protein 1 Is a Sialoglycoprotein With Enzymatic Activity and Is Induced in Diabetic Insulitis. *Am J Pathol* **155**:1613-1624.

Bounous G (1982) Acute Necrosis of the Intestinal Mucosa. *Gastroenterology* **82**:1457-1467.

Bryan PT and Marshall JM (1999) Cellular Mechanisms by Which Adenosine Induces Vasodilatation in Rat Skeletal Muscle: Significance for Systemic Hypoxia. *J Physiol* **514 (Pt 1)**:163-175.

Bulkley GB, Kvietys PR, Perry MA and Granger DN (1983) Effects of Cardiac Tamponade on Colonic Hemodynamics and Oxygen Uptake. *Am J Physiol* **244**:G604-G612.

Calfee CS and Matthay MA (2007) Nonventilatory Treatments for Acute Lung Injury and ARDS. *Chest* **131**:913-920.

Carden DL and Granger DN (2000) Pathophysiology of Ischaemia-Reperfusion Injury. *J Pathol* **190**:255-266.

Castrop H, Huang Y, Hashimoto S, Mizel D, Hansen P, Theilig F, Bachmann S, Deng C, Briggs J and Schnermann J (2004) Impairment of Tubuloglomerular Feedback Regulation of GFR in Ecto-5'-Nucleotidase/CD73-Deficient Mice. *J Clin Invest* **114**:634-642.

Cepkova M and Matthay MA (2006) Pharmacotherapy of Acute Lung Injury and the Acute Respiratory Distress Syndrome. *J Intensive Care Med* **21**:119-143.

Chabot F, Mitchell JA, Gutteridge JM and Evans TW (1998) Reactive Oxygen Species in Acute Lung Injury. *Eur Respir J* **11**:745-757.

Chatterjee A, Dimitropoulou C, Drakopanayiotakis F, Antonova G, Snead C, Cannon J, Venema RC and Catravas JD (2007) Heat Shock Protein 90 Inhibitors Prolong Survival, Attenuate Inflammation, and Reduce Lung Injury in Murine Sepsis. *Am J Respir Crit Care Med* **176**:667-675.

Cheung AM, Tansey CM, Tomlinson G, az-Granados N, Matte A, Barr A, Mehta S, Mazer CD, Guest CB, Stewart TE, Al-Saidi F, Cooper AB, Cook D, Slutsky AS and Herridge MS (2006) Two-Year Outcomes, Health Care Use, and Costs of Survivors of Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* **174**:538-544.

Chu SJ, Lee TY, Yan HC, Lin SH and Li MH (2005) L-Arginine Prevents Air Embolism-Induced Acute Lung Injury in Rats. *Crit Care Med* **33**:2056-2060.

Corbridge TC, Wood LD, Crawford GP, Chudoba MJ, Yanos J and Sznajder JI (1990) Adverse Effects of Large Tidal Volume and Low PEEP in Canine Acid Aspiration. *Am Rev Respir Dis* **142**:311-315.

Cowley HC, Bacon PJ, Goode HF, Webster NR, Jones JG and Menon DK (1996) Plasma Antioxidant Potential in Severe Sepsis: a Comparison of Survivors and Nonsurvivors. *Crit Care Med* **24**:1179-1183.

Crane JK, Olson RA, Jones HM and Duffey ME (2002) Release of ATP During Host Cell Killing by Enteropathogenic E. Coli and Its Role As a Secretory Mediator. *Am J Physiol Gastrointest Liver Physiol* **283**:G74-G86.

Cronstein BN, Kramer SB, Weissmann G and Hirschhorn R (1983) Adenosine: a Physiological Modulator of Superoxide Anion Generation by Human Neutrophils. *J Exp Med* **158**:1160-1177.

Czerny M, Baumer H, Kilo J, Lassnigg A, Hamwi A, Vukovich T, Wolner E and Grimm M (2000) Inflammatory Response and Myocardial Injury Following Coronary Artery Bypass Grafting With or Without Cardiopulmonary Bypass. *Eur J Cardiothorac Surg* **17**:737-742.

de Jong JW, de JR, Keijzer E and Bradamante S (2000) The Role of Adenosine in Preconditioning. *Pharmacol Ther* **87**:141-149.

de Nigris F, Lerman A, Ignarro LJ, Williams-Ignarro S, Sica V, Baker AH, Lerman LO, Geng YJ and Napoli C (2003) Oxidation-Sensitive Mechanisms, Vascular Apoptosis and Atherosclerosis. *Trends Mol Med* **9**:351-359.

Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, Chen JF, Enjyoji K, Linden J, Oukka M, Kuchroo VK, Strom TB and Robson SC (2007) Adenosine Generation Catalyzed by CD39 and CD73 Expressed on Regulatory T Cells Mediates Immune Suppression. *J Exp Med* **204**:1257-1265.

Di Virgilio F, Chiozzi P, Ferrari D, Falzoni S, Sanz JM, Morelli A, Torboli M, Bolognesi G and Baricordi OR (2001) Nucleotide Receptors: an Emerging Family of Regulatory Molecules in Blood Cells. *Blood* **97**:587-600.

Doyle RL, Szaflarski N, Modin GW, Wiener-Kronish JP and Matthay MA (1995) Identification of Patients With Acute Lung Injury. Predictors of Mortality. *Am J Respir Crit Care Med* **152**:1818-1824.

Dreyfuss D, Soler P, Basset G and Saumon G (1988) High Inflation Pressure Pulmonary Edema. Respective Effects of High Airway Pressure, High Tidal Volume, and Positive End-Expiratory Pressure. *Am Rev Respir Dis* **137**:1159-1164.

Eckle T, Fullbier L, Wehrmann M, Khoury J, Mittelbronn M, Ibla J, Rosenberger P and Eltzschig HK (2007a) Identification of Ectonucleotidases CD39 and CD73 in Innate Protection During Acute Lung Injury. *J Immunol* **178**:8127-8137.

Eckle T, Krahn T, Grenz A, Kohler D, Mittelbronn M, Ledent C, Jacobson MA, Osswald H, Thompson LF, Unertl K and Eltzschig HK (2007b) Cardioprotection by Ecto-5'-Nucleotidase (CD73) and A2B Adenosine Receptors. *Circulation* **115**:1581-1590.

Elliott CG, Rasmusson BY, Crapo RO, Morris AH and Jensen RL (1987) Prediction of Pulmonary Function Abnormalities After Adult Respiratory Distress Syndrome (ARDS). *Am Rev Respir Dis* **135**:634-638.

Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjyoji K, Robson SC and Colgan SP (2003) Coordinated Adenine Nucleotide Phosphohydrolysis and Nucleoside Signaling in Posthypoxic Endothelium: Role of Ectonucleotidases and Adenosine A2B Receptors. *J Exp Med* **198**:783-796.

Eltzschig HK, Thompson LF, Karhausen J, Cotta RJ, Ibla JC, Robson SC and Colgan SP (2004) Endogenous Adenosine Produced During Hypoxia Attenuates Neutrophil Accumulation: Coordination by Extracellular Nucleotide Metabolism. *Blood* **104**:3986-3992.

Falke KJ, Pontoppidan H, Kumar A, Leith DE, Geffin B and Laver MB (1972) Ventilation With End-Expiratory Pressure in Acute Lung Disease. *J Clin Invest* **51**:2315-2323.

Fowler AA, Hamman RF, Good JT, Benson KN, Baird M, Eberle DJ, Petty TL and Hyers TM (1983) Adult Respiratory Distress Syndrome: Risk With Common Predispositions. *Ann Intern Med* **98**:593-597.

Frei B (1994) Reactive Oxygen Species and Antioxidant Vitamins: Mechanisms of Action. *Am J Med* **97**:5S-13S.

Frostell C, Fratacci MD, Wain JC, Jones R and Zapol WM (1991) Inhaled Nitric Oxide. A Selective Pulmonary Vasodilator Reversing Hypoxic Pulmonary Vasoconstriction. *Circulation* **83**:2038-2047.

Frostell CG, Blomqvist H, Hedenstierna G, Lundberg J and Zapol WM (1993) Inhaled Nitric Oxide Selectively Reverses Human Hypoxic Pulmonary Vasoconstriction Without Causing Systemic Vasodilation. *Anesthesiology* **78**:427-435.

Gamba G (2005) Molecular Physiology and Pathophysiology of Electroneutral Cation-Chloride Cotransporters. *Physiol Rev* **85**:423-493.

Gattinoni L, Bombino M, Pelosi P, Lissoni A, Pesenti A, Fumagalli R and Tagliabue M (1994) Lung Structure and Function in Different Stages of Severe Adult Respiratory Distress Syndrome. *JAMA* **271**:1772-1779.

Gessler P, Nebe T, Birle A, Mueller W and Kachel W (1996) A New Side Effect of Inhaled Nitric Oxide in Neonates and Infants With Pulmonary Hypertension: Functional Impairment of the Neutrophil Respiratory Burst. *Intensive Care Med* **22**:252-258.

Ghio AJ, Elliott CG, Crapo RO, Berlin SL and Jensen RL (1989) Impairment After Adult Respiratory Distress Syndrome. An Evaluation Based on American Thoracic Society Recommendations. *Am Rev Respir Dis* **139**:1158-1162.

Gnaiger E (2001) Bioenergetics at Low Oxygen: Dependence of Respiration and Phosphorylation

on Oxygen and Adenosine Diphosphate Supply. *Respir Physiol* **128**:277-297.

Granger DN (1988) Role of Xanthine Oxidase and Granulocytes in Ischemia-Reperfusion Injury. *Am J Physiol* **255**:H1269-H1275.

Greene KE, Wright JR, Steinberg KP, Ruzinski JT, Caldwell E, Wong WB, Hull W, Whitsett JA, Akino T, Kuroki Y, Nagae H, Hudson LD and Martin TR (1999) Serial Changes in Surfactant-Associated Proteins in Lung and Serum Before and After Onset of ARDS. *Am J Respir Crit Care Med* **160**:1843-1850.

Grenz A, Zhang H, Eckle T, Mittelbronn M, Wehrmann M, Kohle C, Kloor D, Thompson LF, Osswald H and Eltzschig HK (2007a) Protective Role of Ecto-5'-Nucleotidase (CD73) in Renal Ischemia. *J Am Soc Nephrol* **18**:833-845.

Grenz A, Zhang H, Hermes M, Eckle T, Klingel K, Huang DY, Muller CE, Robson SC, Osswald H and Eltzschig HK (2007) Contribution of E-NTPDase1 (CD39) to Renal Protection From Ischemia-Reperfusion Injury. *FASEB J* **21**:2863-2873.

Grisham MB, Benoit JN and Granger DN (1990) Assessment of Leukocyte Involvement During Ischemia and Reperfusion of Intestine. *Methods Enzymol* **186**:729-742.

Guckelberger O, Sun XF, Sevigny J, Imai M, Kaczmarek E, Enjyoji K, Kruskal JB and Robson SC (2004) Beneficial Effects of CD39/Ecto-Nucleoside Triphosphate Diphosphohydrolase-1 in Murine Intestinal Ischemia-Reperfusion Injury. *Thromb Haemost* **91**:576-586.

Guidet B, Aegerter P, Gauzit R, Meshaka P and Dreyfuss D (2005) Incidence and Impact of Organ Dysfunctions Associated With Sepsis. *Chest* **127**:942-951.

Gutteridge JM and Mitchell J (1999) Redox Imbalance in the Critically Ill. *Br Med Bull* **55**:49-75.

Halliwell B (1994) Free Radicals, Antioxidants, and Human Disease: Curiosity, Cause, or Consequence? *Lancet* **344**:721-724.

Hammermeister KE, Burchfiel C, Johnson R and Grover FL (1990) Identification of Patients at Greatest Risk for Developing Major Complications at Cardiac Surgery. *Circulation* **82**:IV380-IV389.

Hansen KR, Resta R, Webb CF and Thompson LF (1995) Isolation and Characterization of the Promoter of the Human 5'-Nucleotidase (CD73)-Encoding Gene. *Gene* **167**:307-312.

Headrick JP, Hack B and Ashton KJ (2003) Acute Adenosinergic Cardioprotection in Ischemic-Reperfused Hearts. *Am J Physiol Heart Circ Physiol* **285**:H1797-H1818.

Heffner JE, Brown LK, Barbieri CA, Harpel KS and DeLeo J (1995) Prospective Validation of an Acute Respiratory Distress Syndrome Predictive Score. *Am J Respir Crit Care Med* **152**:1518-1526.

Heiss LN, Lancaster JR, Jr., Corbett JA and Goldman WE (1994) Epithelial Autotoxicity of Nitric Oxide: Role in the Respiratory Cytopathology of Pertussis. *Proc Natl Acad Sci U S A* **91**:267-270.

Homma H, Deitch EA, Feketeova E, Lu Q, Berezina TL, Zaets SB, Machiedo GW and Xu DZ (2005) Small Volume Resuscitation With Hypertonic Saline Is More Effective in Ameliorating Trauma-Hemorrhagic Shock-Induced Lung Injury, Neutrophil Activation and Red Blood Cell Dysfunction Than Pancreatitic Protease Inhibition. *J Trauma* **59**:266-272.

Hopkins RO, Weaver LK, Collingridge D, Parkinson RB, Chan KJ and Orme JF, Jr. (2005) Two-Year Cognitive, Emotional, and Quality-of-Life Outcomes in Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* **171**:340-347.

Hudson LD, Milberg JA, Anardi D and Maunder RJ (1995) Clinical Risks for Development of the Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* **151**:293-301.

Hunsucker SA, Mitchell BS and Spychala J (2005) The 5'-Nucleotidases As Regulators of Nucleotide and Drug Metabolism. *Pharmacol Ther* **107**:1-30.

Ichikado K, Suga M, Muranaka H, Gushima Y, Miyakawa H, Tsubamoto M, Johkoh T, Hirata N, Yoshinaga T, Kinoshita Y, Yamashita Y and Sasaki Y (2006) Prediction of Prognosis for Acute Respiratory Distress Syndrome With Thin-Section CT: Validation in 44 Cases. *Radiology* **238**:321-329.

Ichikawa H, Flores S, Kvietys PR, Wolf RE, Yoshikawa T, Granger DN and Aw TY (1997) Molecular Mechanisms of Anoxia/Reoxygenation-Induced Neutrophil Adherence to Cultured Endothelial Cells. *Circ Res* **81**:922-931.

Jaakkola K, Nikula T, Holopainen R, Vahasilta T, Matikainen MT, Laukkanen ML, Huupponen R, Halkola L, Nieminen L, Hiltunen J, Parviainen S,

Clark MR, Knuuti J, Savunen T, Kaapa P, Voipio-Pulkki LM and Jalkanen S (2000) In Vivo Detection of Vascular Adhesion Protein-1 in Experimental Inflammation. *Am J Pathol* **157**:463-471.

Jalkanen S, Karikoski M, Mercier N, Koskinen K, Henttinen T, Elima K, Salmivirta K and Salmi M (2007) The Oxidase Activity of Vascular Adhesion Protein-1 (VAP-1) Induces Endothelial E- and P-Selectins and Leukocyte Binding. *Blood* **110**:1864-1870.

Jalkanen S and Salmi M (2001) Cell Surface Monoamine Oxidases: Enzymes in Search of a Function. *EMBO J* **20**:3893-3901.

Jerome SN, Kong L and Korthuis RJ (1994) Microvascular Dysfunction in Postischemic Skeletal Muscle. *J Invest Surg* **7**:3-16.

Jones SP, Trocha SD, Strange MB, Granger DN, Kevil CG, Bullard DC and Lefer DJ (2000) Leukocyte and Endothelial Cell Adhesion Molecules in a Chronic Murine Model of Myocardial Reperfusion Injury. *Am J Physiol Heart Circ Physiol* **279**:H2196-H2201.

Kakkar AK and Lefer DJ (2004) Leukocyte and Endothelial Adhesion Molecule Studies in Knockout Mice. *Curr Opin Pharmacol* **4**:154-158.

Kamochi M, Kamochi F, Kim YB, Sawh S, Sanders JM, Sarembock I, Green S, Young JS, Ley K, Fu SM and Rose CE, Jr. (1999) P-Selectin and ICAM-1 Mediate Endotoxin-Induced Neutrophil Recruitment and Injury to the Lung and Liver. *Am J Physiol* **277**:L310-L319.

Kas-Deelen AM, Bakker WW, Olinga P, Visser J, de Maar EF, van Son WJ, The TH and Harmsen MC (2001) Cytomegalovirus Infection Increases the Expression and Activity of Ecto-ATPase (CD39) and Ecto-5'Nucleotidase (CD73) on Endothelial Cells. *FEBS Lett* **491**:21-25.

Klinman JP and Mu D (1994) Quinoenzymes in Biology. *Annu Rev Biochem* **63**:299-344.

Kobayashi S, Zimmermann H and Millhorn DE (2000) Chronic Hypoxia Enhances Adenosine Release in Rat PC12 Cells by Altering Adenosine Metabolism and Membrane Transport. *J Neurochem* **74**:621-632.

Kohler D, Eckle T, Faigle M, Grenz A, Mittelbronn M, Laucher S, Hart ML, Robson SC, Muller CE and Eltzschig HK (2007) CD39/Ectonucleoside Triphosphate Diphosphohydrolase 1 Provides Myocardial Protection During Cardiac Ischemia/ Reperfusion Injury. *Circulation* **116**:1784-1794.

Koskinen K, Nevalainen S, Karikoski M, Hanninen A, Jalkanen S and Salmi M (2007) VAP-1-Deficient Mice Display Defects in Mucosal Immunity and Antimicrobial Responses: Implications for Antiadhesive Applications. *J Immunol* **179**:6160-6168.

Koskinen K, Vainio PJ, Smith DJ, Pihlavisto M, Yla-Herttuala S, Jalkanen S and Salmi M (2004) Granulocyte Transmigration Through the Endothelium Is Regulated by the Oxidase Activity of Vascular Adhesion Protein-1 (VAP-1). *Blood* **103**:3388-3395.

Koszalka P, Ozuyaman B, Huo Y, Zernecke A, Flogel U, Braun N, Buchheiser A, Decking UK, Smith ML, Sevigny J, Gear A, Weber AA, Molojavyi A, Ding Z, Weber C, Ley K, Zimmermann H, Godecke A and Schrader J (2004) Targeted Disruption of Cd73/Ecto-5'-Nucleotidase Alters Thromboregulation and Augments Vascular Inflammatory Response. *Circ Res* **95**:814-821.

Kristof AS, Goldberg P, Laubach V and Hussain SN (1998) Role of Inducible Nitric Oxide Synthase in Endotoxin-Induced Acute Lung Injury. *Am J Respir Crit Care Med* **158**:1883-1889.

Kubes P, Suzuki M and Granger DN (1991) Nitric Oxide: an Endogenous Modulator of Leukocyte Adhesion. *Proc Natl Acad Sci U S A* **88**:4651-4655.

Kurahashi K, Kajikawa O, Sawa T, Ohara M, Gropper MA, Frank DW, Martin TR and Wiener-Kronish JP (1999) Pathogenesis of Septic Shock in Pseudomonas Aeruginosa Pneumonia. *J Clin Invest* **104**:743-750.

Kurkijarvi R, Adams DH, Leino R, Mottonen T, Jalkanen S and Salmi M (1998) Circulating Form of Human Vascular Adhesion Protein-1 (VAP-1): Increased Serum Levels in Inflammatory Liver Diseases. *J Immunol* **161**:1549-1557.

Kurkijarvi R, Yegutkin GG, Gunson BK, Jalkanen S, Salmi M and Adams DH (2000) Circulating Soluble Vascular Adhesion Protein 1 Accounts for the Increased Serum Monoamine Oxidase Activity in Chronic Liver Disease. *Gastroenterology* **119**:1096-1103.

Kurose I, Anderson DC, Miyasaka M, Tamatani T, Paulson JC, Todd RF, Rusche JR and Granger DN (1994) Molecular Determinants of Reperfusion-Induced Leukocyte Adhesion and Vascular Protein Leakage. *Circ Res* **74**:336-343.

Lalor PF, Edwards S, McNab G, Salmi M, Jalkanen S and Adams DH (2002) Vascular Adhesion Protein-1 Mediates Adhesion and Transmigration of Lymphocytes on Human Hepatic Endothelial Cells. *J Immunol* **169**:983-992.

Lalor PF, Sun PJ, Weston CJ, Martin-Santos A, Wakelam MJ and Adams DH (2007) Activation of Vascular Adhesion Protein-1 on Liver Endothelium Results in an NF-KappaB-Dependent Increase in Lymphocyte Adhesion. *Hepatology* **45**:465-474.

Lamb NJ, Gutteridge JM, Baker C, Evans TW and Quinlan GJ (1999) Oxidative Damage to Proteins of Bronchoalveolar Lavage Fluid in Patients With Acute Respiratory Distress Syndrome: Evidence for Neutrophil-Mediated Hydroxylation, Nitration, and Chlorination. *Crit Care Med* **27**:1738-1744.

Lang JD, McArdle PJ, O'Reilly PJ and Matalon S (2002) Oxidant-Antioxidant Balance in Acute Lung Injury. *Chest* **122**:314S-320S.

Laufe MD, Simon RH, Flint A and Keller JB (1986) Adult Respiratory Distress Syndrome in Neutropenic Patients. *Am J Med* **80**:1022-1026.

Ledoux S, Runembert I, Koumanov K, Michel JB, Trugnan G and Friedlander G (2003) Hypoxia Enhances Ecto-5'-Nucleotidase Activity and Cell Surface Expression in Endothelial Cells: Role of Membrane Lipids. *Circ Res* **92**:848-855.

Lennon PF, Taylor CT, Stahl GL and Colgan SP (1998) Neutrophil-Derived 5'-Adenosine Monophosphate Promotes Endothelial Barrier Function Via CD73-Mediated Conversion to Adenosine and Endothelial A2B Receptor Activation. *J Exp Med* **188**:1433-1443.

Ley K, Laudanna C, Cybulsky MI and Nourshargh S (2007) Getting to the Site of Inflammation: the Leukocyte Adhesion Cascade Updated. *Nat Rev Immunol* **7**:678-689.

Liew FY, Millott S, Parkinson C, Palmer RM and Moncada S (1990) Macrophage Killing of Leishmania Parasite in Vivo Is Mediated by Nitric Oxide From L-Arginine. *J Immunol* **144**:4794-4797.

Linden J (2001) Molecular Approach to Adenosine Receptors: Receptor-Mediated Mechanisms of Tissue Protection. *Annu Rev Pharmacol Toxicol* **41**:775-787.

Luce JM (2005) The Imperfect Diagnosis of Acute Respiratory Distress Syndrome. *Crit Care Med* **33**:2419-2420.

Luhr OR, Antonsen K, Karlsson M, Aardal S, Thorsteinsson A, Frostell CG and Bonde J (1999) Incidence and Mortality After Acute Respiratory Failure and Acute Respiratory Distress Syndrome in Sweden, Denmark, and Iceland. The ARF Study Group. *Am J Respir Crit Care Med* **159**:1849-1861.

Marini JJ (2006) Limitations of Clinical Trials in Acute Lung Injury and Acute Respiratory Distress Syndrome. *Curr Opin Crit Care* **12**:25-31.

Marnett LJ, Riggins JN and West JD (2003) Endogenous Generation of Reactive Oxidants and Electrophiles and Their Reactions With DNA and Protein. *J Clin Invest* **111**:583-593.

Martelius T, Salaspuro V, Salmi M, Krogerus L, Hockerstedt K, Jalkanen S and Lautenschlager I (2004) Blockade of Vascular Adhesion Protein-1 Inhibits Lymphocyte Infiltration in Rat Liver Allograft Rejection. *Am J Pathol* **165**:1993-2001.

Marttila-Ichihara F, Smith DJ, Stolen C, Yegutkin GG, Elima K, Mercier N, Kiviranta R, Pihlavisto M, Alaranta S, Pentikainen U, Pentikainen O, Fulop F, Jalkanen S and Salmi M (2006) Vascular Amine Oxidases Are Needed for Leukocyte Extravasation into Inflamed Joints in Vivo. *Arthritis Rheum* **54**:2852-2862.

Matthay MA, Pittet JF and Jayr C (1998) Just Say NO to Inhaled Nitric Oxide for the Acute Respiratory Distress Syndrome. *Crit Care Med* **26**:1-2.

McCallion K, Harkin DW and Gardiner KR (2004) Role of Adenosine in Immunomodulation: Review of the Literature. *Crit Care Med* **32**:273-277.

McCleverty JA (2004) Chemistry of Nitric Oxide Relevant to Biology. *Chem Rev* **104**:403-418.

McHugh LG, Milberg JA, Whitcomb ME, Schoene RB, Maunder RJ and Hudson LD (1994) Recovery of Function in Survivors of the Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* **150**:90-94.

Meade MO, Jacka MJ, Cook DJ, Dodek P, Griffith L and Guyatt GH (2004) Survey of Interventions for the Prevention and Treatment of Acute Respiratory Distress Syndrome. *Crit Care Med* **32**:946-954.

Merinen M, Irjala H, Salmi M, Jaakkola I, Hanninen A and Jalkanen S (2005) Vascular Adhesion Protein-1 Is Involved in Both Acute and Chronic Inflammation in the Mouse. *Am J Pathol* **166**:793-800.

Metnitz PG, Bartens C, Fischer M, Fridrich P, Steltzer H and Druml W (1999) Antioxidant Status in Patients With Acute Respiratory Distress Syndrome. *Intensive Care Med* **25**:180-185.

Milberg JA, Davis DR, Steinberg KP and Hudson LD (1995) Improved Survival of Patients With Acute Respiratory Distress Syndrome (ARDS): 1983-1993. *JAMA* **273**:306-309.

Milot J, Perron J, Lacasse Y, Letourneau L, Cartier PC and Maltais F (2001) Incidence and Predictors of ARDS After Cardiac Surgery. *Chest* **119**:884-888.

Modelska K, Pittet JF, Folkesson HG, Courtney B, V and Matthay MA (1999) Acid-Induced Lung Injury. Protective Effect of Anti-Interleukin-8 Pretreatment on Alveolar Epithelial Barrier Function in Rabbits. *Am J Respir Crit Care Med* **160**:1450-1456.

Moncada S, Palmer RM and Higgs EA (1991) Nitric Oxide: Physiology, Pathophysiology, and Pharmacology. *Pharmacol Rev* **43**:109-142.

Montgomery AB, Stager MA, Carrico CJ and Hudson LD (1985) Causes of Mortality in Patients With the Adult Respiratory Distress Syndrome. *Am Rev Respir Dis* **132**:485-489.

Morabito L, Montesinos MC, Schreibman DM, Balter L, Thompson LF, Resta R, Carlin G, Huie MA and Cronstein BN (1998) Methotrexate and Sulfasalazine Promote Adenosine Release by a Mechanism That Requires Ecto-5'-Nucleotidase-Mediated Conversion of Adenine Nucleotides. *J Clin Invest* **101**:295-300.

Motoyama T, Okamoto K, Kukita I, Hamaguchi M, Kinoshita Y and Ogawa H (2003) Possible Role of Increased Oxidant Stress in Multiple Organ Failure After Systemic Inflammatory Response Syndrome. *Crit Care Med* **31**:1048-1052.

Mubagwa K and Flameng W (2001) Adenosine, Adenosine Receptors and Myocardial Protection: an Updated Overview. *Cardiovasc Res* **52**:25-39.

Muller WA (2003) Leukocyte-Endothelial-Cell Interactions in Leukocyte Transmigration and the Inflammatory Response. *Trends Immunol* **24**:327-334.

Murray JF, Matthay MA, Luce JM and Flick MR (1988) An Expanded Definition of the Adult Respiratory Distress Syndrome. *Am Rev Respir Dis* **138**:720-723.

Niemela J, Henttinen T, Yegutkin GG, Airas L, Kujari AM, Rajala P and Jalkanen S (2004) IFN-Alpha Induced Adenosine Production on the Endothelium: a Mechanism Mediated by CD73 (Ecto-5'-Nucleotidase) Up-Regulation. *J Immunol* **172**:1646-1653.

Noda K, Miyahara S, Nakazawa T, Almulki L, Nakao S, Hisatomi T, She H, Thomas KL, Garland RC, Miller JW, Gragoudas ES, Kawai Y, Mashima Y and Hafezi-Moghadam A (2007) Inhibition of Vascular Adhesion Protein-1 Suppresses Endotoxin-Induced Uveitis. *FASEB J*.

O'Farrell PH (2001) Conserved Responses to Oxygen Deprivation. *J Clin Invest* **107**:671-674.

O'Rourke AM, Wang EY, Miller A, Podar EM, Scheyhing K, Huang L, Kessler C, Gao H, Ton-Nu HT, Macdonald MT, Jones DS and Linnik MD (2007a) Anti-Inflammatory Effects of LJP 1586, an Amine-Based Inhibitor of Semicarbazide-Sensitive Amine Oxidase Activity. *J Pharmacol Exp Ther*.

O'Rourke AM, Wang EY, Salter-Cid L, Huang L, Miller A, Podar E, Gao HF, Jones DS and Linnik MD (2007b) Benefit of Inhibiting SSAO in Relapsing Experimental Autoimmune Encephalomyelitis. *J Neural Transm* **114**:845-849.

Ohara M, Unno N, Mitsuoka H, Kaneko H and Nakamura S (2001) Peritoneal Lavage With Oxygenated Perfluorochemical Preserves Intestinal Mucosal Barrier Function After Ischemia-Reperfusion and Ameliorates Lung Injury. *Crit Care Med* **29**:782-788.

Palmer RM, Ferrige AG and Moncada S (1987) Nitric Oxide Release Accounts for the Biological Activity of Endothelium-Derived Relaxing Factor. *Nature* **327**:524-526.

Park PO, Haglund U, Bulkley GB and Falt K (1990) The Sequence of Development of Intestinal Tissue Injury After Strangulation Ischemia and Reperfusion. *Surgery* **107**:574-580.

Parker JC, Townsley MI, Rippe B, Taylor AE and Thigpen J (1984) Increased Microvascular Permeability in Dog Lungs Due to High Peak Airway Pressures. *J Appl Physiol* **57**:1809-1816.

Patel SR, Karmpaliotis D, Ayas NT, Mark EJ, Wain J, Thompson BT and Malhotra A (2004) The Role of Open-Lung Biopsy in ARDS. *Chest* **125**:197-202.

Peng X, Abdulnour RE, Sammani S, Ma SF, Han EJ, Hasan EJ, Tuder R, Garcia JG and Hassoun PM (2005) Inducible Nitric Oxide Synthase

Contributes to Ventilator-Induced Lung Injury. *Am J Respir Crit Care Med* **172**:470-479.

Pepe PE, Potkin RT, Reus DH, Hudson LD and Carrico CJ (1982) Clinical Predictors of the Adult Respiratory Distress Syndrome. *Am J Surg* **144**:124-130.

Pepke-Zaba J, Higenbottam TW, nh-Xuan AT, Stone D and Wallwork J (1991) Inhaled Nitric Oxide As a Cause of Selective Pulmonary Vasodilatation in Pulmonary Hypertension. *Lancet* **338**:1173-1174.

Petty TL and Ashbaugh DG (1971) The Adult Respiratory Distress Syndrome. Clinical Features, Factors Influencing Prognosis and Principles of Management. *Chest* **60**:233-239.

Pittet JF, Mackersie RC, Martin TR and Matthay MA (1997) Biological Markers of Acute Lung Injury: Prognostic and Pathogenetic Significance. *Am J Respir Crit Care Med* **155**:1187-1205.

Powers KA, Kapus A, Khadaroo RG, He R, Marshall JC, Lindsay TF and Rotstein OD (2003) Twenty-Five Percent Albumin Prevents Lung Injury Following Shock/Resuscitation. *Crit Care Med* **31**:2355-2363.

Pratt PC, Vollmer RT, Shelburne JD and Crapo JD (1979) Pulmonary Morphology in a Multihospital Collaborative Extracorporeal Membrane Oxygenation Project. I. Light Microscopy. *Am J Pathol* **95**:191-214.

Prescott SM, McIntyre TM and Zimmerman G (1999) Two of the Usual Suspects, Platelet-Activating Factor and Its Receptor, Implicated in Acute Lung Injury. *J Clin Invest* **104**:1019-1020.

Prewitt RM, McCarthy J and Wood LD (1981) Treatment of Acute Low Pressure Pulmonary Edema in Dogs: Relative Effects of Hydrostatic and Oncotic Pressure, Nitroprusside, and Positive End-Expiratory Pressure. *J Clin Invest* **67**:409-418.

Pugin J, Verghese G, Widmer MC and Matthay MA (1999) The Alveolar Space Is the Site of Intense Inflammatory and Profibrotic Reactions in the Early Phase of Acute Respiratory Distress Syndrome. *Crit Care Med* **27**:304-312.

Quinlan GJ, Lamb NJ, Evans TW and Gutteridge JM (1996) Plasma Fatty Acid Changes and Increased Lipid Peroxidation in Patients With Adult Respiratory Distress Syndrome. *Crit Care Med* **24**:241-246.

Raijmakers PG, Groeneveld AB, Rauwerda JA, Teule GJ and Hack CE (1997) Acute Lung Injury After Aortic Surgery: the Relation Between Lung and Leg Microvascular Permeability to 111indium-Labelled Transferrin and Circulating Mediators. *Thorax* **52**:866-871.

Richard C, Lemonnier F, Thibault M, Couturier M and Auzepy P (1990) Vitamin E Deficiency and Lipoperoxidation During Adult Respiratory Distress Syndrome. *Crit Care Med* **18**:4-9.

Rimar S and Gillis CN (1993) Selective Pulmonary Vasodilation by Inhaled Nitric Oxide Is Due to Hemoglobin Inactivation. *Circulation* **88**:2884-2887.

Rosengren S, Arfors KE and Proctor KG (1991) Potentiation of Leukotriene B4-Mediated Inflammatory Response by the Adenosine Antagonist, 8-Phenyl Theophylline. *Int J Microcirc Clin Exp* **10**:345-357.

Rossaint R, Falke KJ, Lopez F, Slama K, Pison U and Zapol WM (1993) Inhaled Nitric Oxide for the Adult Respiratory Distress Syndrome. *N Engl J Med* **328**:399-405.

Rubenfeld GD, Caldwell E, Granton J, Hudson LD and Matthay MA (1999) Interobserver Variability in Applying a Radiographic Definition for ARDS. *Chest* **116**:1347-1353.

Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ and Hudson LD (2005) Incidence and Outcomes of Acute Lung Injury. *N Engl J Med* **353**:1685-1693.

Rudkowski JC, Barreiro E, Harfouche R, Goldberg P, Kishta O, Orleans-Juste P, Labonte J, Lesur O and Hussain SN (2004) Roles of INOS and NNOS in Sepsis-Induced Pulmonary Apoptosis. *Am J Physiol Lung Cell Mol Physiol* **286**:L793-L800.

Salmi M and Jalkanen S (1992) A 90-Kilodalton Endothelial Cell Molecule Mediating Lymphocyte Binding in Humans. *Science* **257**:1407-1409.

Salmi M and Jalkanen S (2005) Cell-Surface Enzymes in Control of Leukocyte Trafficking. *Nat Rev Immunol* **5**:760-771.

Salmi M, Kalimo K and Jalkanen S (1993) Induction and Function of Vascular Adhesion Protein-1 at Sites of Inflammation. *J Exp Med* **178**:2255-2260.

Salmi M, Stolen C, Jousilahti P, Yegutkin GG, Tapanainen P, Janatuinen T, Knip M, Jalkanen S and Salomaa V (2002) Insulin-Regulated Increase

of Soluble Vascular Adhesion Protein-1 in Diabetes. *Am J Pathol* **161**:2255-2262.

Salmi M, Yegutkin GG, Lehvonen R, Koskinen K, Salminen T and Jalkanen S (2001) A Cell Surface Amine Oxidase Directly Controls Lymphocyte Migration. *Immunity* **14**:265-276.

Salter-Cid LM, Wang E, O'Rourke AM, Miller A, Gao H, Huang L, Garcia A and Linnik MD (2005) Anti-Inflammatory Effects of Inhibiting the Amine Oxidase Activity of Semicarbazide-Sensitive Amine Oxidase. *J Pharmacol Exp Ther* **315**:553-562.

Sapirstein LA, Sapirstein EH and Bredemeyer A (1960) Effect of Hemorrhage on the Cardiac Output and Its Distribution in the Rat. *Circ Res* **8**:135-148.

Sato Y, Walley KR, Klut ME, English D, D'yachkova Y, Hogg JC and van Eeden SF (1999) Nitric Oxide Reduces the Sequestration of Polymorphonuclear Leukocytes in Lung by Changing Deformability and CD18 Expression. *Am J Respir Crit Care Med* **159**:1469-1476.

Schlensak C, Doenst T and Beyersdorf F (2000) Lung Ischemia During Cardiopulmonary Bypass. *Ann Thorac Surg* **70**:337-338.

Schlensak C, Doenst T, Preusser S, Wunderlich M, Kleinschmidt M and Beyersdorf F (2001) Bronchial Artery Perfusion During Cardiopulmonary Bypass Does Not Prevent Ischemia of the Lung in Piglets: Assessment of Bronchial Artery Blood Flow With Fluorescent Microspheres. *Eur J Cardiothorac Surg* **19**:326-331.

Schlensak C, Doenst T, Preusser S, Wunderlich M, Kleinschmidt M and Beyersdorf F (2002) Cardiopulmonary Bypass Reduction of Bronchial Blood Flow: a Potential Mechanism for Lung Injury in a Neonatal Pig Model. *J Thorac Cardiovasc Surg* **123**:1199-1205.

Sheridan BC, McIntyre RC, Jr., Meldrum DR and Fullerton DA (1999) L-Arginine Attenuates Endothelial Dysfunction in Endotoxin-Induced Lung Injury. *Surgery* **125**:33-40.

Silliman CC, Ambruso DR and Boshkov LK (2005) Transfusion-Related Acute Lung Injury. *Blood* **105**:2266-2273.

Sloane PJ, Gee MH, Gottlieb JE, Albertine KH, Peters SP, Burns JR, Machiedo G and Fish JE (1992) A Multicenter Registry of Patients With Acute Respiratory Distress Syndrome. Physiology and Outcome. *Am Rev Respir Dis* **146**:419-426.

Slutsky AS and Tremblay LN (1998) Multiple System Organ Failure. Is Mechanical Ventilation a Contributing Factor? *Am J Respir Crit Care Med* **157**:1721-1725.

Smith DJ, Salmi M, Bono P, Hellman J, Leu T and Jalkanen S (1998) Cloning of Vascular Adhesion Protein 1 Reveals a Novel Multifunctional Adhesion Molecule. *J Exp Med* **188**:17-27.

Springer TA (1994) Traffic Signals for Lymphocyte Recirculation and Leukocyte Emigration: the Multistep Paradigm. *Cell* **76**:301-314.

Sprung CL, Caralis PV, Marcial EH, Pierce M, Gelbard MA, Long WM, Duncan RC, Tendler MD and Karpf M (1984) The Effects of High-Dose Corticosteroids in Patients With Septic Shock. A Prospective, Controlled Study. *N Engl J Med* **311**:1137-1143.

Stamler JS, Singel DJ and Loscalzo J (1992) Biochemistry of Nitric Oxide and Its Redox-Activated Forms. *Science* **258**:1898-1902.

Stapleton RD, Wang BM, Hudson LD, Rubenfeld GD, Caldwell ES and Steinberg KP (2005) Causes and Timing of Death in Patients With ARDS. *Chest* **128**:525-532.

Steinberg KP, Hudson LD, Goodman RB, Hough CL, Lanken PN, Hyzy R, Thompson BT and Ancukiewicz M (2006) Efficacy and Safety of Corticosteroids for Persistent Acute Respiratory Distress Syndrome. *N Engl J Med* **354**:1671-1684.

Stevens T, Garcia JG, Shasby DM, Bhattacharya J and Malik AB (2000) Mechanisms Regulating Endothelial Cell Barrier Function. *Am J Physiol Lung Cell Mol Physiol* **279**:L419-L422.

Stolen CM, Madanat R, Marti L, Kari S, Yegutkin GG, Sariola H, Zorzano A and Jalkanen S (2004) Semicarbazide Sensitive Amine Oxidase Overexpression Has Dual Consequences: Insulin Mimicry and Diabetes-Like Complications. *FASEB J* **18**:702-704.

Stolen CM, Marttila-Ichihara F, Koskinen K, Yegutkin GG, Turja R, Bono P, Skurnik M, Hanninen A, Jalkanen S and Salmi M (2005) Absence of the Endothelial Oxidase AOC3 Leads to Abnormal Leukocyte Traffic in Vivo. *Immunity* **22**:105-115.

Stuart RB, Ovadia B, Suzara VV, Ross PA, Thelitz S, Fineman JR and Gutierrez JA (2003) Inhaled Nitric Oxide Increases Surfactant Protein Gene Expression in the Intact Lamb. *Am J Physiol Lung Cell Mol Physiol* **285**:L628-L633.

Stuart-Smith K and Jeremy JY (2001) Microvessel Damage in Acute Respiratory Distress Syndrome: the Answer May Not Be NO. *Br J Anaesth* **87**:272-279.

Suchyta MR, Clemmer TP, Elliott CG, Orme JF, Jr. and Weaver LK (1992) The Adult Respiratory Distress Syndrome. A Report of Survival and Modifying Factors. *Chest* **101**:1074-1079.

Suchyta MR, Clemmer TP, Orme JF, Jr., Morris AH and Elliott CG (1991) Increased Survival of ARDS Patients With Severe Hypoxemia (ECMO Criteria). *Chest* **99**:951-955.

Suzuki M, Asako H, Kubes P, Jennings S, Grisham MB and Granger DN (1991) Neutrophil-Derived Oxidants Promote Leukocyte Adherence in Postcapillary Venules. *Microvasc Res* **42**:125-138.

Swank GM and Deitch EA (1996) Role of the Gut in Multiple Organ Failure: Bacterial Translocation and Permeability Changes. *World J Surg* **20**:411-417.

Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, Hansen KR, Thompson LF and Colgan SP (2002) Ecto-5'-Nucleotidase (CD73) Regulation by Hypoxia-Inducible Factor-1 Mediates Permeability Changes in Intestinal Epithelia. *J Clin Invest* **110**:993-1002.

Szabo C (2003) Multiple Pathways of Peroxynitrite Cytotoxicity. *Toxicol Lett* **140-141**:105-112.

Sznajder JI (1999) Strategies to Increase Alveolar Epithelial Fluid Removal in the Injured Lung. *Am J Respir Crit Care Med* **160**:1441-1442.

Sznajder JI, Fraiman A, Hall JB, Sanders W, Schmidt G, Crawford G, Nahum A, Factor P and Wood LD (1989) Increased Hydrogen Peroxide in the Expired Breath of Patients With Acute Hypoxemic Respiratory Failure. *Chest* **96**:606-612.

Takeyama N, Shoji Y, Ohashi K and Tanaka T (1996) Role of Reactive Oxygen Intermediates in Lipopolysaccharide-Mediated Hepatic Injury in the Rat. *J Surg Res* **60**:258-262.

Tassiopoulos AK, Carlin RE, Gao Y, Pedoto A, Finck CM, Landas SK, Tice DG, Marx W, Hakim TS and McGraw DJ (1997) Role of Nitric Oxide and Tumor Necrosis Factor on Lung Injury Caused by Ischemia/Reperfusion of the Lower Extremities. *J Vasc Surg* **26**:647-656.

Thompson LF, Eltzschig HK, Ibla JC, Van De Wiele CJ, Resta R, Morote-Garcia JC and Colgan SP (2004) Crucial Role for Ecto-5'-Nucleotidase (CD73) in Vascular Leakage During Hypoxia. *J Exp Med* **200**:1395-1405.

Tohka S, Laukkanen M, Jalkanen S and Salmi M (2001) Vascular Adhesion Protein 1 (VAP-1) Functions As a Molecular Brake During Granulocyte Rolling and Mediates Recruitment in Vivo. *FASEB J* **15**:373-382.

Tonz M, Mihaljevic T, von Segesser LK, Fehr J, Schmid ER and Turina MI (1995) Acute Lung Injury During Cardiopulmonary Bypass. Are the Neutrophils Responsible? *Chest* **108**:1551-1556.

Toung T, Reilly PM, Fuh KC, Ferris R and Bulkley GB (2000) Mesenteric Vasoconstriction in Response to Hemorrhagic Shock. *Shock* **13**:267-273.

Vatner SF (1974) Effects of Hemorrhage on Regional Blood Flow Distribution in Dogs and Primates. *J Clin Invest* **54**:225-235.

Vejchapipat P, Leawhiran N, Poomsawat S, Theamboonlers A, Chittmittrapap S and Poovorawan Y (2006) Amelioration of Intestinal Reperfusion Injury by Moderate Hypothermia Is Associated With Serum SICAM-1 Levels. *J Surg Res* **130**:152-157.

Verheij J, van LA, Raijmakers PG, Rijnsburger ER, Veerman DP, Wisselink W, Girbes AR and Groeneveld AB (2006) Effect of Fluid Loading With Saline or Colloids on Pulmonary Permeability, Oedema and Lung Injury Score After Cardiac and Major Vascular Surgery. *Br J Anaesth* **96**:21-30.

Virag L, Szabo E, Gergely P and Szabo C (2003) Peroxynitrite-Induced Cytotoxicity: Mechanism and Opportunities for Intervention. *Toxicol Lett* **140-141**:113-124.

von Andrian UH and Mempel TR (2003) Homing and Cellular Traffic in Lymph Nodes. *Nat Rev Immunol* **3**:867-878.

Ware LB (2005) Prognostic Determinants of Acute Respiratory Distress Syndrome in Adults: Impact on Clinical Trial Design. *Crit Care Med* **33**:S217-S222.

Ware LB and Matthay MA (2000) The Acute Respiratory Distress Syndrome. *N Engl J Med* **342**:1334-1349.

Webb HH and Tierney DF (1974) Experimental Pulmonary Edema Due to Intermittent Positive

Pressure Ventilation With High Inflation Pressures. Protection by Positive End-Expiratory Pressure. *Am Rev Respir Dis* **110**:556-565.

Weinberger B, Laskin DL, Heck DE and Laskin JD (2001) The Toxicology of Inhaled Nitric Oxide. *Toxicol Sci* **59**:5-16.

Wheeler AP and Bernard GR (2007) Acute Lung Injury and the Acute Respiratory Distress Syndrome: a Clinical Review. *Lancet* **369**:1553-1564.

Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B, Connors AF, Jr., Hite RD and Harabin AL (2006) Comparison of Two Fluid-Management Strategies in Acute Lung Injury. *N Engl J Med* **354**:2564-2575.

Wiener-Kronish JP, Albertine KH and Matthay MA (1991) Differential Responses of the Endothelial and Epithelial Barriers of the Lung in Sheep to Escherichia Coli Endotoxin. *J Clin Invest* **88**:864-875.

Wu R, Dong W, Zhou M, Zhang F, Marini CP, Ravikumar TS and Wang P (2007) Ghrelin Attenuates Sepsis-Induced Acute Lung Injury and Mortality in Rats. *Am J Respir Crit Care Med* **176**:805-813.

Xu HL, Salter-Cid L, Linnik MD, Wang EY, Paisansathan C and Pelligrino DA (2006) Vascular Adhesion Protein-1 Plays an Important Role in Postischemic Inflammation and Neuropathology in Diabetic, Estrogen-Treated Ovariectomized Female Rats Subjected to Transient Forebrain Ischemia. *J Pharmacol Exp Ther* **317**:19-29.

Yasuhara H (2005) Acute Mesenteric Ischemia: the Challenge of Gastroenterology. *Surg Today* **35**:185-195.

Yegutkin G, Bodin P and Burnstock G (2000) Effect of Shear Stress on the Release of Soluble Ecto-Enzymes ATPase and 5'-Nucleotidase Along With Endogenous ATP From Vascular Endothelial Cells. *Br J Pharmacol* **129**:921-926.

Yoong KF, McNab G, Hubscher SG and Adams DH (1998) Vascular Adhesion Protein-1 and ICAM-1 Support the Adhesion of Tumor-Infiltrating Lymphocytes to Tumor Endothelium in Human Hepatocellular Carcinoma. *J Immunol* **160**:3978-3988.

Yu PH, Lu LX, Fan H, Kazachkov M, Jiang ZJ, Jalkanen S and Stolen C (2006) Involvement of Semicarbazide-Sensitive Amine Oxidase-Mediated Deamination in Lipopolysaccharide-Induced Pulmonary Inflammation. *Am J Pathol* **168**:718-726.

Yu PH and Zuo DM (1997) Aminoguanidine Inhibits Semicarbazide-Sensitive Amine Oxidase Activity: Implications for Advanced Glycation and Diabetic Complications. *Diabetologia* **40**:1243-1250.

Zhang H, Slutsky AS and Vincent JL (2000) Oxygen Free Radicals in ARDS, Septic Shock and Organ Dysfunction. *Intensive Care Med* **26**:474-476.

Zilberberg MD and Epstein SK (1998) Acute Lung Injury in the Medical ICU: Comorbid Conditions, Age, Etiology, and Hospital Outcome. *Am J Respir Crit Care Med* **157**:1159-1164.

Zimmermann H (1992) 5'-Nucleotidase: Molecular Structure and Functional Aspects. *Biochem J* **285 (Pt 2)**:345-365.