Chinese Journal of Physiology 49(5): 266-274, 2006

# Effects of Anti-Tumor Necrosis Factor-α and Anti- Intercellular Adhesion Molecule-1 Antibodies on Ischemia/Reperfusion Lung Injury

**Chi-Huei Chiang**

*Chest Department, Taipei Veterans General Hospital*
*School of Medicine, National Yang-Ming University*
*Taipei, Taiwan, ROC*

## Abstract

**Inhibition of neutrophil activation and adherence to endothelium by antibodies to tumor necrosis factor-α (TNF-α) and intercellular adhesion molecules (ICAM-1), respectively, might attenuate ischemia-reperfusion injury(I/R). I/R was conducted in an isolated rat lung model. Anti-TNF-α antibody and/or anti-ICAM-1 antibody were added before ischemia or after reperfusion. Hemodynamic changes, lung weight gain (LWG), capillary filtration coefficients ($K_{fc}$), and pathologic changes were assessed to evaluate the severity of I/R. The LWG, $K_{fc}$, pathological changes and lung injury score of treatment groups with anti-TNF-α antibody treatment, either pre-ischemia or during reperfusion, were less than those observed in control groups. Similar findings were found in group treated with anti-ICAM-1 antibody or combination therapy during reperfusion. In contrast, pre-I/R treatment with anti-ICAM-1 antibody induced severe lung edema and failure to complete the experimental procedure. No additional therapeutic effect was found in combination therapy. We conclude that TNF-α and ICAM-1 play important roles in I/R. Anti-TNF-α antibody has therapeutic and preventive effects on I/R. However, combined therapy with anti-TNF-α antibody and anti-ICAM-1 antibody may have no additive effect and need further investigation.**

**Key Words: tumor necrosis factor-α (TNF-α); intercellular adhesion molecules (ICAM-1); ischemia and reperfusion lung injury**

## Introduction

Microvascular injury produced by ischemia/reperfusion (I/R) is a pathophysiologic event with broad clinical relevance such as in organ transplantation, myocardial infarction or ischemia, cerebral infarction, and shock. The pathogenesis of I/R is still unclear. Ischemia is characterized by the absence of blood flow into the lung, which can cause lipid peroxidation and oxidant injury. Endothelial cells are highly sensitive to physical forces resulting from blood flow variation and are able to transform these mechanical forces into electrical and biochemical signals (mechanotransduction) (27). The absence of the mechanical component of flow during lung ischemia stimulates membrane depolarization of endothelial cells with the activation of NADPH oxidase, nuclear factor-κB, and calcium/calmodulin-dependent nitric oxide synthase (NOS) (3, 14). Other cells such as macrophages and/or marginated neutrophils, which are known to have a high NADPH oxidase activity, could also contribute to the lung oxidant burden induced during the ischemic period.

Consequences of ischemia and reperfusion include upregulation of molecules on cell surface membrane (2) and cytokines (5). Leukocyte emigration involves the sequential events of activation, rolling, adherence, and extravasation. Leukocyte is activated by reactive oxygen species (ROS) and cytokine tumor necrosis

Corresponding author: Chi-Huei Chiang, M.D., Division of Pulmonary Immunology and Infectious Diseases, Chest Department, Taipei Veterans General Hospital. No. 201, Section 2, Shih-Pai Road, Taipei, Taiwan, ROC. Tel: 886-2- 28757713, Fax: 886-2-28765323, E-mail: chiang01@vghtpe.gov.tw
Received: March 27, 2006; Revised (Finally): August 26, 2006; Accepted: September 4, 2006.

factor-α (TNF-α)(11, 12). Leukocyte rolling is dependent on selectin-mediated interaction between endothelial cells (P-selectin and E-selectin) and leukocytes (L-selectin). Firm adherence and activation of leukocytes occur when leukocyte $\beta_1$-integrin or $\beta_2$-integrin binds to endothelial cells expressing intercellular adhesion molecule-1(ICAM-1) or vascular endothelial adhesion molecule-1, respectively (14). Finally, leukocyte extravasation into the tissue is dependent on integrin-immunoglobulin interactions, involving ICAM-1 and platelet endothelial cell adhesion molecule-1(14).

Based on above review of previous literatures (5, 6, 32, 37), we postulated the pathway of pathogenesis as follows: hypoxia (stop ventilation) and mechanotransduction (no blood flow) during ischemia induces macrophages, endothelial cell or other cells to generate ROS and proinflammatory cytokines (5, 6, 32, 37) as well as upregulation of molecules on cell surface membrane. During reperfusion (re-oxygenation), cytokines (e.g., TNF-α) and ROS mediate polymorphonuclear neutrophil (PMN) activation, rolling and adherence to endothelial cells which further promote release of their oxygen radicals, cytokines and other mediators beginning a complex cascade resulting in vascular injury (1, 18, 36) and migration of PMNs into interstitium and alveoli. This sequence is followed by more inflammatory cells being recruited into interstitial spaces and alveoli.

Our previous study showed that cytokine TNF-α is up-regulated in ischemic/reperfusion lung injury (5). Furthermore, a significant correlation was observed between TNF-α and severity of I/R lung injury (5, 6) indicating TNF-α is an important proinflammatory cytokine in modulating the I/R lung injury. TNF-α release is required to produce the damage, most likely by upregulating ICAM-1 and P-selectin on endothelial cells and CD-18 on neutrophils (24, 28). ·TNF-α triggers degranulation and oxygen radical release in adherent neutrophils (25). Therefore, TNF-α might be one of the important mediators for neutrophil activation and expression of adhesion molecules on neutrophils and endothelium in the lung. Previous studies showed pretreatment with anti-TNF-α antibody to attenuate I/R lung injury (12, 24, 26, 46) but the information of post-I/R treatment (therapeutic effect) with anti-TNF-α antibody on I/R lung injury is still limited.

Adhesion molecules, particularly CD11/CD18 and ICAM-1, play important roles in the I/R lung injury (13, 19, 30). ICAM-1 is one of the important adhesion molecules that modulate neutrophil adhesion to endothelium of pulmonary capillary (33).

In small animal models cobra venom factor induces lung injury, and administration of monoclonal antibodies to ICAM-1 decreases PMN migration into the lung (15). Moore *et al*. observed pretreatment with ICAM-1 antibodies has attenuation effect on I/R lung injury. However, pretreatment with anti-ICAM-1 monoclonal antibody did not prevent lung injury in gram-negative sepsis (45). Therefore, effects of pretreatment with ICAM-1 antibodies are still inconclusive.

We hypothesized that treatment with both anti-TNF-α antibody to neutralize the TNF-α (for preventing the neutrophil activation) and anti-ICAM-1 antibody (to inhibit neurophil adherence to endothelial cells for modulating leukocyte-endothelial cell adhesion) would be beneficial and synergistically attenuate I/R.

## Materials and Methods

*Preparation of Isolated and Perfused Rat Lungs*

This study was approved by the Institutional Review Board for the care of animal subjects, and the care and handling of the animals were in accord with National Institutes of Health guidelines for ethical animal research. Our isolated-perfused lung *in situ* I/R model has been previously described (3,8). Briefly, male Sprague-Dawley rats (250 to 350 g body weight) were anesthetized intraperitoneally with sodium pentobarbital (20-25 mg). A tracheotomy was performed to permit ventilation with a Harvard rodent ventilator (Model 683, Harvard Apparatus, South Natick, MA, USA) at 55 breaths/min at a tidal volume at 2.5 ml and a positive end-expiratory pressure of 2 cm $H_2O$. The inspired gas mixture contained 5% $CO_2$ and 95% air. After a median sternotomy was performed, heparin (1 U/g) was injected into the right ventricle. Blood was drawn from the right ventricle and discarded. A cannula was placed into the pulmonary artery through a puncture into the right ventricle, and a tight ligature was placed around the main trunk of the pulmonary artery. A large catheter was inserted into the left atrium through the left ventricle and mitral valve, fixed by ligature at the apex of the heart and used to divert pulmonary venous outflow into a reservoir. A third ligature was placed above the atrioventricular junction to prevent perfusate flowing back into the ventricles. The lungs were perfused with the chosen perfusate using a peristaltic pump (Minipulse 2, Gilson Medical Electronic, Middleton, WI, USA) at a constant flow of 0.03 ml/min/g body weight. An initial 75 ml of chosen perfusate, which contained residual blood cells and plasma, were discarded and not recirculated. An additional 25 ml of the chosen perfusate was recirculated in the lung. Pulmonary artery (Ppa) and pulmonary venous (Ppv) pressures were continuously monitored with pressure transducers (P23 ID; Statham, Oxnard, CA) from a sidearm of the inflow and outflow cannulas and continuously recorded on a polygraph recorder (Gould Instruments, Cleveland, OH, USA). The Ppv was set at 2.5 mm Hg by adjusting the height of the

venous outflow reservoir and zone III flow conditions (arterial>venous>alveolar pressures) were maintained in all experiments.

The isolated perfused lung remained *in situ*, and the weight of the whole rat was monitored on an electronic balance and recorded on an oscillograph after digital-to-analog conversion. Any change in the preparation weight (body weight) was considered a result of changes in lung weight (5-9). Three criteria were used to continue the isolated lung preparation experiment: [1] no leakage was observed at the sites of cannula insertion, [2] no evidence of edema was present, and [3] the lung attained an isogravimetric state, i.e. the lung was neither gaining nor losing weight.

*Perfusates*

Physiological salt solution (PSS) was the perfusate and it contained 4% bovine serum albumin (Sigma Chemical, St. Louis, MO, USA) and (in mM) 119 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 22.6 $NaHCO_3$, 1.18 $KH_2PO_4$, 3.2 $CaCl_2$, and 5.5 glucose. It was used as perfusate in all of studied groups. The therapeutic agents were anti-TNF-α poly clonal rat antibody (Peprotech, Rocky Hill, NJ, USA) and anti-ICAM-1 antibody (Peprotech, Rocky Hill, NJ, USA).

*Determination of Pulmonary Capillary Pressure*

The pulmonary capillary pressure (Ppc) was estimated using the double-occlusion method (16). Arterial inflow and venous outflow lines were simultaneously occluded and the equilibrium Ppa and Ppv were measured. This equilibration pressure is the same as the isogravimetric measure of Ppc and also reflects the prevailing capillary pressure when the lungs are damaged.

*Calculation of Pulmonary Vascular Resistance*

The pulmonary arterial (Ra) and venous (Rv) resistances were calculated using the following equations: Ra = (Ppa - Ppc)/Q, and Rv = (Ppc - Ppv)/Q, respectively, where Q is perfusate flow and Ppa and Ppv are the arterial and venous pressures.

*Measurement of Microvascular Permeability*

Pulmonary capillary filtration coefficient ($K_{fc}$) has been used as an index of microvascular permeability to solvent in many published studies (16). The $K_{fc}$ was measured using a method described previously. Briefly, after an isogravimetric state was attained in the lung, Ppv was rapidly elevated to 6 to 8 cm $H_2O$ for 15 min. The increase in lung weight was recorded. The recording showed a characteristic rapid weight gain (vascular filling) which was followed by a slower rate of weight gain. The rate of weight change ($\Delta W/\Delta t$) occurring in the 6- to 14-min interval was analyzed using linear regression of the log10-transformed rates of weight changes calculated at each minute. The initial rate of weight gain was then determined by extrapolation of ($\Delta W/\Delta t$) to zero time. $K_{fc}$ was then calculated by dividing ($\Delta W/\Delta t$) at time 0 by the change in Ppc that was imposed after venous outflow pressure was increased. The $K_{fc}$ value was normalized using the baseline wet lung weight and expressed as ml/ min /cm $H_2O$/ 100 g lung tissue.

*Experimental Protocols*

Acute lung injury was induced by 45 min of ischemia (I) followed by 90 min of reperfusion (R) with PSS as perfusates as control group (group 1). Experimental groups were divided into five groups. The anti-TNF-α antibody (group 2), anti- ICAM-1 antibody (group 3), or combination (anti-TNF-α+ anti- ICAM-1 antibody) (group 4) were added at 15 min of reperfusion for study the therapeutic effect on I/R. The anti-TNF-α antibody (group 5), and anti-ICAM-1 antibody (group 6) were added before 15 min of ischemia for study the prevention on I/R lung injury. The concentration of anti-TNF-α antibody was 1200 pg/ml in 40 ml PSS perfusate.

The concentration of anti-TNF-α antibody based on our previous study which showed this concentration of anti-TNF-α antibody as additives to promote protection by University Wisconsin solution in I/R injury (10) and anti-ICAM-1 antibody (4 μg/ml in 40 ml PSS perfusate). The concentration of anti-ICAM-1 antibody based on the previous study (9, 46).

The isolated lungs were perfused with PSS as perfusates and challenged by 45 min ischemia followed by 90 min reperfusion. The closed system of circulation was maintained at constant flow, volume and temperature. The experiment was initiated after hemodynamic stability for 15 min in the extracorporeal isolated lung circulation was achieved. The protocols of I/R injury challenge were as follows: the isolated lung was not ventilated and perfused (ischemia) for 45 min, then followed by reinstitution of ventilation and perfusion (reperfusion) for 90 min at room temperature.

*Lung Histopathology*

After termination of each experiment, the middle lobes of the right lungs were dissected and immediately fixed in 10% neutral buffered formalin. After fixation, the right middle lobes were dehydrated through a graded series of alcohol, cleared in xylene, and embedded in paraffin. All sections were cut at 5 μm and stained with hematoxylin-eosin. The severity of perivascular,

**Table 1. Lung weight gains (LWG)**

| Group | N | LWG (g) |
|---|---|---|
| PSS | 7 | 3.33 ± 0.80 |
| **After I and during R** | | |
| PSS+anti-TNF-α | 7 | 0.86 ± 0.32* |
| PSS+anti-ICAM | 7 | 1.10 ± 0.43* |
| PSS+ anti-TNF-α+ anti-ICAM | 7 | 0.87 ± 0.47* |
| **Before I/R** | | |
| PSS+ anti-TNF-α | 7 | 0.53 ± 0.23* |
| PSS+anti-ICAM | 7 | More than 8 g just restart reperfusion in few mins |

Value are mean ±SD, PSS: physiological salt solution; I: ischemia; R: reperfusion; I/R: ischemia/reperfusion lung injury; *: $P < 0.05$ compared with PSS.

peribronchial, septal, alveolar edema and perivascular, interstitial , alveolar cell infiltration were examined by score system. According previous study (5), the score method to measure the severity of acute lung injury as followings: perivascular edema=1, peribronchial edema =2, interstitial edema=2 and alveolar edema=3, perivascular cell infiltration=2, interstitial cell infiltration =3 and alveolar cell infiltration= 4. 20 scope views were examined in each lung tissue. Sum of all pathologic scores was the score of each scope, and then we calculated the mean score of 20 scopes as the injury score of this lung tissue. After a blinded review by two pathologists, mean of two scores of two pathologists was the final score.

*Statistical Analysis*

Values are expressed as "mean ± SD". Comparisons among all groups for a given variable were done using a one-way analysis of variance and Dunnett's method of post-hoc testing. A $P < 0.05$ was considered a statistically significant difference.

## Results

*Lung Weight Gains (LWG)*

There was less LWG in therapeutic group treated with single agent (anti-TNF-α antibody or anti-ICAM-1 antibody) or combined agents (anti-TNF-α antibody + anti-ICAM-1 antibody) during reperfusion, than those in control groups with PSS as control (Table 1). Furthermore the group with anti-ICAM-1 antibody was given before ischemia developed pulmonary edema immediately and could not complete 90 min reperfusion challenge. LWG of groups treated with combined agents (anti-TNF-α antibody + anti-ICAM-1 antibody) showed no significant difference compared with groups treated with single agent (anti-TNF-α antibody). This indicates anti-TNF-α antibody has prevention and therapeutic effect on pulmonary edema, but anti- ICAM-1 antibody seemed to have only therapeutic effect. Furthermore, pretreatment of anti-ICAM-1 antibody induced severe pulmonary edema. Although combination therapy of both agents attenuated pulmonary edema, no additive effect was found.

*Capillary Filtration Coefficient ($K_{fc}$)*

In groups challenged by 45 min of ischemia then 60 min reperfusion, we found that less $K_{fc}$ in therapeutic group treated with anti-TNF-α antibody before ischemia or during reperfusion than those in control groups with PSS as control (Table 2). $K_{fc}$ of the groups treated with anti-ICAM-1 antibody or combined (anti-TNF-α antibody and anti-ICAM-1 antibody) during reperfusion was less than those in control group. These findings indicated anti-TNF-α antibody prevents and treats the capillary leakage. Furthermore, no additive therapeutic effect of combined therapy on capillary leakage was found in I/R lung injury.

*Hemodynamic Changes*

In control group with PSS as perfusate showed an increase of Pa, Pc, Ra and Rv after I/R lung injury but not statistically significant. However, Pa, Pc, Ra and Rv of treated groups with treatment with anti-ICAM antibody and anti-TNF-α showed a decrease of these values.

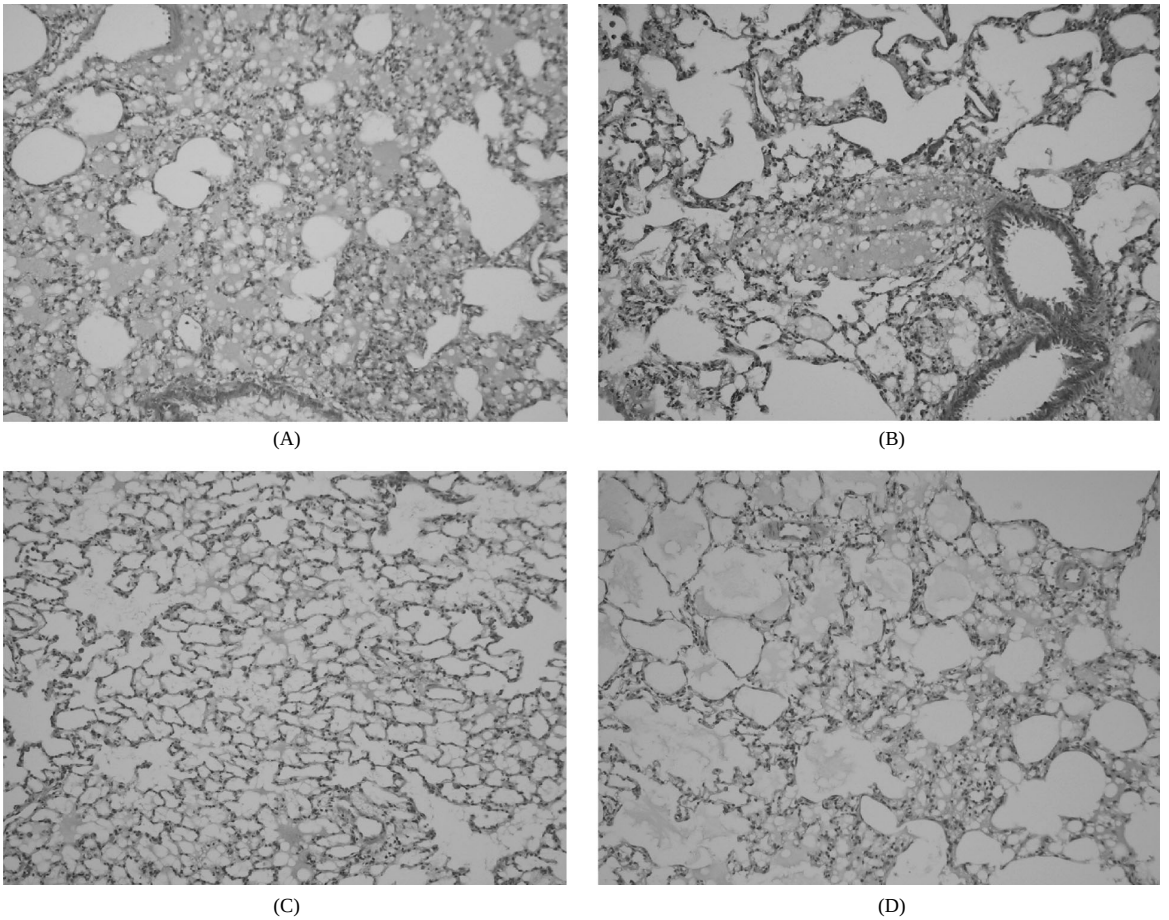
*Histological Findings*

The histological findings following I/R lung injury

**Table 2. Capillary filtration coefficients ($K_{fc}$: $ml.cmH_2O^{-1}.100g^{-1}$ lung weight)**

| Group | N | Baseline | After I/R |
|---|---|---|---|
| PSS | 7 | 0.11 ± 0.09 | 0.50 ± 0.11 |
| **After I and during R** | | | |
| PSS+anti-TNF-α | 7 | 0.07 ± 0.08 | 0.13 ± 0.11* |
| PSS+anti-ICAM-1 | 7 | 0.06 ± 0.07 | 0.20 ± 0.08* |
| PSS+ anti-TNF-α +anti-ICAM-1 | 7 | 0.06 ± 0.03 | 0.16 ± 0.05* |
| **Before I/R** | | | |
| PSS+ anti-TNF-α | 7 | 0.05 ± 0.04 | 0.14 ± 0.15* |
| PSS+ anti-ICAM | 7 | Severe edema can not measure | |

Value are mean ± SD, PSS+anti-TNF-α (After I and during R, and Before I/R) were significantly less than the PSS. (*: $P < 0.05$).



(A) (B) (C) (D)

Fig. 1. Light micrograph of lung tissue suffering ischemia/reperfusion lung injury, stained with hematoxylin/eosin. Using physiologic salt solution (PSS) as perfusate, isolated rat lung were challenge by 45 min ischemia then 90 min reperfusion. (A and B) In the control group with PSS as perfusate only (no protective agents were added), there was marked perivascular edema, focal interstitial and intra-alveolar leukocyte infiltration, and proteinaceous exudate. (C) In the groups with anti-TNF-α antibody added during perfusion, less perivascular edema, proteinaceous exudate and leukocyte infiltration were found and it indicated the histological changes were greatly alleviated. Similar findings were found in the group with anti-TNF-α antibody treatment before I/R, anti- ICAM-1 antibody during I/R and combed anti- TNF-α antibody and ICAM-1 antibody treatment (not shown) (D) In the group with ICAM-1 antibody treatment before I/R was similar to PSS group and showed perivascular edema, proteinaceous exudate and leukocyte infiltration. Original magnification x200.

**Table 3. Hemodynamic**

| Group | N | Pap | Pv | Pc | Ra | Rv |
|---|---|---|---|---|---|---|
| | | | Before Ischemia | | | |
| PSS | 7 | 9.90 ± 1.88 | 1.20 ± 0.75 | 5.03 ± 0.79 | 0.10 ± 0.03 | 0.08 ± 0.02 |
| **After I and during R** | | | | | | |
| PSS+anti-TNF-α | 7 | 9.40 ± 1.47 | 0.90 ± 0.65 | 4.64 ± 0.55 | 0.10 ± 0.02 | 0.08 ± 0.02 |
| PSS+anti-ICAM-1 | 7 | 9.50 ± 2.08 | 0.60 ± 0.25 | 4.53 ± 0.81 | 0.10 ± 0.03 | 0.08 ± 0.02 |
| PSS+ anti-TNF-α +anti-ICAM-1 | 7 | 11.87 ± 2.32 | 0.75 ± 0.29 | 5.64 ± 1.06 | 0.13 ± 0.03 | 0.08 ± 0.02 |
| **Before I/R** | | | | | | |
| PSS+ anti-TNF-α | 7 | 10.25 ± 3.50 | 0.86 ± 0.25 | 5.00 ± 1.49 | 0.11 ± 0.04 | 0.08 ± 0.03 |
| | | | After I/R | | | |
| PSS | 7 | 10.90 ± 1.67 | 0.90 ± 0.65 | 5.30 ± 0.49 | 0.11 ± 0.03 | 0.09 ± 0.02 |
| **After I and during R** | | | | | | |
| PSS+anti-TNF-α | 7 | 8.00 ± 1.27[a] | 0.90 ± 0.42 | 4.02 ± 0.61[a] | 0.08 ± 0.02[a] | 0.07 ± 0.01[a] |
| PSS+anti-ICAM-1 | 7 | 8.37 ± 1.37[a] | 0.75 ± 0.29 | 4.10 ± 0.64[a] | 0.09 ± 0.02[a] | 0.07 ± 0.01[a] |
| PSS+ anti-TNF-α +anti-ICAM-1 | 7 | 11.87 ± 2.39 | 0.75 ± 0.29 | 5.64 ± 1.13 | 0.14 ± 0.03 | 0.08 ± 0.02 |
| **Before I/R** | | | | | | |
| PSS+ anti-TNF-α | 7 | 8.50 ± 2.38[a] | 0.06 ± 0.25[a] | 4.09 ± 0.95 | 0.09 ± 0.03[a] | 0.07 ± 0.02[a] |

Value are mean ± SD; Ppa and Ppv (mmHg): pulmonary arterial and venous pressure, respectively; Ppc (mmHg): isogravimetric capillary pressure; Ra and Rv (cmHg · min · $ml^{-1}$): arterial and venous resistance, respectively; I: ischemia; R: reperfusion; I/R: ischemia/reperfusion lung injury; PSS+anti-TNF-α (after I and during R ,and before I/R) and PSS+anti-ICAM-1 (after I and during R) was significant less than the PSS after I/R group ([a]: $P < 0.05$).

showed perivascular edema, focally marked intra-alveolar hemorrhage, proteinaceous exudates, interstitial leukocyte infiltrate and intra-alveolar debris, including macrophage and pneumocyte (Fig. 1). Less edema and leukocyte infiltration was found in pre or post-I/R treatment with anti-TNF-α antibody and similar findings in post-I/R treatment of anti-ICAM-1 antibody and combined therapy (Table 3). The severity of these findings was documented by the pathological score (showed in Table 4). The inflammatory cell infiltration in interstitial and alveoli was less in pre or post-I/R treatment with anti-TNF-α antibody than the control group. It indicates anti-TNF-α antibody might have an inhibitory effect on leukocyte adhesion to endothelium or migration into interstitium or alveoli.

## Discussion

Based on the attenuation of I/R lung injury by LWG, $K_{fc}$, and injury score by pathologic finding, we found that anti-TNF-α antibody has both preventive and therapeutic effects on I/R lung injury. In contrast, anti-ICAM-1 antibody only has therapeutic effect.

**Table 4. Acute injury score based on pathological findings**

| Group | N | Score |
|---|---|---|
| PSS | 5 | 9.6 ± 1.5 |
| **After I and during R** | | |
| PSS+anti-TNF-α | 7 | 5.3 ± 1.5* |
| PSS+anti-ICAM-1 | 7 | 6.0 ± 1.4 |
| PSS+ anti-TNF-α +anti-ICAM-1 | 7 | 4.7 ± 1.7* |
| **Before I/R** | | |
| PSS+ anti-TNF-α | 7 | 4.5 ± 0.6* |

Value are mean ± SD, PSS+anti-TNF-α (before ischemia), PSS+ anti-TNF-α+anti-ICAM-1 (after ischemia and during reperfusion) and PSS+ anti-TNF-α (after ischemia and during reperfusion) were significantly less than the PSS. (*: $P < 0.05$)

The combined treatment of anti-TNF-α antibody and anti-ICAM-1 antibody produced attenuation of I/R

lung injury without additive effect.

The present study shows clearly that I/R lung injury includes increased capillary permeability, as reflected by increases in $K_{fc}$ and formation of pulmonary edema, as reflected by LWG consistent with previous findings (5-9). Histological data such as perivascular edema, and interstitial and intra-alveolar hemorrhage, proteinaceous exudate, and interstitial and intra-alveolar leukocyte infiltrates were consistent with our previous studies (5-9).

Pre-I/R, treatment with anti-TNF-α antibody inducing attenuation on I/R lung injury in this study was similar to previous studies (24, 26, 46). In addition, our results showed the groups with post- I/R treatment with anti-TNF-α antibody to have less I/R than control. This indicates that anti-TNF-α antibody might have preventive and therapeutic effects on I/R. Furthermore, less amount of leukocyte infiltration was found in group with anti-TNF-α antibody pre-I/R and post I/R treatment, indicating that anti-TNF-α antibody can prevent lung capillary leakage (reflected by less LWG and $K_{fc}$) and leukocyte migration (reflected by less leukocyte infiltration in interstitium and alveoli). Similarly. Sorkine and colleagues noted that TNF blockade reduced the sequestration of neutrophils and microvascular leakage in the lungs of animals undergoing intestinal I/R (40).

The exact mechanism of action of anti-TNF-α antibody in attenuating I/R injury remains unclear. TNF-α plays an important role in acute lung injury, reminiscent of acute respiratory distress syndrome in many animal models (5, 32, 38). Infusion of appropriate doses of recombinant human TNF-α produces multiple-organ system injury, hypotension, and death in a pattern indistinguishable from endotoxemia (42). Our earlier study showed that elevated TNF-α was found in I/R lung injury and that a significant correlation existed between severity of I/R lung injury and TNF-α expression (5, 6). Our current data showing that pretreatment with anti-TNF-α antibody significantly attenuates lung injury caused by I/R supports the concept of our previous reports (5-9), Yao's and Taylor's studies (24, 47). They also provide evidences that TNF-α might be the main cytokine to participate in the modulation of the early events of lung injury evoked by I/R injury. TNF-α seems to mediate increased vascular permeability through both neutrophil-dependent (17) and neutrophil-independent mechanisms (20). Oxygen radicals have also been demonstrated to be involved in TNF-α-mediated injury. It seems that the accumulation of neutrophils through the action of TNF-α caused by I/R perturbation is involved in the up-regulation of adhesion molecules on both neutrophil and endothelial cells (34). TNF-α was shown to induce the expression of both ICAM-1 and endothelial expression of E-selectin (34, 38), and to mediate neutrophil attachment to endothelial cells resulting in release of neutrophil-derived oxygen metabolites, which leads to vascular and tissue injury (1). Indeed, Seekamp *et al*. (38) reported that anti-TNF-α antibodies attenuate E-selectin expression in the lung vasculature in a rat hind limb I/R model. Similarly, Colletti *et al*. (11) found that TNF blockade reduced pulmonary ICAM-1 expression after hepatic I/R, while Squadrito *et al*. (41) reported that TNF blockade reduced ICAM-1 expression in the aorta and SMA.

Our results showed that anti-ICAM-1 antibody treatment after ischemia attenuates I/R lung injury, but pretreatment with anti-ICAM-1 antibody before I/R did not prevent injury.

The endothelial cell plays a vital role in neutrophil binding through a series of adhesion molecules expressed on its surface. ICAM-1 is an important adhesion molecule, which leads to neutrophil adhesion to endothelium (3, 23), the release of $O_2$ radicals and damage to endothelium (23). Anti-adhesion antibodies have been demonstrated to decrease organ damage and improve survival following hemorrhagic shock and resuscitation in rabbits (31); reduce infarct size in animal models of coronary artery occlusion and reperfusion (21, 47); and decrease liver injury in rats following ischemia-reperfusion injury (22). In animal models of unilateral warm lung I/R injury induced by temporary artery clamping, anti-β2 and anti- ICAM-1 antibodies reduced neutrophil sequestration and pulmonary edema in the reperfused lung (13). Taylor's studies also showed that pretreatment with anti-ICAM antibodies ameliorated I/R lung injury in the isolated rat lung model (30). However, Welty-Wolf's study showed that monoclonal antibodies to ICAM-1 did not ameliorate acute lung injury in E. coli sepsis, and chances of survival are lower in septic primates given anti- ICAM-1 (45). More recently, Lu's study showed pre-I/R treatment with anti-ICAM-1 antibodies reversed I/R-induced increase in pulmonary microvascular leakage. The same study showed that anti-ICAM-1 antibodies did not affect lung myeloperoxidase activity and the circulating neutrophil count (29).

Our result showed that pre-I/R and post-I/R with anti-ICAM-1 antibody have different effect on I/R. We suggest that the increase of ICAM-1 expression occurred after I/R (23) then anti-ICAM-1 antibody to neutralize ICAM-1 might be beneficial for attenuation on I/R with anti-ICAM-1 post-I/R treatment. Why pretreatment with anti-ICAM antibody immediately induced severe pulmonary edema during reperfusion in our study is unknown. One potential reason for the adverse physiologic outcome after anti-ICAM antibody treatment pre-I/R, is a change in proinflammatory cytokine profiles. Welty-Wolf's study reported that

anti-ICAM antibody increased peak levels of interleukin 1 (IL-1) and increased both peak and duration of the elevations in IL-6, IL-8, and tumor necrosis factor receptor in sepsis (44). Previous studies (35, 39, 43), pretreatment of anti-ICAM-1 antibody may have altered host response to I/R lung injury through effects of the antibody on other immune or nonimmune cell function. Blockade of ICAM-1 might redirect immune responses toward more intense inflammation with excessive "bystander" injury (39). We, therefore, suggest that treatment with anti-ICAM-1 antibodies should be given with an appropriate dose at right dosing time during the development of I/R.

In conclusion, TNF-α and ICAM-1 play important roles in the development of the lung injury caused by I/R. Anti-TNF-α antibody alone have therapeutic and preventive effect on I/R lung injury. The combination therapy by using anti TNF-α antibody and anti-ICAM-1 antibody produced no synergistic effect. The effect of various timing and dosing of anti-ICAM-1 antibody on I/R need to be investigated further.

## Acknowlegments

This work was supported by the grant from the Tri-Service General Hospital (TSGH C90-05) and grant from the National Science Council (NSC 90-2314-B-075-141), Taiwan, ROC.

## References

1. Adkins, W.K. and Taylor, A.E. Role of xanthine oxidase and neutrophils in ischemia-reperfusion injury in rabbit lung. *J. Appl. Physiol*. 69: 2012-2018, 1990.
2. Albelda, S.M., Smith, C.W. and Ward P.A. Adhesion molecules and inflammatory injury. *FASEB J*. 8: 504-512, 1994.
3. Almehdi, A.B., Zhao, G., Dodia, C., Tozawa, K., Costa, K., Muzykantov, V., Ross, C., Blecha, F., Dinauer, M. and Fisher, A.B. Endothelial NADPH oxidase as the source of oxidants in lungs exposed to ischemia or high $K^+$. *Circ. Res*. 83: 730-737, 1998.
4. Chang, D.M., Hsu, K., Ding, Y.A. and Chiang, C.H. Interleukin-1 in ischemia-reperfusion acute lung injury. *Am. J. Respir. Crit. Care Med*. 156: 1230-1234, 1997.
5. Chiang, C.H., Yu, C.P., Yan, H.C., Perng, W.C. and Wu, C.P. Cytokine upregulation in ischemia-reperfused lungs perfused with University of Wisconsin solution and normal saline. *Clin. Sci. 101*: 285-294, 2001.
6. Chiang, C.H., Hsu, K., Yan, H.C., Harn, H.J. and Chang, D.M. PGE1, dexamethasone, U-74389, or Bt2-cAMP as an additive to promote protection by UW solution in I/R injury. *J. Appl. Physiol.* 83: 583-590, 1997.
7. Chiang, C.H., Wu, K., Yu. C.P., Perng, W.C., Yan, H.C., Wu, C.P. and Chang, D.M. Protective agents used as additives to promote the protection of ischemia-reperfusion in University of Wisconsin solution (UW). *Clin. Sci*. 95: 369-376, 1998.
8. Chiang, C.H., Wu, K., Yu. C.P., Yan, H.C., Perng, W.C. and Wu, C.P. Hypothermia and prostaglandin E1 produce synergistic attenuation of ischemia-reperfusion lung injury. *Am. J. Respir. Crit. Care Med*. 160: 1319-1323, 1999.
9. Chiang, C.H., Wu, C.P., Peerng, W.C., Yan, H.C. and Yu, C.P. Dexamethasone and pentastarch produce synergistic attenuation on ischemia-reperfusion lung injury. *Clin. Sci*. 99: 413-419, 2000.
10. Chiang, C.H., Wu, C.P., Peerng, W.C., Yan, H.C. and Yu, C.P. TNF-α antibody or 3-deazaadenosine as additives to promote protection by UW solution in I/R injury. *Clin. Sci*. 99: 215-222, 2000.
11. Colletti, L.M., Cortis, A., Lukacs, N., Kunkel, S.L., Green, M. and Strieter, R.M. Tumor necrosis factor up-regulates intracellular adhesion molecule 1, which is important in the neutrophil-dependent lung and liver injury associated with hepatic ischemia and reperfusion in the rat. *Shock* 10: 182-191, 1998.
12. Colletti, L., Remick, D., Burtch, G., Kunkel, S., Strieter, R. and Campbell, D. Role of tumor necrosis factor-alpha in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in rat. *J. Clin. Invest*. 85: 1936-1943, 1990.
13. DeMeester, S.R., Molinari, M.A., Shiraish, T., Okabayashi, K., Manchester, J.K., Wick, M.R., Cooper, J.D. and Patterson, G.A. Attenuation of rat lung isograft reperfusion injury with a combination of anti-ICAM-1 and anti-β2 integrin monoclonal antibodies. *Transplantation* 62: 1477-1485, 1996.
14. DePerrot, M., Liu, M., Wakkell, T.K. and Keshavjee, S. Ischemia reperfusion induced lung injury. *Am. J. Respir. Crit. Care Med*. 167: 490-511, 2003.
15. Doerschuk, C.M., Quinlan, W.M., Doyle, N.A., Bullard, D.C., Vestweber, D., Jones, M.L., Takei, F., Ward, P.A. and Beaudet, A.L. The role of P-selectin and ICAM-1 in acute lung injury as determined using blocking antibodies and mutant mice. *J. Immunol.* 157: 4609-4614, 1996.
16. Drake, R., Gaar, K.A. and Taylor, A.E. Estimation of the filtration coefficient of pulmonary exchange vessels. *Am. J. Physiol*. 234: H266- H274, 1978.
17. Ferrante, A., Nandoskar, M., Walz, A., Goh, A.H. and Kowanko, I. Effects of tumor necrosis factor alpha and interleukin-1 alpha and beta on human neutrophil migration. Respiratory burst and degranulation. *Int. Arch. Allergy Appl. Immunol*. 86: 82-91, 1988.
18. Hogg, J.L. Neutrophil kinetics and lung injury. *Physiol. Rev*. 67: 1249-1287, 1987.
19. Horgan, M.J., Ge, M., Gu, J., Rothlein, R. and Malik, A.B. Role of ICAM-1 in neutrophil-mediated lung vascular injury after occlusion and reperfusion. *Am. J. Physiol. (Heart. Circ. Physiol.)* 261: H1578-H1584, 1991.
20. Horvath, C.J., Ferro, T.J., Jesmok, G. and Malik, A.B. Recombinant tumor necrosis factor increase pulmonary vascular permeability independent of neutrophils. *Proc. Natl. Acad. Sci., USA* 85: 9219-9223, 1988.
21. Ioculano, M., Squadrito, F., Altavilla, D., Canale, P., Squadrito, G., Campo, G.M., Saitta, A. and Caputi, A.P. Antibodies against intercellular adhesion molecule-1 protect against myocardial ischemia-reperfusion injury in rat. *Eur. J. Pharmacol*. 264: 143-149, 1994.
22. Jaeschke, H., Farhood, A., Bautista, A.P., Spolarics, Z., Spitzer, J.J.M. and Smith, C.W. Functional inactivation of neutrophils with a Mac-1 (CD11b/CD18) monoclonal antibody protects against ischemia-reperfusion injury in rat liver. *Hepatoloygy* 17: 915-923, 1993 .
23. Kamochi, M., Kamoch, F., Kin, Y.B., Sawh, S., Sanders, J.M., Sarembock, I., Green, S., Young, J.S., Ley, K., Fu, S.M. and Rose, Jr. C.E. P-selectin and ICAM-1 mediate endotoxin-induced neutrophil recruitment and injury to the lung and liver. *Am. J. Physiol*. 277: L310-L319, 1999.
24. Khimenko, P.L., Bagby, G.J., Fuseler, J. and Taylor, A.E. Tumor necrosis factor-α in ischemia and reperfusion injury in rat lung. *J. Appl. Physiol*. 85: 2005-2011, 1998.
25. Kilpatrick, L.E., Song, Y.H., Rossi, M.W. and Korchak, H.M. Serine phosphorylation of p60 tumor necrosis factor receptor by PKC-delta in TNF-alpha-activated neutrophils. *Am. J. Physiol. (Cell Physiol*.) 79: C2011-C2018, 2000.
26. Koksoy, C., Kuzu, M. A., Kuzu, I., Ergun, H. and Gurhan, I. Role of tumour necrosis factor in lung injury caused by intestinal is-

chemia-reperfusion. *Brit. J. Surg*. 88: 464-468, 2001.
27. Lansman, J.B. Endothelial mechanosensors: going with the flow. *Nature* 331: 481-482, 1988.
28. Lo, S.K., Everitt, J.G. and Malik, A.B. Tumor necrosis factor mediates experimental pulmonary edema by ICAM-1 and CD18-dependent mechanism. *J. Clin. Invest.* 89: 981-988, 1992.
29. Lu, Y.T., Chen, P.G. and Liu, S.F. Time course of lung ischemia-reperfusion induced ICAM-1 expression and its role in ischemia-reperfusion lung injury. *J. Appl. Physiol*. 93: 620-628, 2002.
30. Moore, T.M., Khimenko. P., Adkins, W.K., Miyasaka, M. and Taylor, A.E. Adhesion molecules contribute to ischemia and reperfusion- induced injury in the isolated rat lung. *J. Appl. Physiol.* 78: 2245-2252, 1995.
31. Ossa, J.C.D., Malago, M. and Gewertz, B.L. Current research review neutrophil-endothelial cell binding in neutrophil-mediated tissue injury. *J. Surg. Res.* 53: 103-107, 1992.
32. Palace, C.P., Del Vecchio, P.J., Horgan, M.J. and Malik, A.B. Release of tumor necrosis factor after pulmonary artery occlusion and reperfusion. *Am. Rev. Respir. Dis*. 147: 143-147, 1993.
33. Panes, J., Perry, M. and Granger, D.N. Leukocyte-endothelial cell adhesion: avenues for therapeutic intervention. *Brit. J. Pharmacol*. 126: 537-550, 1999.
34. Pohlman, T.H., Stanness, K.A., Beatty, P.G., Ochs, H.D. and Harlan, J.M. An endothelial cell surface factor(s) induced *in vitro* by lipopolysaccharide, interleukin-1 and tumor necrosis factor-$\alpha$ increases neutrophil adherence by a CDw18-dependent mechanism. *J. Immunol*. 136: 4548-4553, 1986.
35. Salomon, B. and Bluestone, J.A. Cutting edge: LFA-1 interaction with ICAM-1 and ICAM-2 regulated Th2 cytokine production. *J. Immunol*. 161: 5138-5142, 1998.
36. Schmeling, D.J., Gaty, M.G., Oldham, K.T., Guice, K.S. and Hinshaw, D.B. Evidence for neutrophil-related acute lung injury after intestinal ischemia reperfusion. *Surgery* 106: 195-202, 1989.
37. Serrick, C., Franchesca, S.L., Giaid, A. and Shennib, H. Cytokine interleukin-2, tumor necrosis factor-alpha, and interferon-$\gamma$ release after ischemia/reperfusion injury in a novel lung autograft animal model. *Am. J. Respir. Crit. Care Med*. 152: 277-282, 1995.
38. Seekamp, A., Warren, J.S., Remick, D.G., Till, G.O. and Ward, P.A. Requirements for tumor necrosis factor-[alpha] and interleukin-1 in limb ischemia/reperfusion injury and associated lung injury. *Am. J. Pathol*. 143: 453-463, 1993.
39. Sieg, S., Smith, D. and Kaplan, D. Differential activity of soluble versus cellular Fas ligand: regulation by an accessory molecule. *Cell Immunol*. 195: 89-95, 1999.
40. Sorkine, P., Setton, A., Halpern, P., Miller, A., Rudick, V. and Marmor, S. Soluble tumor necrosis factor receptors reduce bowel ischemia-induced lung permeability and neutrophil sequestration. *Crit. Care Med*. 23: 1377-1381, 1995.
41. Squadrito, F., Altavilla, D., Squadrito, G., Ferlito, M., Campo, G.M. and Arlotta, M. Tacrolimus suppresses tumour necrosis factor-alpha and protects against splanchnic artery occlusion shock. *Brit. J. Pharmacol*. 127: 498-504, 1999.
42. Tracey, K.J., Beutler, B., Lowry, S.F., Merryweather, J., Wolpe, S., Milsark, I.W., Harriri, R.J. and Fahey, T.J. Shock and tissue injury induced by recombinant human cachectin. *Science* 234: 470-474, 1986.
43. Van de Stolpe, A. and Van der Saag, P.T. Intercellular adhesion molecule-1. *J. Mol. Med*. 74: 13-33, 1996.
44. Welty-Wolf, K.E., Carraway, M.S., Ghio, A., Kantrow, S.P., Huang, Y.C.T. and Piantadosi, C.A. Pro-inflammatory cytokines increase in sepsis after anti-adhesion molecule therapy. *Shock* 13: 404-409, 2000.
45. Welty-Wolf, K.E., Carraway, M.S., Huang, Y.U.T., Simonson, S.G., Kantrow, S.P., Kishimoto, T.K. and Piantadosi, C.A. Antibody to intercellular adhesion molecule 1 (CD54) decreases survival and not lung injury in baboons with sepsis. *Am. J. Respir. Crit. Care Med*. 163: 665-673, 2001.
46. Yao, Y.M., Bahrami, S., Redl, M. and Schlag, G. Monoclonal antibody to tumor necrosis factor-alpha attenuates hemodynamic dysfunction secondary to intestinal ischemia / reperfusion in rats. *Crit. Care Med*. 24: 1547-1553, 1996.
47. Yamazaki, T., Seko, Y., Tamatani, T., Miyasaka, M., Yagita, H., Okumura, K., Nagai, R. and Yazaki, Y. Expression of intercellular adhesion molecule-1 in rat heart with ischemia/reperfusion and limitation of infarct size by treatment with antibodies against cell adhesion molecules. *Am. J .Pathol*. 143: 410-418, 1993.