Table 1. Baseline Clinical Characteristics and Hemodynamics in the Patients with PAH

	1983-2012, n=103	1983-2004, n=66	2005-2012, n=37
Female/male	86/17	53/13	33/4
Age(yrs)	46.9±15.0	46.0±15.4	48.3±14.4
Survivor, n (%)	50 (47.6%)	21 (31.8%)	29 (78.4%)
Type of PAH			
Idiopathic and heritable PAH, n (%)	44 (42.7%)	31 (47.0%)	13 (35.1%)
PAH associated with connective tissue disease, n (%)	39 (37.9%)	25 (37.9%)	14 (37.8%)
PAH associated with congenital heart disease, n (%)	8 (7.8%)	3 (4.5%)	5 (13.5%)
PAH associated with portal hypertension, n (%)	12 (11.7%)	7 (10.6%)	5 (13.5%)
Hemodynamics			
mRAP, mmHg	5.2±6.1	4.3±4.7	6.9±7.8
mPAP, mmHg	47.9 ±13.2	48.8 ±13.9	46.2 ±11.7
mPCWP, mmHg	6.4±3.0	5.7±2.9	7.6±2.7*
CO, L/min	4.2±1.4	3.9±1.2	4.7±1.5*
CI, L/min per m^2	2.7±0.9	2.5±0.8	3.1±1.0*
PVR, dyne sec cm^{-5}	898.7±504.6	984.6±521.2	754.8±446.1*
mSAP, mmHg	89.3±14.9	90.5±13.4	87.2±17.2
Heart rate, beats/min	78.2±13.8	79.3±13.9	76.4±13.8
SvO_2, %	68.4±8.6	68.3±9.0	68.5±8.0
WHO functional class			
I, n (%)	1 (1.2%)	0 (0%)	1 (2.7%)
II, n (%)	32 (37.2%)	17 (32.7%)	15 (40.5%)
III, n (%)	47 (54.7%)	30 (57.7%)	17 (46.0%)
IV, n (%)	6 (7.0%)	5 (9.6%)	1 (2.7%)
Treatment			
Bosentan, n (%)	22 (21.4%)	8 (12.1%)	14 (37.8%)
Ambrisentan, n (%)	1 (1.0%)	0 (0.0%)	1 (2.7%)
Sildenafil, n (%)	20 (19.4%)	3 (4.5%)	17 (45.9%)
Tadalafil, n (%)	2 (1.9%)	0 (0.0%)	2 (5.4%)
Epoprostenol, n (%)	4 (3.9%)	3 (4.5%)	1 (2.7%)
Oral beraprost, n (%)	36 (35.0%)	20 (30.3%)	16 (43.2%)

*p<0.05; vs 1983-2004

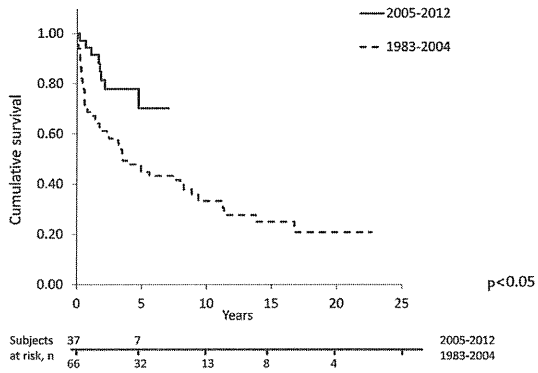

Figure 1. The Kaplan-Meier survival estimates for the PAH patients. The survival rate for patients treated between 2005 and 2012 (solid line) was 70.1% at 5-years compared with 44.8% for patients treated between 1983 and 2004 (dashed line; p<0.05 by the Cox-Mantel log-rank test).

41.5±61.3 months (range: 1 to 276). The mean pulmonary arterial pressure (PAP) and mean pulmonary vascular resistance (PVR) were 47.9 ±13.2 mmHg and 898.7±504.6 dyne. sec. cm^{-5}, respectively.

Survival

Patients diagnosed between 1983 and 2004 (n=66) vs. those diagnosed between 2005 and 2012 (n=37)

We divided the patients into two groups based on the timing of diagnosis (Table 1). We found that the patients diagnosed between 2005 and 2012 had a better survival rate than the patients diagnosed between 1983 and 2004 (5-year survival: 70.1% vs. 44.8%) (p<0.05) (Fig. 1). However, cardiac output (CO) and pulmonary vascular resistance (PVR) at baseline significantly differed between the two groups (Table 1), and these differences make it difficult to attribute the superior outcome to the introduction of ERAs and PDE5 inhibitors.

Outcomes of patients treated with ERAs and/or PDE5 inhibitors (n=36) and those treated with conventional therapy and/or oral beraprost (n=67)

To elucidate the absolute benefits of ERAs and/or PDE5 inhibitors on the survival of Japanese patients with PAH, an analysis was completed comparing the results of patients treated with ERAs and/or PDE5 inhibitors (n=36) and the

Table 2. Baseline Clinical Characteristics and Hemodynamics in the Patients with PAH

	The PAH patients, n=106		The idiopathic and heritable PAH patients, n=44		The associated PAH patients, n=59	
	ERA and/or PDE5 Inhibitors therapy, n=36	PAH therapies without ERA and PDE5 inhibitor, n=67	ERA and/or PDE5 Inhibitors therapy, n=16	PAH therapies without ERA and PDE5 inhibitor, n=28	ERA and/or PDE5 Inhibitors therapy, n=20	PAH therapies without ERA and PDE5 inhibitor, n=39
Female/male	33/3	53/14	13/3	19/9	20/0	34/5
Age(yrs)	47.3±14.4	46.7±15.5	44.8±14.0	41.7±15.2	49.2±14.7	50.2±14.9
Survivor, n (%)	28 (69.4%)	21 (29.9%)	14 (87.5%)	5 (17.9%)	14 (70.0%)	16 (41.0%)
Hemodynamics						
mRAP, mmHg	5.5±7.9	5.1±4.9	3.9±2.7	5.3±6.6	6.8±410.2	5.0±3.4
mPAP, mmHg	44.9 ±11.8	49.5 ±13.7	44.4 ±11.1	54.5±16.7*	45.3 ±12.6	46.2±10.1
mPCWP, mmHg	6.7±2.4	6.2±3.2	6.6±2.5	6.0±3.4	6.8±2.4	6.4±3.2
CO, L/min	4.4±1.1	4.1±1.5	4.4±0.9	3.7±1.4	4.4±1.3	4.4±1.6
CI, L/min per m^2	2.9±0.7	2.7±1.0	2.7±0.5	2.4±0.8	3.0±0.9	2.8±1.1
PVR, dyne $\cdot$ sec $\cdot$ cm^{-5}	771.4±440.3	971.4±527.6*	736.7±338.9	1166.7±609.7*	799.2±514.4	842.9±427.2
mSAP, mmHg	88.8±14.2	89.6±15.3	84.4±14.9	89.2±14.1	92.3±12.9	89.9±16.3
Heart rate, beats/min	75.9±12.7	79.6±14.3	73.1±13.3	83.0±14.3	78.1±12.1	77.2±14.0
SvO_2, %	68.1±7.7	68.5±9.1	67.5±6.1	66.1±9.5	68.7±8.9	70.1±8.7
WHO functional class						
I, n (%)	0 (0.0%)	1 (1.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.6%)
II, n (%)	15 (41.7%)	16 (23.9%)	6 (37.5%)	5 (17.9%)	9 (45.0%)	11 (28.2%)
III, n (%)	12(33.3%)	32(47.8%)	8 (50.0%)	14 (50.0%)	7 (35.0%)	18(46.2%)
IV, n (%)	1 (2.8%)	5 (7.5%)	0 (0.0%)	3 (10.7%)	1 (5.0%)	2 (5.1%)
Treatment						
Bosentan, n (%)	22 (61.1%)	0 (0.0%)	11 (68.8%)	0 (0.0%)	11 (55.0%)	0 (0.0%)
Ambrisentan, n (%)	1 (2.8%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Sildenafil, n (%)	18 (50.0%)	0 (0.0%)	7 (43.8%)	0 (0.0%)	11 (55.0%)	0 (0.0%)
Tadalafil, n (%)	2 (5.6%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	1 (5.0%)	0 (0.0%)
Epoprostenol, n (%)	3 (8.3%)	1 (1.5%)	3 (18.8%)	0 (0.0%)	0 (0.0%)	1 (2.6%)
Oral beraprost, n (%)	14 (38.9%)	22 (32.8%)	7 (43.8%)	8 (28.6%)	7 (35.0%)	14 (35.9%)

*p<0.05; vs ERA and/or PDE5 Inhibitors therapy

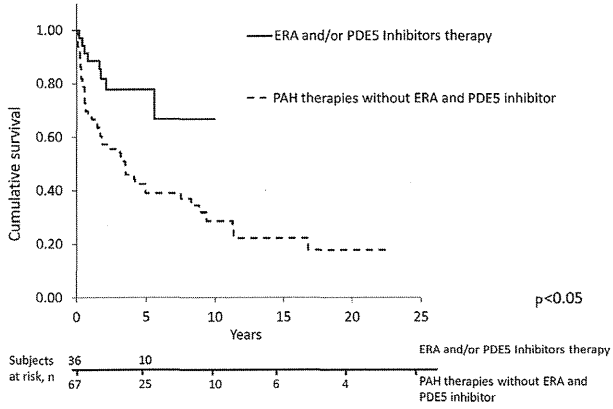

Figure 2. The Kaplan-Meier survival estimates for the PAH patients. The survival rates for patients treated with ERA and/or PDE5 inhibitor therapy (solid line) were 77.8% and 66.7% at five and eight years compared with 39.0% and 37.0% for patients treated with PAH therapies without ERA or PDE5 inhibitors (dashed line; p<0.05 by the Cox-Mantel log-rank test).

results of patients treated without ERAs and/or PDE5 inhibitors (n=67) (Table 2). A significant difference was observed between the two groups in the Kaplan-Meier survival curve (77.8% and 66.7% vs. 39.0% and 37.0%, respectively) (p< 0.05) (Fig. 2) and in PVR (Table 2). In particular, the patients with idiopathic and heritable PAH treated with ERAs and/or PDE5 inhibitors (n=16) showed significantly better survival outcomes than those not treated with these drugs (n=28) (Table 2) (5- and 8-year survival: 92.9% and 69.6% vs. 26.0% and 20.8%, respectively) (p<0.05) (Fig. 3). However, in the associated PAH patients (Table 2), no significant differences were observed between the groups (Fig. 4).

A univariate Cox proportional hazard analysis showed that cardiac index (CI), mean pulmonary arterial pressure (mPAP) and the use of ERA and/or PDE5 inhibitor therapy were associated with cumulative survival. Moreover, CI and ERA and/or PDE5 therapy were the significant predictors of survival in the multivariate analysis. The use of ERA and/or PDE5 inhibitor therapy was an independent predictor for superior outcomes (Table 3).

Discussion

The data presented here show that the patients treated be-

Table 3. Univariate and Multivariate Cox Proportional Hazards Models for the Predictors of Survival

Factors	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
CI (L/min per m^2)	0.56 (0.423-0.747)	0.0001	0.60 (0.458-0.810)	0.0008
mPAP (mmHg)	1.03 (1.005-1.048)	0.0160	1.02 (0.992-1.038)	0.1875
ERA and/or PDE5 Inhibitors therapy (vs. PAH therapies without ERA and PDE5 inhibitor)	0.32 (0.137-0.636)	0.0008	0.39 (0.168-0.796)	0.0084

ERAs: endothelin receptor antagonists, PDE5: phosphodiesterase type 5 (PDE5)

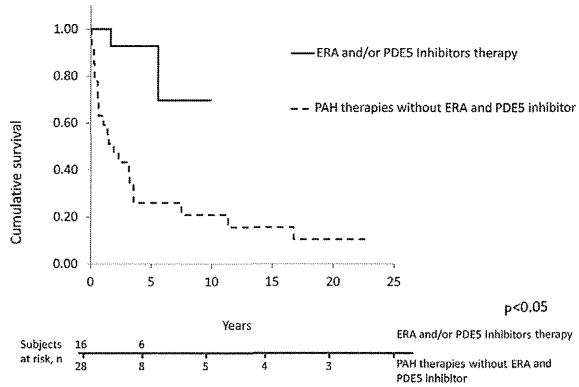

Figure 3. The Kaplan-Meier survival estimates for the idiopathic and heritable PAH patients. The survival rates for patients treated with ERA and/or PDE5 inhibitor therapy (solid line) were 92.9% and 69.6% at five and eight years compared with 26.0% and 20.8% for patients treated with PAH therapies without ERA or PDE5 inhibitors (dashed line; p<0.05 by the Cox-Mantel log-rank test).

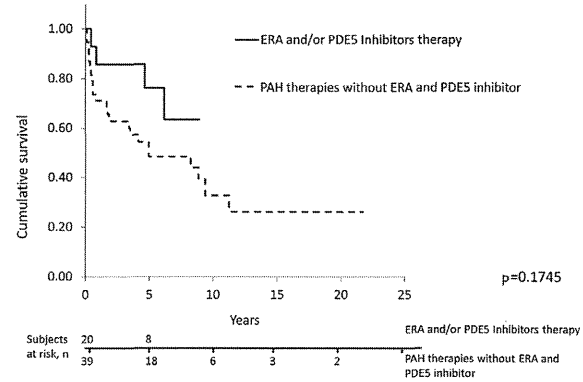

Figure 4. The Kaplan-Meier survival estimates for the associated PAH patients. The survival rates for patients treated with ERA and/or PDE5 inhibitor therapy (solid line) were 76.2% and 63.5% at five and eight years compared with 48.5% and 32.6% for patients treated with PAH therapies without ERA or PDE5 inhibitors (dashed line; p=0.1745 by the Cox-Mantel log-rank test).

tween 2005 and 2012 had a better survival rate (p<0.05) (Fig. 1); however, these patients had significantly less serious hemodynamic alterations (Table 1). The hemodynamic difference observed between the groups makes it difficult to attribute the superior outcome to the introduction of ERAs and PDE5 inhibitors (Fig. 1).

Although it is beyond the scope of this paper to argue delaying the diagnosis, the presence of less serious hemodynamic alterations in the patients diagnosed between 2005 and 2012 suggests that earlier detection of PAH in patients with mild/moderate hemodynamic changes was achieved more often in that group than in the group diagnosed between 1983 and 2004. Since signs and symptoms of PAH do not generally manifest until hemodynamic changes are advanced, there are significant delays in diagnosing this disease. Although primary pulmonary hypertension (PPH) registry data from 1987 show that, at that time, the time from symptom onset to diagnosis with catheterization was 2.3 years (12), there have not been any recent advances in the diagnostic processes. The registry to evaluate early and long-term PAH (REVEAL) study showed that the average time from onset to diagnosis is still more than two years (13). The advances observed in this study may be based on the development and approval of oral therapies and increased doctor recognition of PAH following the introduction of these drugs.

This single-center, uncontrolled study demonstrated that idiopathic and heritable PAH patients treated with ERAs and/or PDE5 inhibitors (n=13) have a higher survival rate than those treated with conventional therapy and/or oral beraprost (Fig. 3, Table 2). In line with previous reports (3), this result may support the concept that the use of these drugs provides benefits for the survival of Japanese patients with PAH. Nevertheless, the benefits of ERAs and/or PDE5 inhibitors on survival may be restricted to idiopathic and heritable PAH patients because no significant differences were observed between the groups in the associated PAH patients (Table 2, Fig. 4).

Because oral beraprost has a weak recommendation in the PAH evidence-based treatment algorithm (4) and has so far only been approved in Japan and Korea (10), this study was conducted without regard to oral beraprost therapy. However, this is a limitation of this study. It is impossible to deny that oral beraprost has a beneficial effect on the treatment of PAH. A randomized and properly controlled dose-response study of beraprost is currently underway (14).

There may be beneficial effects of high-dose oral beraprost on exercise capacity and hemodynamics in patients with PAH.

Epoprotenol was approved in Japan in 1999, and 23 patients treated after 1999 died in our center. In this study, only four patients (three between 1983 and 2004 and one between 2005 and 2012) were treated with epoprostenol and all survived. Although epoprotenol is strongly recommended by the WHO/NYHA class IV according to recent guidelines, we were unable to administer intravenous treatments in some of the 23 non-survivor cases because the patients were elderly (>70 years of age) (n=2), had comorbidities (n=5) or were unwilling (n=6) to undergo intravenous treatments. However, the early administration of epoprostenol therapy is suggested to improve survival in patients with a PVR >1,000 dynes.sec.cm^{-5}.

The data presented in this study were limited because this was an observational study from a single center and the PAH patients were not treated in a randomized manner according to hemodynamics and comorbidities, i.e., this study included patients treated with conventional therapy, which may have favorably biased the results. We realize the limitations of interpreting our results. We interpreted the results of this study as part of a hypothesis-generating analysis, which suggested that there are beneficial effects of treatment with ERAs and/or PDE5 inhibitors on overall survival in idiopathic and heritable PAH patients. This hypothesis will need to be further investigated in a large confirmatory long-term trial in the future.

This study has evolved over the 28-year time period of our practice. The results of six minute walk distance (6 MWD) tests and brain natriuretic peptide (BNP) tests were not obtained as consistently in the past as they have been in the most recent eight years. For this reason, in this observational study, we evaluated survival benefits only in PAH patients treated with PAH-specific therapy in comparison to patients treated with conventional therapy, instead of comparing the 6MWD and BNP results.

In conclusion, this study suggests that superior survival rates are observed in patients with idiopathic and heritable PAH after the introduction of ERAs and PDE5 inhibitors, and the use of these drugs provides a survival benefit in patients with idiopathic and heritable PAH.

Author's disclosure of potential Conflicts of Interest (COI).
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CD40 amplifies Fas-mediated apoptosis: a mechanism contributing to emphysema

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Departments of [1]Respirology, [3]Medical Oncology, and [4]Developmental Genetics, Graduate School of Medicine, Chiba University, Chiba, Japan; [2]Laboratory for Immune Diversity, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Kanagawa, Japan; [5]Department of Pathology, School of Medicine, Toho University, Tokyo, Japan; [6]Department of Dermatology, Peking University First Hospital, Beijing, China; [7]Department of Pathology, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan; [8]Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University, Nashville, Tennessee

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Shigeta A, Tada Y, Wang J-Y, Ishizaki S, Tsuyusaki J, Yamauchi K, Kasahara Y, Iesato K, Tanabe N, Takiguchi Y, Sakamoto A, Tokuhisa T, Shibuya K, Hiroshima K, West J, Tatsumi K. CD40 amplifies Fas-mediated apoptosis: a mechanism contributing to emphysema. *Am J Physiol Lung Cell Mol Physiol* 303: L141–L151, 2012. First published May 18, 2012; doi:10.1152/ajplung.00337.2011.—Excessive apoptosis and prolonged inflammation of alveolar cells are associated with the pathogenesis of pulmonary emphysema. We aimed to determine whether CD40 affects alveolar epithelial cells and endothelial cells, with regard to evoking apoptosis and inflammation. Mice were repeatedly treated with agonistic-anti CD40 antibody (Ab), with or without agonistic-anti Fas Ab, and evaluated for apoptosis and inflammation in lungs. Human pulmonary microvascular endothelial cells and alveolar epithelial cells were treated with agonistic anti-CD40 Ab and/or anti-Fas Ab to see their direct effect on apoptosis and secretion of proinflammatory molecules in vitro. Furthermore, plasma soluble CD40 ligand (sCD40L) level was evaluated in patients with chronic obstructive pulmonary disease (COPD). In mice, inhaling agonistic anti-CD40 Ab induced moderate alveolar enlargement. CD40 stimulation, in combination with anti-Fas Ab, induced significant emphysematous changes and increased alveolar cell apoptosis. CD40 stimulation also enhanced IFN-γ-mediated emphysematous changes, not via apoptosis induction, but via inflammation with lymphocyte accumulation. In vitro, Fas-mediated apoptosis was enhanced by CD40 stimulation and IFN-γ in endothelial cells and by CD40 stimulation in epithelial cells. CD40 stimulation induced secretion of CCR5 ligands in endothelial cells, enhanced with IFN-γ. Plasma sCD40L levels were significantly increased in patients with COPD, inversely correlating to the percentage of forced expiratory volume in 1 s and positively correlating to low attenuation area score by CT scan, regardless of smoking history. Collectively CD40 plays a contributing role in the development of pulmonary emphysema by sensitizing Fas-mediated apoptosis in alveolar cells and increasing the secretion of proinflammatory chemokines.

alveolus; inflammation; IFN-γ; chemokine regulated on activation normal T cells expressed and secreted (RANTES)

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is characterized by the progressive development of airflow limitation that is not fully reversible. COPD is a leading global cause of morbidity and mortality, and the number of patients with COPD is expected to increase as the world population continues to age. The pathogenesis of COPD is complex, and several mechanisms are involved, including chronic airway inflammation, protease/antiprotease imbalance, cell senescence, and deficiency of growth and/or angiogenic factors (3, 6, 8). Several animal models of COPD have been established, including chronic inhalation of lipopolysaccharide (5), repeated exposure to ozone (35), interferon (IFN)-γ transgenic mice (39), and the vascular endothelial growth factor (VEGF) signaling blockade model (22). In each of these models excessive apoptosis of alveolar component cells is closely associated with the pathogenesis of COPD, especially of pulmonary emphysema.

CD40 is a member of the tumor necrosis factor receptor superfamily and binds to CD40 ligand (CD40L). The CD40/CD40L plays a central role in the activation of adaptive immunity (18) but is also involved in the induction of apoptosis in overactivated cells (14, 38). Whether CD40 stimulation induces or protects from apoptosis depends on the cell type. Thus CD40 stimulation protects airway epithelial cells from oxidant-induced apoptosis (27); conversely, it enhances Fas-mediated apoptosis in hepatocytes and salivary gland epithelial cells (1, 31). Recent studies demonstrated that the CD40/CD40L system is upregulated in response to cigarette smoking (19), viral infection (36), pulmonary hypertension (12), and hypoxia (7), all of which are associated with COPD. Analysis of CD40L in patients with COPD in the Framingham study showed that high expression was a significant risk factor but only in the context of high levels of smoking (37). Therefore, it has been suggested that CD40L is a context-specific risk factor for COPD, but the effect of CD40 on apoptosis of alveolar component cells, such as alveolar epithelial cells and pulmonary arterial endothelial cells, as well as on inducing sustained inflammation, has not been determined. The aim of this study is to assess whether CD40/CD40L plays a contributing role in COPD, either alone or in combination with other factors.

MATERIALS AND METHODS

Animal treatment protocols. Male C57BL/6J mice (8 wk old) were purchased from CLEA (Tokyo, Japan). All mice were housed in specific pathogen-free animal facility with free access to food and water. In the first set of experiments, mice were divided into eight groups (n = 5): *1*) control (isotype control antibody), *2*) anti-CD40

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antibody (Ab), *3*) IFN-γ, *4*) anti-CD40 Ab + IFN-γ, *5*) anti-Fas Ab, *6*) anti-Fas Ab + anti-CD40 Ab, *7*) anti-Fas Ab + IFN-γ, and *8*) anti-Fas Ab + anti-CD40 Ab + IFN-γ. Agonistic anti-mouse monoclonal antibody to CD40 (FGK45) (Alexis Biochemicals, San Diego, CA) (30 μg) and/or recombinant mouse IFN-γ (Bender MedSystems, Vienna, Austria) (100 ng) and/or anti-mouse monoclonal antibody to Fas (Jo2) (BD Biosciences, San Jose, CA) (0.1 μg/g) were dissolved in a total volume of 60 μl of PBS and injected intratracheally by MicroSprayer (Penn-Century, Philadelphia, PA) every 3 days for a total of eight times. An initial dose-ranging study was also performed to determine the minimum effective dose for each compound. For example, CD40 Ab (30 μg) and IFN-γ (100 ng) were used because these doses increased the cellularity in mice lung in the preliminary study. Furthermore, anti-Fas Ab was used at 0.1 μg/g because this dose did not induce acute lung injury seen in 2-μg/g inhalation in our study and previous report (29). Isotype-matched antibodies were used as controls, including purified NA/LE Rat IgG2a κ isotype control (BD Biosciences) (30 μg) and purified NA/LE Hamster IgG2 λ isotype control (BD Biosciences) (0.1 μg/g). All animal protocols were approved by the Committee on Animal Welfare of Chiba University.

Expression of CD40 and Fas in vivo. After treatment, mice were killed by intraperitoneal injection of pentobarbital sodium (100 mg/kg) and phenotyped on *day 27*. The left lung was filled with 0.5% low melting agarose at a constant pressure of 25 cm H_2O, allowing homogenous expansion of the lung parenchyma. The lungs were fixed in 10% formalin for 48 h, embedded in paraffin, and sectioned sagittally (2 μm) for histological analysis. Immunohistochemistry was performed using the following primary antibodies; anti-CD40 antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-CD95 antibody (1:100; Abcam, Cambridge, UK). A total of 200 cells per mouse ($n = 5$) randomly selected by independent two pathologists and CD40 or Fas-positive cells were counted under a light microscope.

Evaluation of alveolar enlargement. Sections (2 μm) were stained with hematoxylin and eosin, and then the evaluation of mean linear intercept (MLI) was performed as described (9). In brief, 5 fields of 500 × 100 μm grid per mouse were selected randomly, following that the total length of each line divided by the number of alveolar intercepts gave the average distance between alveolate surfaces. All samples were assessed by light microscopy (Nikon ECLIPSE E400).

Evaluation of apoptosis in vivo. Evaluation of apoptotic cells in mouse lung were determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) and immunohistochemical staining of activated caspase-3.

TUNEL staining was performed using an ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Millipore, Billerica, MA), according to the manufacturer's instructions. Briefly, after deparaffinization and rehydration, sections were digested with proteinase K at a concentration of 20 μg/ml for 15 min. Endogenous peroxidase activity was quenched with 3% H_2O_2 for 5 min. The slides were incubated in a humid atmosphere at 37°C for 60 min with terminal deoxynucleotidyl transferase (TdT) buffer containing TdT and digoxigenin-dNTP. The slides were then washed with PBS and incubated with anti-digoxigenin-peroxidase for 30 min. After being rinsed with PBS, the slides were immersed in diaminobenzidene solution. The slides were counterstained for 10 min with 0.5% methyl green.

Caspase-3 staining was performed using cleaved caspase-3 antibody (1:100; Cell Signaling, Beverly, MA) as a primary antibody. After deparaffinization and rehydration, sections were heated at 120°C in autoclaved sterilizer for 10 min and naturally cooled for 30 min. They were then exposed to 1% hydrogen peroxide/methanol for 30 min to block endogenous peroxidase activity and rinsed in TBS. Next they were treated with 8% skimmed milk for 30 min. Cleaved caspase-3 antibody in TBS was applied to the sections in a moisture chamber at 4°C overnight. They were then sequentially treated with biotinylated secondary antibody and peroxidase-labeled streptavidin.

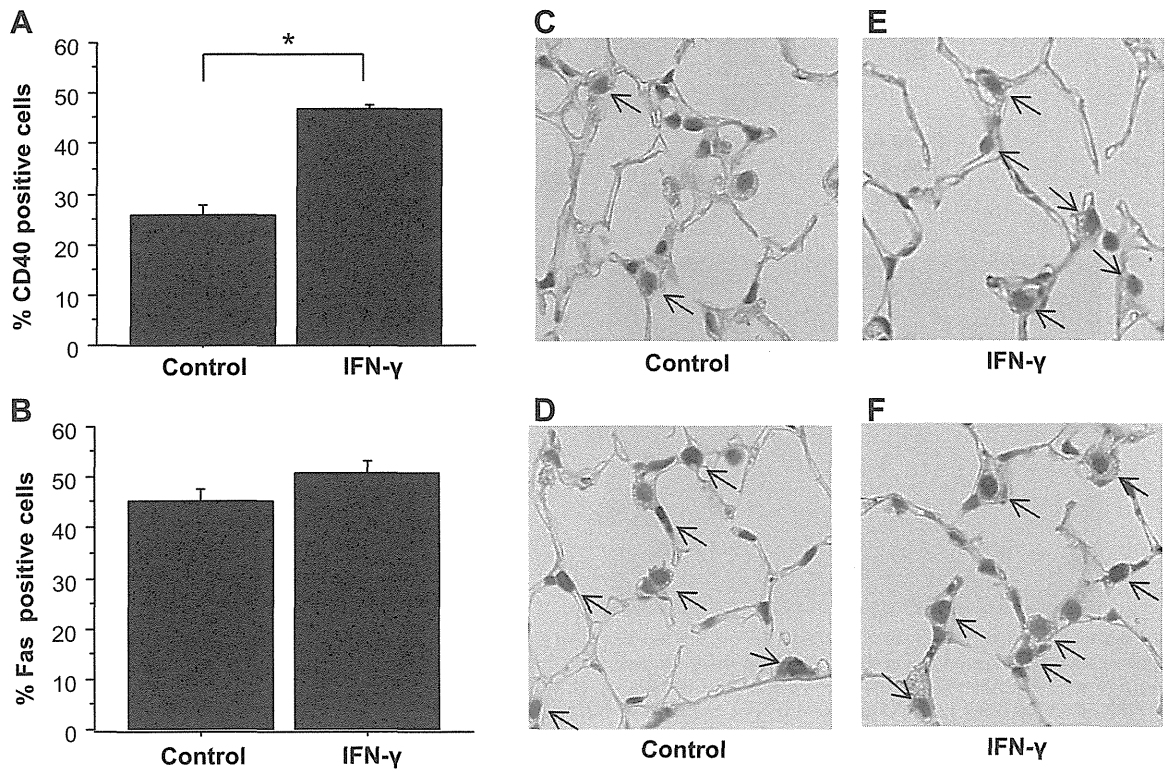


Fig. 1. CD40 (*A*) and CD95 (Fas, *B*) expression in mouse lung alveoli; arrows point to positive cells. Expression of CD40 and Fas in mouse lung alveoli after intratracheal injection with isotype control (*C* and *D*) or IFN-γ (*E* and *F*). IFN-γ stimulation significantly induced enhanced CD40 expression (*A*: isotype control 26.0 ± 1.8%, IFN-γ 46.8 ± 1.0%, $P < 0.0001$; $n = 5$). *$P < 0.05$.

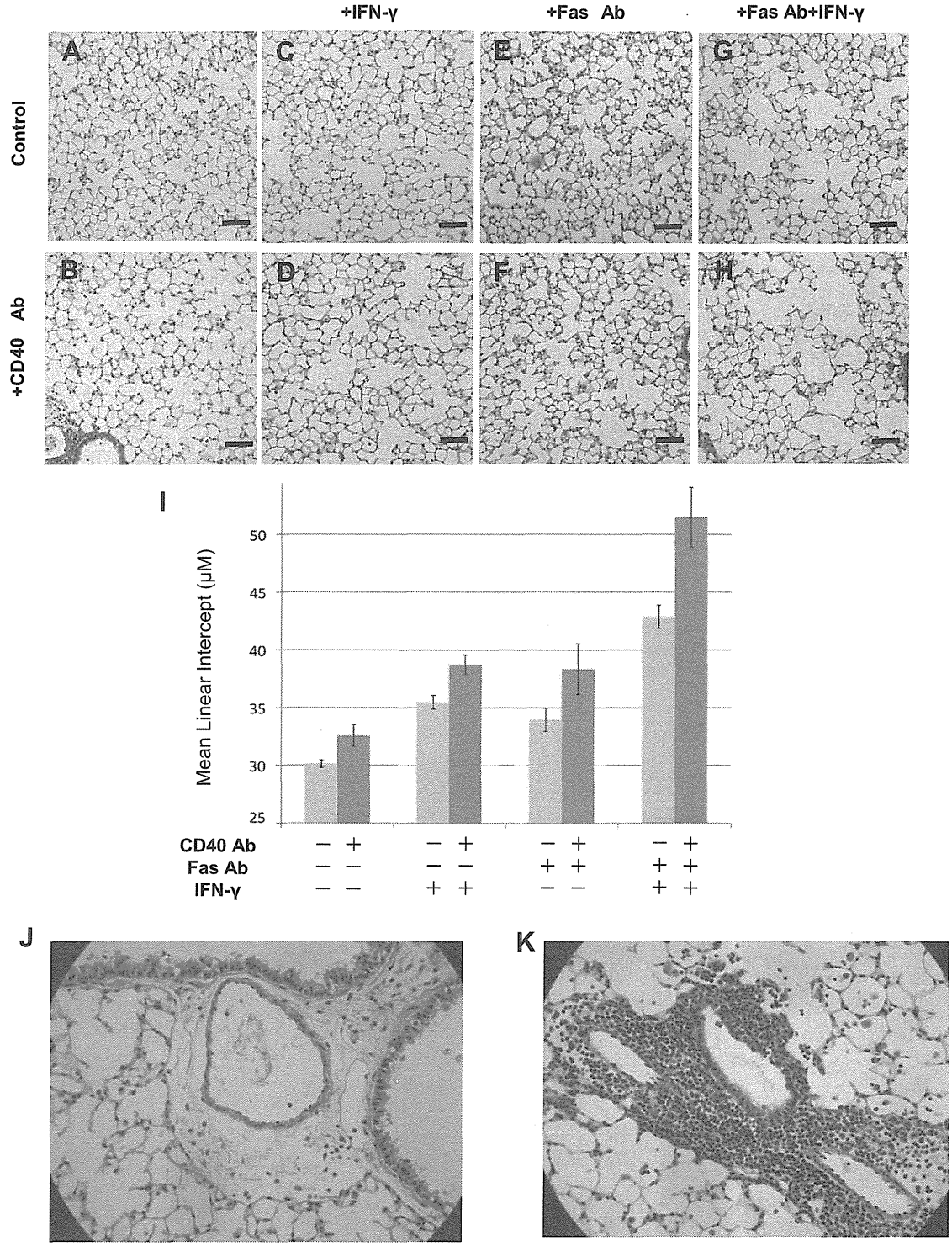


Fig. 2. Repeated inhalation of act-CD40 mAb (CD40 Ab), IFN-γ, and act-Fas mAb (Fas Ab) induced alveolar wall destruction and emphysematous changes in mice. *A–H*: representative hematoxylin and eosin-stained lung tissue sections from 5 mice (×100, scale bar = 50 μm). *A*: CD40 Ab(−) IFN-γ(−) Fas Ab(−); *B*: CD40 Ab(+) IFN-γ(−) Fas Ab(−); *C*: CD40 Ab(−) IFN-γ(+) Fas Ab(−); *D*: CD40 Ab(+) IFN-γ(+) Fas Ab(−); *E*: CD40 Ab(−) IFN-γ(−) Fas Ab(+); *F*: CD40 Ab(+) IFN-γ(−) Fas Ab(+); *G*: CD40 Ab(−) IFN-γ(+) Fas Ab(+); *H*: CD40 Ab(+) IFN-γ(+) Fas Ab(+). *I*: quantification of mean linear intercept (MLI) in mice. Data are means ± SE from 5 experiments. The effects of IFN-γ, CD40 Ab, and Fas Ab as individual factors were significant at $P < 0.0001$ by multiple-factor ANOVA. By Fisher's least-significant-difference (LSD) post hoc analysis, CD40 had a significant effect ($P < 0.05$) in increasing MLI in combination with either Fas Ab or the combination of Fas Ab and IFN-γ, but not when used alone. Mononuclear cell accumulation at perivascular site in mouse lungs after repeated inhalation of CD40 Ab (*K*), compared with isotype control (*J*).

Finally, they were visualized by diaminobenzidene reaction and counterstained with hematoxylin.

A total of 200 cells per mouse (n = 5) randomly selected by two independent pathologists was examined under a light microscope, and TUNEL- and caspase-3-positive cells were counted.

Evaluation of inflammation in lungs by bronchoalveolar lavage. Bronchoalveolar lavage (BAL) of mice was performed as describe elsewhere (40). In brief, the trachea was exposed and lavaged three times with 1 ml of PBS. The BAL fluid (BALF) was centrifuged at 300 *g* for 5 min at 4°C. Total cell counts and the differential cell counts were determined using hemocytometer by counting 200 cells per mouse stained with Diff-Quick (Sysmex, Kobe, Japan). Resulting supernatants were stored at −80°C and used for measurement of CCR5 ligands [macrophage inflammatory protein (MIP)-1α, MIP-1β, and chemokine regulated on activation normal T cells expressed and secreted (RANTES)] by commercial ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instruction.

Cell culture. Human pulmonary microvascular endothelial cells (HPMVEC) and human pulmonary alveolar epithelial cells (HPAEpiC) were purchased from Lonza (Walkersville, MD) and ScienCell Research Laboratories (Carlsbad, CA), respectively. HPAEpiC was composed of 95% of alveolar type I cell and 5% of alveolar type II cell. HPMVEC were placed in BD BioCoat well plates coated with type I collagen (Becton Dickinson, Franklin Lakes, NJ) and maintained in EGM-2 BulletKit medium (Lonza), consisting of basal medium (CCMD-130) with the following supplements: fetal bovine serum (2.0%), hydrocortisone (0.04%), human epidermal growth factor (0.1%), VEGF (0.1%), human fibroblast growth factor-basic (with heparin, 0.4%), long recombinant 3-insulin-like growth factor-1 (0.1%), gentamicin/amphotericin-B (GA-1000, 0.1%), ascorbic acid (0.1%), and heparin (0.1%). HPAEpiC were placed in BioCoat poly-D-lysine-coated well plates (Becton Dickinson) and maintained in Alveolar Epithelial Cell Medium (ScienCell Research), consisting of basal medium with the following supplements: fetal bovine serum (10 μg/ml), apo-transferrin (10 μg/ml), insulin (5 μg/ml), epidermal growth factor (10 ng/ml), fibroblast growth factor-2 (2 ng/ml), epinephrine (500 ng/ml), hydrocortisone (1 μg/ml), retinoic acid (10^{-7} M), penicillin G sodium salt (100 U/ml), and streptomycin (100 μg/ml). These cells were incubated at 37°C in 5% CO_2 overnight and grown to ~60–80% confluence before being used for further analyses. Cells at *passages 4–6* were used for all experiments.

Surface expression of CD40 and Fas on HPMVEC and HPAEpiC. HPMVEC and HPAEpiC were incubated with phycoerythrin-conjugated anti-human CD40 mAb (R&D Systems) and FITC-conjugated mouse anti-human CD95 mAb (R&D Systems). FITC-conjugated mouse anti-human IgG (R&D Systems) was used as control antibody. Surface expression of CD40 and Fas (CD95) was assessed on a BD FACS Calibur flow cytometer using CELL Quest software (Becton Dickinson). In some experiments, recombinant human IFN-γ (BD Biosciences) (1,000 U/ml) was added to the cultures of HPMVEC and HPAEpiC for 24 h before analysis.

Evaluation of apoptosis in vitro. Functional anti-human CD40 Ab (eBioscience, San Diego, CA) at 0.2, 2.0, and 20.0 μg/ml and/or anti-human Fas Ab (eBioscience) at 2.0 μg/ml were added to HPMVEC and HPAEpiC cultures. After a 12-h incubation, cells were stained with FITC-conjugated Annexin V (Invitrogen, Carlsbad, CA) and examined by flow cytometry. In some experiments, recombinant human IFN-γ (1,000 U/ml) was added, together with anti-human CD40 Ab and/or anti-human Fas Ab.

Secretion of CCR5 ligands into culture supernatants. Culture medium was removed from 60% confluent HPMVEC and HPAEpiC, and cells were washed three times with PBS. Cells were incubated with serum-free medium and functional anti-human CD40 Ab (eBioscience) (2 μg/ml) for 12 h. In some experiments, recombinant human IFN-γ (1,000 U/ml) was added to the cultures. The concentrations of CCR5 ligands, i.e., MIP-1α, MIP- 1β, and RANTES, were determined using a Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions.

Patients. The patient group consisted of 69 subjects with smoking-related COPD, who were recruited from the respiratory outpatient clinic at Chiba University Hospital. On the basis of past history, physical examination, and spirometric data, COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (30), that is, forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio < 0.70 with $\%FEV_1$ < 80%. Participants matched for age, sex, and pack/year smoking history without impaired respiratory function on spirometry were enrolled as healthy smokers (n = 19). All the subjects are not current smokers, and patients with COPD had been clinically stable for more than 2 mo, without any symptoms of acute exacerbation. Subjects with liver disease, renal disease, cancer, autoimmune disease, and infection were excluded from the study.

All studies were approved by the Institutional Review Board of Chiba University Graduate School of Medicine, in the name of "No. 2217 Evaluation of QOL and prognosis in COPD. Written informed consent was obtained from all participants in this study.

CT scan. Evaluation for the presence of emphysema was done using a high-resolution computed tomography (HRCT) at full inspiration (Aquilion 64 scanner; TOSHIBA Medical Systems, Tokyo, Japan). HRCT images were photographed with a window setting appropriate for the lungs [window level −700 to −900 Hounsfield units (HU) and width 800 to 1,000 HU]. The presence of emphysema on HRCT was defined as well-demarcated areas of decreased attenuation compared with a contiguous normal lung and marginated by either a very thin wall (>1 mm), no wall at all, and/or multiple bullae

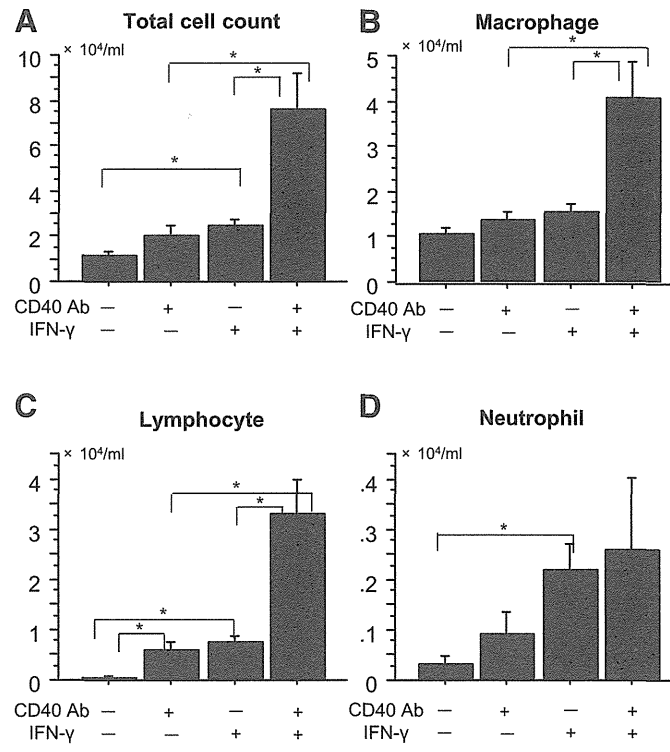


Fig. 3. *A–D*: total and differential cell counts in bronchoalveolar lavage fluid (BALF) after intratracheal injection. Sole CD40 activation significantly increased the number of lymphocytes compared with control (P = 0.0093; n = 4) but further enhanced when combined with IFN-γ. *P < 0.05.

(<1 cm) with an upper lung field predominance. Emphysema was scored visually in bilateral upper, middle, and lower lung fields according to the method used by Goddard et al. (17, 32). Briefly, the score in each field was calculated for the dimensions according to the ratio of low-attenuation area (LAA) as follows: score 0, LAA $< 5\%$; score 1, $5\% \leq$ LAA $< 25\%$; score 2, $25\% \leq$ LAA $\leq 50\%$; score 3, $50\% \leq$ LAA $\leq 75\%$; score 4, $75\% \leq$ LAA. The severity of emphysema was graded in accordance with the sum of scores for six dimensions (minimum score 0 to maximum score 24). CT images were analyzed independently by two pulmonologists with no information of the patients' clinical status.

Measurement of plasma sCD40L. All samples were collected at the time of diagnosis, namely before any intervention for COPD was initiated. Peripheral venous blood samples were collected by single puncture with 18-gauge needle by physicians. All samples were centrifuged at 3,000 *g* for 10 min and stored at −80°C until analysis. Soluble CD40L in plasma were determined using commercial ELISA kit (R&D Systems) according to the manufacturer's instruction.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was performed using Stat View 5.0 (SAS Institute, Cary, NC). Group comparisons were made by using χ^2 test and unpaired Student's *t*-test. Pearson's correlation coefficients and multiple-regression analysis were used to identify the variables that influenced plasma sCD40L level. Multiple-factor analysis of variance was used to assess the independent and synergistic effects of treatment with combinations of CD40 Ab, Fas Ab, and IFN-γ. In these tests, rather than compare any two specific conditions, the statistical test deter-

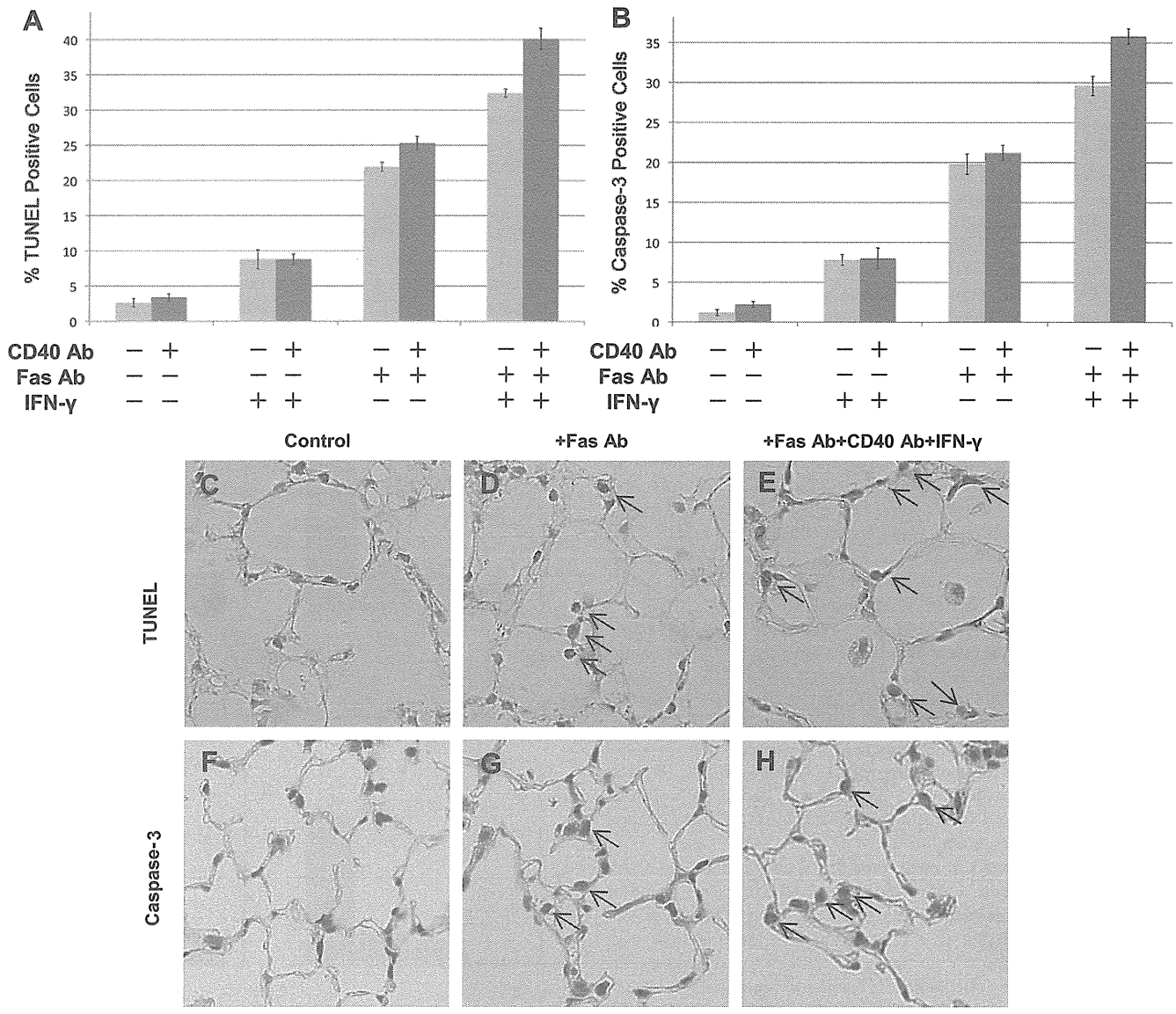


Fig. 4. Quantification of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)- (*A*) and Caspase-3-positive (*B*) cells in mouse lung. Data are means ± SE from 5 experiments. Multiple-factor ANOVA was used to test the effects of IFN-γ, CD40 Ab, and Fas Ab. *C–H*: localization of TUNEL- and Caspase-3-positive cells (white-encircled) in lung tissue sections from 5 mice. By multiple-factor ANOVA, the effects on the TUNEL$^+$ staining of all 3 singly were significant at $P < 0.0001$, and there was synergy between CD40 Ab and Fas Ab ($P = 0.0004$) and also between IFN-γ and Fas Ab ($P < 0.0001$). By Fisher's LSD post hoc analysis, CD40 had a significant effect ($P < 0.05$) in increasing TUNEL staining in combination with either Fas Ab or the combination of Fas Ab and IFN-γ, but not when used alone. By multiple-factor ANOVA, effects of IFN-γ, CD40 Ab, or Fas Ab on the Caspase-3 staining were significant at $P < 0.0001$, $P = 0.003$, and $P < 0.0001$, respectively. Interactions between Fas Ab and both CD40 Ab and IFN-γ were significant at $P = 0.03$ and $P < 0.0001$ respectively. By Fisher's LSD post hoc analysis, CD40 significantly ($P < 0.05$) increased Caspase-3 staining in combination with Fas Ab and IFN-γ, but not under other conditions.

mines whether each factor has significant effect across conditions and then determines whether those effects are altered in an additive or synergistic way when combined with other factors. It is these statistical values that are usually reported, rather than comparisons of any two conditions; where post hoc tests were performed, they were done by Fisher's least-significant difference and reported as such. The statistical significance of dose-response curves was determined by the correlation z-test using log-transformed doses, i.e., apoptosis rate plotted against log (dose).

RESULTS

CD40 activation induced emphysematous changes in mice. We first determined whether CD40 activation, alone or in combination with other factors, was capable of inducing an emphysematous phenotype in mice. IFN-γ and Fas activation were chosen as the two cofactors, IFN-γ because it is well known to be involved in the pathogenesis of emphysema (3, 6, 8) and produces an accepted mouse model of emphysema (39), and Fas because synergy between CD40/CD40L and Fas has been demonstrated previously (1, 38) and Fas-driven apoptosis may be mechanistically relevant. Mice received activating antibodies to CD40 (CD40 Ab), IFN-γ, and activating antibodies to Fas (Fas Ab), individually or in combination, by inhalation every 3 days for 24 days. Inhalation of activating Fas Ab has been reported to cause acute lung injury associated with the infiltration of massive leukocyte (23, 29). To see the chronic effects for alveolar destruction, we started Fas Ab instillation from a smaller dose and gradually escalated to determine the final dose.

Before performing inhalation experiments, we first confirmed constitutive expression of CD40 and Fas in alveoli. Both CD40 and Fas were expressed by mouse lung alveoli, and IFN-γ stimulation enhanced the expression of CD40 (Fig. 1). In assessment of emphysematous change, IFN-γ and Fas-activation each independently increased MLI. Although CD40 activation alone did not show a strong effect on MLI, it increased MLI induced by Fas Ab, IFN-γ, or Fas Ab + IFN-γ (Fig. 2, *A–I*). These data indicate that CD40 activation is capable of enhancing alveolar wall destruction by Fas and by IFN-γ, resulting in emphysematous changes in mice lung.

In addition to the presence of dilation of alveoli and an enlargement of respiratory bronchiole alveolar duct complex, a decrease in the numbers of alveoli recognized as a closed curve means an enlargement of continuous terminal airway with disruption of septa, which has been known as the essential of remodeling in case of emphysema. Of interest, mononuclear cell accumulation, predominantly lymphocytes, at the perivascular site was observed only in the CD40-activation group (Fig. 2, *J* and *K*). This implies that CD40 stimulation elicits lymphocyte activation or induction of chemotactic molecule secretion from local tissues. In vivo, CD40 activation, as well as IFN-γ, significantly increased inflammatory cell accumulation in lung, especially lymphocytes, in BALF (Fig. 3). CD40 activation dramatically enhanced the accumulation of inflammatory cell when combined with IFN-γ.

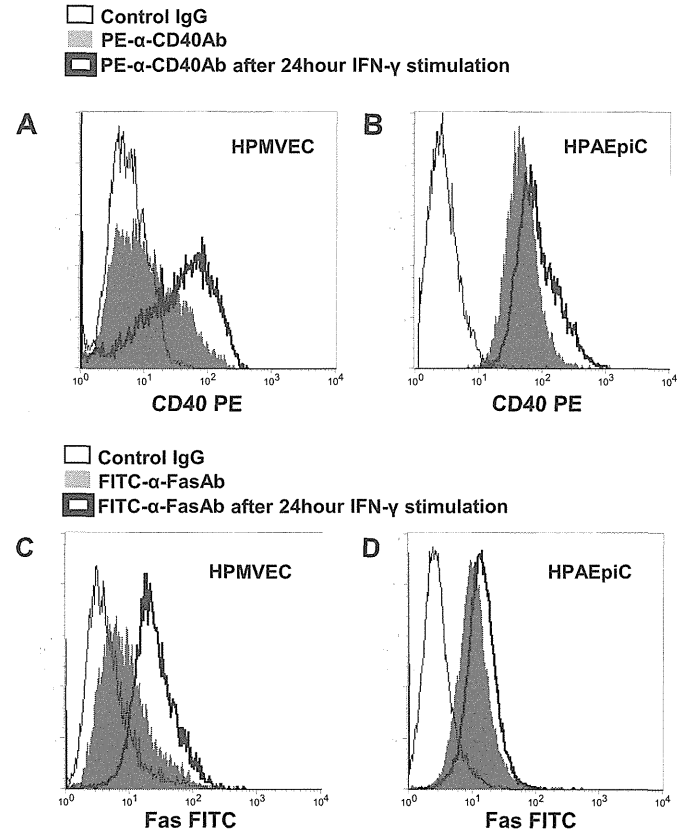


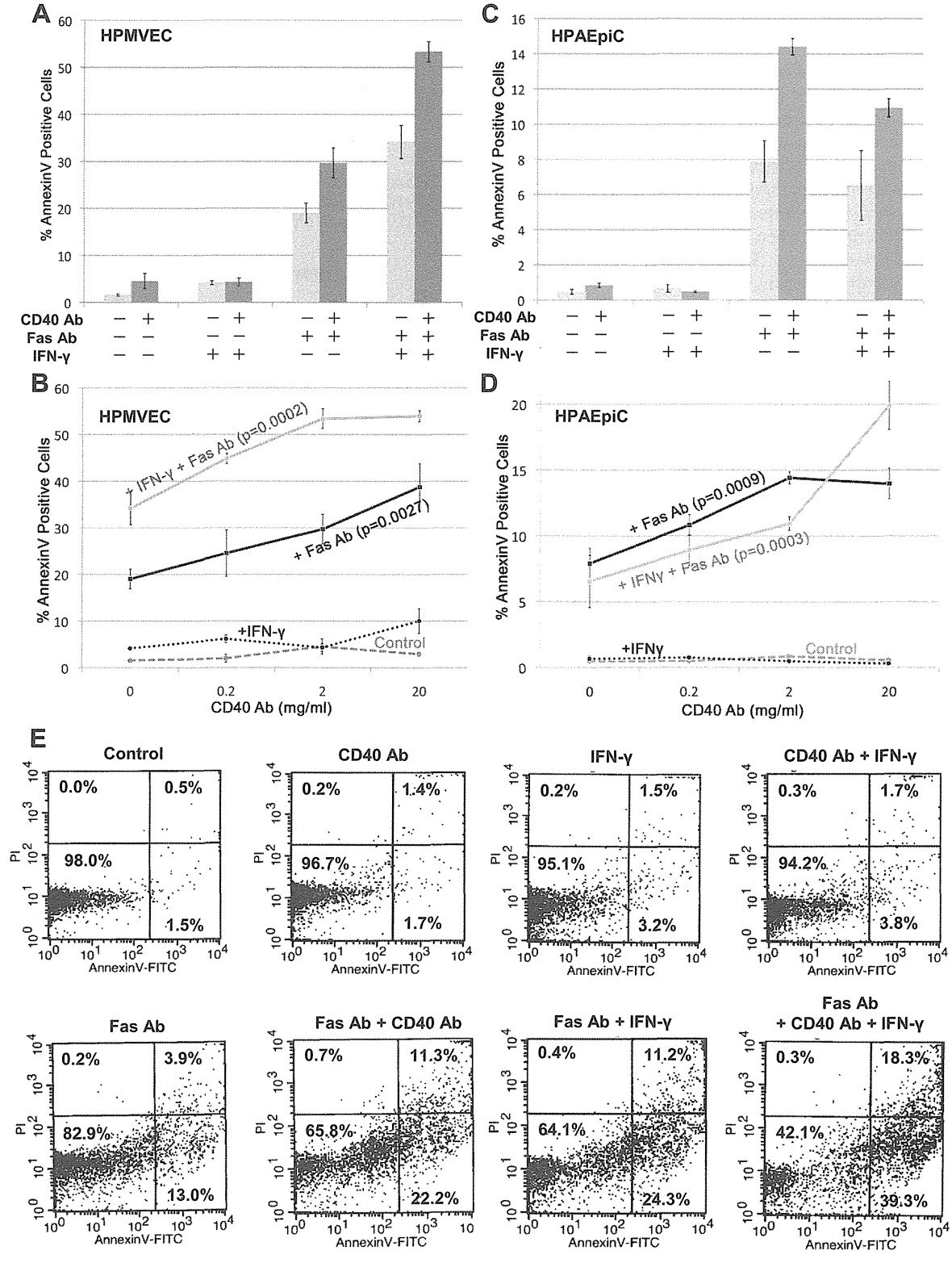
Fig. 5. *A* and *B*: expression of CD40 in human pulmonary microvascular endothelial cells (HPMVEC) (*A*) and human pulmonary alveolar epithelial cells (HPAEpiC) (*B*). *C* and *D*: expression of Fas in HPMVEC (*C*) and HPAEpiC (*D*). Data for control IgG, in the absence of IFN-γ, and in the presence of IFN-γ are shown as white histogram with thin line, gray shaded histogram, and white histogram with thick line, respectively. PE, phycoerythrin.

CD40 activation increased apoptotic cells in alveoli. Apoptosis is thought to play a major role in emphysema (2) and has been shown to drive emphysematous changes in mouse models (5, 22). To determine whether the increase in MLI in mice treated with activating antibody to CD40 correlated with increased apoptosis, TUNEL and caspase-3 staining were performed on lung sections, with positive cells counted as a proportion of the total cells. In TUNEL staining, CD40 activation, IFN-γ, and Fas activation each independently increased

Fig. 6. HPMVEC (*A* and *B*) and HPAEpiC (*C* and *D*) apoptosis following CD40, IFN-γ, and Fas stimulation. Data are means ± SE from 3 experiments. Multiple-factor ANOVA was used in *A* and *C* to test the effects of IFN-γ, CD40 Ab, and Fas Ab. The correlation z-test was used in *B* and *D* to test the dose response for increasing concentrations of activating mAb to CD40 in combination with Fas Ab and/or IFN-γ. *A*: effects of all 3 singly were significant at $P < 0.0001$; there was synergy between Fas Ab and CD40 Ab ($P = 0.0004$) and between Fas Ab and IFN-γ ($P < 0.0001$). *B*: there was no effect of CD40 alone; however, increasing CD40 activation, in combination with Fas Ab and Fas Ab + IFN-γ, increased the percentage of apoptotic cells. *C*: both CD40 Ab and Fas Ab increased apoptosis, and this was significant at $P < 0.0003$. IFN-γ did not significantly affect apoptosis in this model, either as a single factor or in combination with other factors. The combined effects of Fas Ab and CD40 Ab were synergistic ($P = 0.0004$). *D*: there was no effect of CD40 alone; however, in combination with Fas Ab, increasing CD40 activation significantly increased the percentage of apoptotic cells (P values indicated). By multiple-factor ANOVA, IFN-γ had no effect on apoptosis in this model. *E*: representative flow cytometry plots for Annexin V and phosphatidyl inositol in HPMVEC.

the numbers of apoptotic nuclei, and there was a mild synergy between CD40 and Fas, as well as between IFN-γ and Fas (Fig. 4*A*). In caspase-3 staining, CD40 activation slightly increased the number of caspase-3-positive cells, whereas IFN-γ and Fas activation significantly increased caspase-3-positive cells. Moreover, in accordance with the TUNEL results, there was a mild synergy between CD40 and Fas (Fig. 4*B*). These results indicate that CD40 activation slightly increased cell death in lung although its major effect in apoptosis was to enhance the death signal by Fas.

CD40 and Fas were constitutively expressed on HPMVEC and HPAEpiC. We next determined whether CD40 directly affects alveolar endothelial and epithelial cells, alone or in



combination with cofactors in vitro. Previous results on CD40 were based on experiments using human umbilical vein endothelial cells, and little is known about CD40 in lung-derived cells. Flow cytometry was therefore performed to confirm the cell surface expression of CD40 and Fas in HPMVEC and in HPAEpiC. Both CD40 (Fig. 5, *A* and *B*) and Fas (Fig. 5, *C* and *D*) were constitutively expressed in both cell types, although at a lower level in endothelium. CD40 and Fas were both upregulated by incubation with IFN-γ in both cell types, although to a greater extent in endothelium than in epithelium. Enhancement of Fas expression by CD40 activation, which occurs in B cells (38), was not observed in either cell type (data not shown).

Sensitization of Fas-mediated cell death by CD40 and/or IFN-γ stimulation. After confirming the expression of CD40 and Fas on both cell types, we determined whether CD40 would increase apoptosis in cultured cells; if this were so, it implies that the effect is on the cells themselves, rather than being mediated through bystander inflammation. HPMVEC or HPAEpiC were incubated for 12 h in medium containing activating anti-CD40 Ab, activating anti-Fas Ab, IFN-γ, or combinations of these, and apoptotic cells were evaluated by flow cytometry.

In endothelial cells (HPMVEC), the effects of both CD40 Ab and IFN-γ, as individual factors, on Annexin V-positive cell counts were weak. Fas Ab induced cell death in ~20% of cells. However, both CD40 Ab and IFN-γ enhanced Fas-mediated cell death ($P = 0.0004$ and $P < 0.0001$ by multiple-factor ANOVA, respectively, Fig. 6*A*). The enhanced effect of CD40 Ab in combination with Fas Ab and Fas Ab + IFN-γ was further assessed in a dose-response study (Fig. 6*B*). CD40 activation, either alone or in combination with IFN-γ, had no significant effect on cell death. However, increased CD40 activation, in combination with either Fas Ab or Fas Ab + IFN-γ, resulted in significantly increased cell death, suggesting that cell death is primarily triggered by Fas ligation.

In epithelial cells (HPAEpiC), the induction of cell death was generally less pronounced than in HPMVEC. In HPAEpiC, IFN-γ had no significant effect on cell death, either alone or in combination with Fas Ab or CD40 Ab. CD40 activation had little effect alone but significantly enhanced Fas-mediated cell death (Fig. 6*C*). CD40 activation also showed a strong and significant dose-response effect in the amplification of Fas-mediated cell death but had no effect alone or in combination with IFN-γ (Fig. 6*D*).

Taken together, these data indicate that CD40 potentiates Fas-mediated cell death in both endothelial and epithelial cells but that the effect of IFN-γ is specific to endothelial cells. It is plausible that the effect of IFN-γ on cell death is entirely the result of its effects on CD40 and Fas, which it induces to a much greater extent in endothelial cells than in epithelial cells (Fig. 5, *A–D*).

Induction of CCR5 ligands by CD40 stimulation. The findings thus far indicate that CD40 amplified Fas-mediated apoptosis in mice lungs (Fig. 4) and in pulmonary endothelial and epithelial cells (Fig. 6) but showed little effect on apoptosis, either alone or in combination with IFN-γ (Figs. 4 and 6). When combined with IFN-γ, however, CD40 did drive the loss of alveoli in mice (Fig. 2*I*). This suggests that, although amplification of Fas-mediated apoptosis is one effect of CD40 in emphysema, other independent effects may also exist.

In other systems, CD40 has been shown to regulate proinflammatory molecules (13, 26, 36) involved in sustained inflammation, resulting in mild alveolar loss in the lung. Among them, we noticed the important role of CCR5 ligands such as MIP-1α, MIP-1β, and RANTES because these molecules may be involved in IFN-γ-induced inflammation and remodeling in the pathogenesis of pulmonary emphysema. CCR5 ligands play a role in the progression of emphysema by an IFN-γ-dependent mechanism that involves the regulation of cell death, as well as caspase, matrix metalloproteinase, and antiprotease activities (3, 24). Thus their concentrations were measured in culture supernatants from HPMVEC and HPAEpiC treated by activating CD40 Ab and/or IFN-γ. In HPMVEC, both CD40 Ab and IFN-γ, as independent factors, significantly induced RANTES and MIP-1β secretion in culture supernatant (Fig. 7). Synergy between CD40 Ab and IFN-γ was observed for secretion of RANTES. In HPAEpiC, the levels of CCR5 ligands were below the threshold of detection (data not shown). RANTES is a potent chemoattractant for monocyte and T cell, and its expression is often upregulated, especially in acute exacerbations of COPD (8, 24). We measured RANTES in BALF from treated mice and confirmed that RANTES was also increased in vivo by sole CD40 stimulation or in combination with IFN-γ (Fig. 7*D*).

Plasma sCD40L was increased in patients with COPD. Circulating levels of soluble CD40 ligand (sCD40L) are upregulated in systemic vascular diseases, such as ischemic heart disease, stroke, pulmonary hypertension, and even in cigarette smokers (12, 16, 19, 20). Furthermore, we found that CD40 stimulation induces emphysematous change in mouse lung. Therefore, we lastly wanted to determine whether or not sCD40L was upregulated in patients with COPD. The COPD group showed significant decreases in $\%FEV_1$ and FEV_1/FVC as expected. Body mass index was also decreased in the COPD group (Table 1). Plasma sCD40L levels were significantly higher in the COPD group compared with the healthy smoker group (COPD 2.1 ± 0.2 ng/ml, healthy smoker 0.8 ± 0.2 ng/ml: $P = 0.008$) (Fig. 8). Notably, plasma sCD40L level in patients was oppositely correlated to $\%FEV_1$ ($R = 0.68$, $P < 0.0001$) and positively correlated to LAA score ($R = 0.54$, $P < 0.0001$) regardless of pack/year smoking (Fig. 9, *A* and *B*, Table 2). These findings may indicate that high concentration of plasma sCD40L is associated with impaired lung function, as well as alveolar structural destruction, regardless of smoking history.

DISCUSSION

In the present study, we showed in mice that CD40 agonists, both alone and in concert with Fas agonists and IFN-γ, drive the enlargement of airspaces (Fig. 2*I*). In concert with the mouse data, patients with COPD have elevated plasma levels of sCD40L, which was correlated to impaired lung function and alveolar destruction (Fig. 9). A likely mechanism is the amplification of Fas-mediated apoptosis by CD40, as demonstrated both in mice (Fig. 4) and in human pulmonary endothelial and epithelial cells (Fig. 6). IFN-γ enhanced the airspace enlargement caused by CD40 agonist (Fig. 2*I*) although

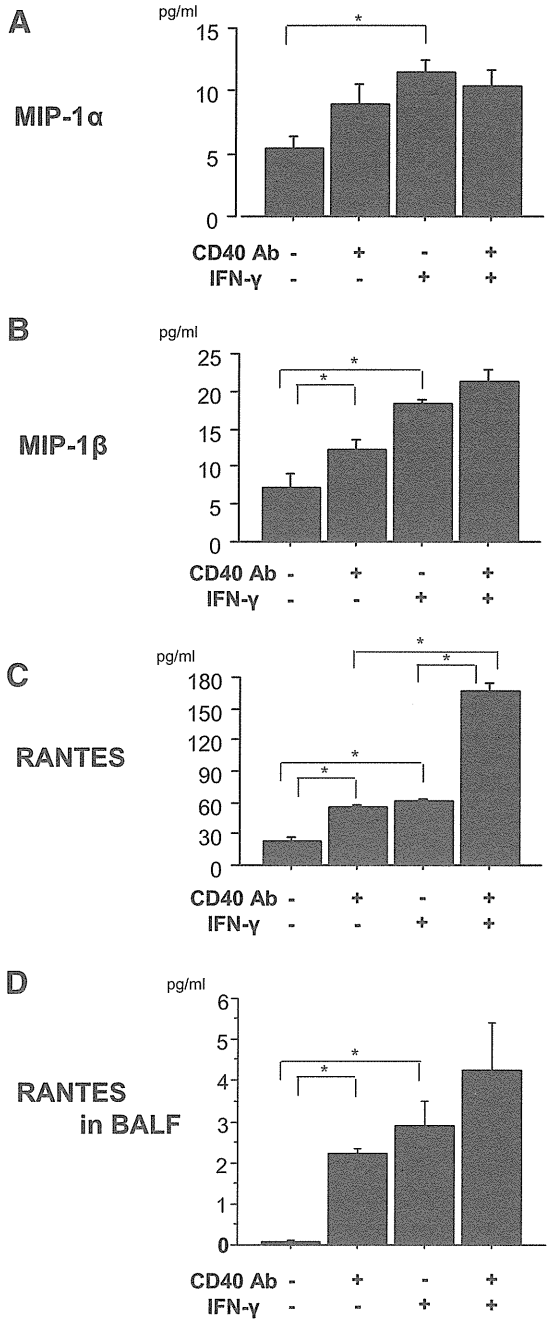


Fig. 7. *A–C*: CCR5 ligand levels in cell supernatants as assessed using the Bioplex system (n = 5), after a 12-h incubation with CD40 agonist and/or IFN-γ in HPMVEC. *D*: chemokine regulated on activation normal T cells expressed and secreted (RANTES) level in BALF from mice with treatments (n = 4). *P < 0.05. MIP, macrophage inflammatory protein.

synergy between CD40 and IFN-γ in apoptotic induction was not obvious (Figs. 4 and 6). It might indicate that collaboration between CD40 and IFN-γ in mediating emphysema probably does not occur through enhanced apoptosis but rather through enhanced inflammation (Fig. 7).

Fas-mediated apoptosis of alveolar cells has previously been reported only in acute lung injury and pulmonary fibrosis (23, 29). However, in the context of emphysema, endothelial and epithelial cell apoptosis in COPD were thought to be driven by

Table 1. *Participant characteristics*

	Healthy Smoker (n = 19)	COPD (n = 69)	P Value
Age, yr	72.4 ± 2.2	73.3 ± 0.7	n.s.
Male/Female , n	19/0	62/7	n.s.
BMI	23.2 ± 0.6	19.9 ± 0.3	< 0.0001
FEV_1/FVC, %	79.4 ± 0.3	45.3 ± 1.1	< 0.0001
$\%FEV_1$, %	104.7 ± 4.1	44.5 ± 1.8	< 0.0001
Pack/Year	27.9 ± 4.9	34.6 ± 2.1	n.s.
Plasma sCD40L, ng/ml	0.8 ± 0.2	2.1 ± 0.2	0.008

Applicable values are means ± SE. COPD, chronic obstructive pulmonary disorder; BMI, body mass index; n.s., not significant; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

the release of perforin and granzyme from $CD8^+$T cells (3, 8). The interaction of Fas/FasL system in the development of COPD has long been discussed, only in the context of circulating FasL levels in the patient's serum (34, 41), and it remains controversial. However, because $CD8^+$ T cells are a major source of functional FasL, it is quite possible that these cells also mediate alveolar or endothelial apoptosis through the Fas/FasL pathway, in addition to perforin and granzyme release, thus reconciling these two mechanisms. Sensitization of Fas by CD40 stimulation has only been reported in activated B cells and a few other cell types (1, 31, 38). Although it is not surprising that the same mechanism appears to function in pulmonary cells, this finding is relevant and of potential importance in the pathophysiology of emphysema. The role of IFN-γ in emphysema has been studied in a mouse transgenic overexpression model. In that model, the pathogenesis of emphysema was attributed to overactivation of matrix metalloproteinase-12 although alveolar apoptosis was not confirmed (39). This is consistent with the present data, which showed that IFN-γ did not induce apoptosis on its own, either in epithelial or endothelial cells. The present findings suggest a potential mechanism for the effect of IFN-γ in emphysema, namely the induction of CD40 and Fas. However, the major mechanism for the effect of IFN-γ and the interaction between CD40 and IFN-γ appeared to be through the induction of

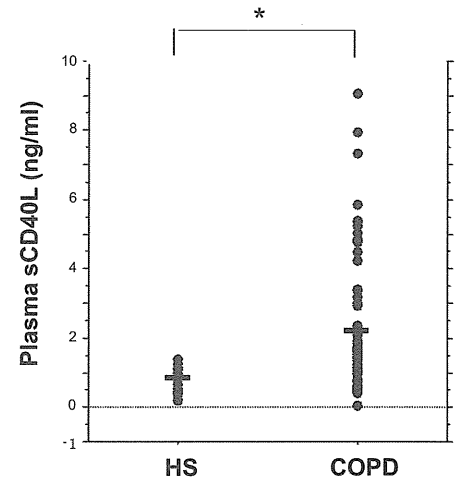


Fig. 8. Plasma sCD40L level in patients with chronic obstructive pulmonary disease (COPD) (n = 69) was increased compared with that of in healthy smoker (HS; n = 19). COPD 2.1 ± 0.2 ng/ml, HS 0.8 ± 0.2 ng/ml, P = 0.008. Values are means ± SE.

CCR5 ligands, such as MIP-1β and RANTES. In the present study, the increase in RANTES attributable to CD40 activation was comparable to that stimulated by high concentrations of IFN-γ, and this was further increased by the combination of CD40 with IFN-γ. Thus, in concert with IFN-γ, CD40 plays a role in sustaining inflammation by increasing the secretion of RANTES.

One of the limitations of this study was that we have not investigated the recruitment of professional antigen-presenting cells, such as dendritic cells and macrophages, although these cells have been suggested to play important roles in emphysema (3, 6). Surface expression of CD40 on pulmonary dendritic cells has been reported to be increased by smoke inhalation in mice (11). However, Matute et al. (4, 25) reported that, in acute lung injury, Fas directly affects alveolar cells, not via myeloid cells such as alveolar macrophages. Indeed, we did not detect increased numbers of apoptotic immune cells in lungs of our mouse models. CD40 signaling is an effective driver of antigen-presenting cells for maturation and producing Th1/Tc1 cytokines as IFN-γ (15, 33); thus we speculate that the effects on immune cell by these molecules did not alter the present results. Increased lymphocyte recruitment was observed in the CD40 agonist model systems and was consistent with the findings of increased chemokine secretion in vitro and in vivo. This observation suggested that increased recruitment of inflammatory cells may be one of the mechanisms through which CD40 facilitates IFN-γ-induced emphysematous change.

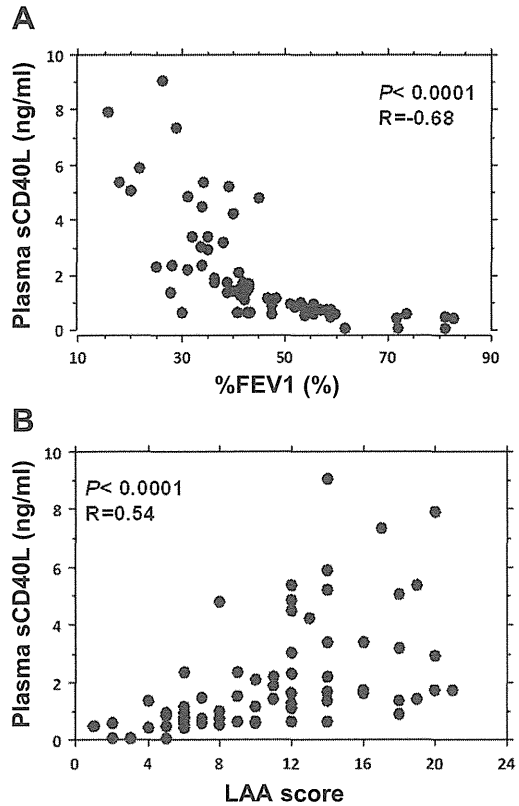


Fig. 9. Correlations between the percentage of forced expiratory volume in 1 s (%FEV_1) or low-attenuation area (LAA) score, and plasma sCD40L level. Plasma sCD40L level in patients was oppositely correlated to %FEV_1 (R = 0.68, $P < 0.0001$, *A*) and positively correlated to LAA score (R = 0.54, $P <$ 0.0001, *B*).

Table 2. *Relationship between sCD40L and emphysema-associated factors*

Variable	SRC	*P* value
%FEV_1	−0.656	<0.0001
Pack/Year	0.14	0.12
LAA score	0.51	<0.0001
Pack/Year	0.11	0.32

Multiple-regression analysis to find which is more associated, pack/year or % FEV_1 and low-attenuation area (LAA), with plasma sCD40L level in patients with COPD (n = 69). SRC, standardized regression coefficients.

We analyzed only type-1-dominant alveolar cell (HPAEpiC: composed of 95% of alveolar type I cell) in the present study. However, alveolar type II cells are considered to be local progenitor cells that have the ability to compensate for the loss of type I cells and are resistant to apoptotic signals (28). Because growing evidence suggests that impaired repair of alveoli is a critical mechanism in emphysema (10, 21), it will be interesting to analyze the response of both type I and type II alveolar cells.

Taking all the findings together, we conclude that CD40 enhances Fas-mediated apoptosis in alveolar cells and secretion of proinflammatory chemokines, both of which are associated with the development of pulmonary emphysema.

This study adds insight to reconsider the role of TNF-family molecules in the pathogenesis of the disease. Blocking or normalization of the CD40 signaling pathway might be an alternative therapeutic strategy for the treatment of pulmonary emphysema.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: A. Shigeta, Y. Tada, and K.T. conception and design of research; A. Shigeta, S.I., J.T., K.Y., A. Sakamoto, K.S., and K.H. performed experiments; A. Shigeta and J.W. analyzed data; A. Shigeta, Y. Tada, J.-Y.W., A. Sakamoto, K.S., K.H., and J.W. interpreted results of experiments; A. Shigeta and J.W. prepared figures; A. Shigeta and Y. Tada drafted manuscript; A. Shigeta, Y. Tada, J.-Y.W., S.I., J.T., K.Y., Y.K., K.I., N.T., Y. Takiguchi, A. Sakamoto, T.T., K.S., K.H., J.W., and K.T. approved final version of manuscript; Y. Tada, J.-Y.W., Y.K., K.I., N.T., Y. Takiguchi, A. Sakamoto, T.T., K.S., K.H., J.W., and K.T. edited and revised manuscript.

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Long-term outcome after pulmonary endarterectomy for chronic thromboembolic pulmonary hypertension

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Objectives: Pulmonary endarterectomy is the treatment of choice for chronic thromboembolic pulmonary hypertension. Although several reports demonstrated excellent medium-term survival after pulmonary endarterectomy, long-term outcomes remain unclear. We reviewed long-term outcomes and determined risk factors for early and late adverse events.

Methods: Seventy-seven patients were studied. Mean pulmonary arterial pressure was 47 ± 10 mm Hg and pulmonary vascular resistance was 868 ± 319 dyne · s · cm^{-5}. Disease was classified as chronic thromboembolic pulmonary hypertension type 1 (n = 61), type 2 (n = 12), or type 3 (n = 4). Median and maximum follow-up periods were 5.6 and 20 years, respectively.

Results: There were 11 in-hospital deaths. Nonsurvivors had significantly higher mean pulmonary arterial pressure and pulmonary vascular resistance than did survivors (54 ± 10 vs 46 ± 10 mm Hg; $P = .02$; 1124 ± 303 vs 824 ± 303 dyne · s · cm^{-5}; $P < .01$). In multivariate analysis, preoperative pulmonary vascular resistance was associated with in-hospital death (odds ratio, 1.003; 95% confidence interval, 1.001–1.005; $P < .01$). During follow-up, there were 10 all-cause deaths, including 5 related to chronic thromboembolic pulmonary hypertension. Freedom from adverse events, including disease-specific death or New York Heart Association functional class III, was 70% at 10 years. In the Cox proportional hazard model, postoperative mean pulmonary arterial pressure was associated with adverse events (hazard ratio, 1.12; 95% confidence interval, 1.03–1.21; $P < .01$). Receiver operating characteristic curve analysis showed mean pulmonary arterial pressure of 34 mm Hg as cutoff for adverse events.

Conclusions: Pulmonary endarterectomy had sustained favorable effects on long-term survival. High pulmonary vascular resistance was associated with in-hospital death, and postoperative mean pulmonary arterial pressure was an independent predictor of adverse events. (J Thorac Cardiovasc Surg 2012;144:321-6)

Chronic thromboembolic pulmonary hypertension (CTEPH) is a life-threatening disease caused by unresolved, organized thrombi obstructing the pulmonary arteries.[1,2] Progressive pulmonary hypertension severely compromises both clinical functional status and exercise capacity as a result of ventilation–perfusion mismatch and decreased cardiac output. In patients who do not undergo operation, the prognosis is disappointing and is determined by the severity of the pulmonary hypertension: if mean pulmonary arterial pressure (mPAP) exceeds 30 mm Hg, the 5-year survival is less than 30%, and if it exceeds 50 mm Hg, the 5-year survival is as low as 10%.[3]

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Pulmonary endarterectomy (PEA), which has been established as a standard surgical treatment for CTEPH, is performed worldwide, mostly at centers with experience.[1,4] PEA offers immediate and substantial decreases in both mPAP and pulmonary vascular resistance (PVR) and an increase in the cardiac index.[4,5] Medium-term follow-up results for PEA reveal favorable effects on survival and clinical functional status[6-10]; however, the long-term outcomes remain unclear because of the limited available data. We began our PEA program in 1986 and have thus been performing the operation now for more than 20 years. Early on in our series, we performed PEA through a lateral thoracotomy; however, we have performed PEA through median sternotomy with intermittent deep hypothermic circulatory arrest according to the San Diego group procedure since 1990.[4,11,12] Here we review the long-term follow-up outcomes of a consecutive series of patients who underwent PEA through median sternotomy, and we seek to determine the factors influencing both early and late survival and functional status.

MATERIALS AND METHODS

Between 1990 and 2010, a total of 77 patients underwent PEA at Chiba University Hospital and affiliated hospitals. We retrospectively reviewed

Abbreviations and Acronyms

CTEPH = chronic thromboembolic pulmonary hypertension
mPAP = mean pulmonary arterial pressure
NYHA = New York Heart Association
PEA = pulmonary endarterectomy
PVR = pulmonary vascular resistance

this cohort of patients. All 77 patients in our cohort underwent preoperative pulmonary angiography, right heart catheterization, and computed tomographic scan. Surgical indications were decided as follows: PVR greater than 300 dyne · s · cm^{-5}, mPAP greater than 30 mm Hg, New York Heart Association (NYHA) functional class greater than II, and absence of significant comorbidities. Postoperative pulmonary hemodynamics were evaluated by right heart catheterization 1 month after PEA in 61 of the 66 PEA survivors. Perioperative data were collected from hospital records. Follow-up data, which were obtained by calling the patients or their primary physicians, were available for 74 patients (96% complete). The median and maximum follow-up periods were 5.6 years and 20 years, respectively.

Baseline patient characteristics are shown in Table 1. Fifty-five patients (56%) were female, and thus the gender distribution was female dominant. Twenty-four patients (31%) had a coagulation abnormality (antithrombin III deficiency, 5 patients; protein C or S deficiency, 5 patients; antiphospholipid syndrome, 9 patients), and 37 patients (48%) had a deep vein thrombosis. Most of the patients (79%) were in NYHA functional class III or IV, and all patients required home oxygen therapy. The mPAP was 47 ± 10 mm Hg, with 42% of the patients having mPAP values greater than 50 mm Hg. PVR was 868 ± 319 dyne · s · cm^{-5}, with 30% of the patients having PVR values greater than 1000 dyne · s · cm^{-5}.

On the basis of the location and morphology of the thromboembolic and vascular wall disease found at the time of surgery, the disease was classified as follows: type 1 (n = 61) consisted of fresh (acute) thrombus in the main lobar pulmonary arteries; type 2 (n = 12) consisted of intimal thickening and fibrosis with or without organized thrombus proximal to the segmental arteries; type 3 (n = 4) consisted of fibrosis, intimal webbing, and thickening with or without an organized thrombus within distal segmental arteries alone; and type 4 (n = 0) consisted of microscopic distal arteriolar vasculopathy without visible thromboembolic disease.[13]

PEA was performed through a median sternotomy with intermittent deep hypothermic circulatory arrest according to the procedure of University of California, San Diego group.[4,11,12] An inferior vena cava filter was put in place in 65 patients (84%). All PEA survivors received permanent anticoagulation therapy (Coumadin). Pulmonary vasodilator therapy (bosentan or sildenafil citrate [INN sildenafil]) was not routinely used, except for patients in NYHA functional class III.

Statistics

Results are expressed as mean ± SD. All analyses were performed with SPSS 18 software (IBM Corporation, Armonk, NY). The Wilcoxon signed-rank test was used for the comparison of preoperative and postoperative pulmonary hemodynamic data. Pulmonary hemodynamic data obtained by right heart catheterization 1 month after PEA were used for the calculation of postoperative mPAP or PVR, except in the case of patients who did not undergo postoperative right heart catheterization; for these patients, the data obtained before removal of the thermodilution catheter were used. Predictors of in-hospital mortality were analyzed by univariate analysis and multivariate stepwise logistic regression analysis. The univariate analysis was performed for continuous variables with a *t* test or a Mann-Whitney test and for categoric variables with the χ^2 test or the Fisher's Exact test. We calculated the incidence rates for all-cause mortality, disease-specific mortality, and adverse events, including disease-specific mortality and impaired functional status in NYHA functional class III, and we used the Kaplan-Meier method to estimate survivals. Univariate and multivariate stepwise analyses with the Cox proportional hazard model were performed to identify the risk factors for disease-specific death and adverse events. The optimal PVR cutoff point for in-hospital death and the optimal mPAP cutoff value for adverse events were determined with the aid of a receiver operating characteristic curve. Results are expressed as hazard ratios with 95% confidence intervals.

RESULTS

Early Results

There were 11 in-hospital deaths among the cohort studied (14%). The causes of death were as follows: right heart failure (n = 6), pulmonary hemorrhage (n = 2), cardiac tamponade (n = 2), and reperfusion lung edema (n = 1). With the exception of the 2 patients who died of cardiac tamponade, the other 9 patients had persistent pulmonary hypertension develop, and there were 5 patients who could not be weaned from cardiopulmonary bypass. Among the 66 PEA survivors, mPAP and PVR decreased (from 47 ± 10 mm Hg to 25 ± 10 mm Hg, $P < .0001$; from 868 ± 319 dyne · s · cm^{-5} to 313 ± 206 dyne · s · cm^{-5}, $P < .0001$; respectively), and cardiac index increased (from 2.5 ± 0.7 L/[min · m^2] to 3.1 ± 0.6 L/[min · m^2]; $P < .0001$). Comparisons between survivors and nonsurvivors in terms of preoperative data are shown in Table 2. In the multivariate analysis, only preoperative PVR (odds ratio, 1.003; 95% confidence interval, 1.001–1.005; $P < .01$) was an independent predictor of in-hospital death. To determine the cutoff point for the influence of preoperative PVR on in-hospital death, we performed receiver operating characteristic curve analysis. This revealed a preoperative PVR of 1052 dyne · s · cm^{-5} as the cutoff value for in-hospital death (area under the curve, 0.76; sensitivity, 0.64; specificity, 0.83).

Late Results

During the follow-up period, there were 10 all-cause deaths. Four deaths of right heart failure and 1 sudden cardiac death were regarded as being related to CTEPH. These patients exhibited NYHA functional class III symptoms as a result of persistence or worsening of pulmonary hypertension: 3 patients had persistent pulmonary hypertension with less than 10% decrease in PVR, and 2 patients had worsening of pulmonary hypertension despite early postoperative decrease in PVR (from 618 dyne · s · cm^{-5} to 386 dyne · s · cm^{-5} and from 1288 dyne · s · cm^{-5} to 507 dyne · s · cm^{-5}). Other causes of death included suffocation hematemesis, interstitial pneumonia, brain hemorrhage, and stroke. Among the operative survivors, clinical functional status improved markedly relative to the preoperative status. At the most recent follow-up at a mean of 6.5 years, 56 patients

TABLE 1. Baseline patient characteristics

Variable	Preoperative
Male (no.)	34 (44%)
Age (y, mean ± SD)	55 ± 11
Coagulation abnormality (no.)	24 (31%)
Deep vein thrombosis (no.)	37 (48%)
Insertion of inferior vena cava filter (no.)	65 (84%)
Duration of illness (mo, mean ± SD)	49.6 ± 40.4
New York Heart Association functional class (no.)	
I	0
II	16 (21%)
III	54 (70%)
IV	7 (9%)

(92%) were in NYHA functional class I or II, and 35 patients (63%) had been weaned from home oxygen therapy. Among 5 patients in NYHA functional class III, 4 patients had worsening of pulmonary hypertension at follow-up. Their PVR decreased from 795 ± 245 dyne · s · cm^{-5} to 398 ± 146 dyne · s · cm^{-5} early after surgery, but it rose again to 738 ± 214 dyne · s · cm^{-5} at follow-up.

Figure 1 shows freedoms from all-cause death, disease-specific death, and adverse events (including disease-specific death and impaired functional status, NYHA functional class III). The values for freedom from disease-specific death at 5 and 10 years were 84% and 82%, respectively, whereas those for freedom from adverse events were 78% and 70%.

We next sought to determine risk factors for late adverse events. In the individual variable model, age and postoperative mPAP and PVR were associated with adverse events; however, only postoperative mPAP was found to be significant in the multivariable analysis (hazard ratio, 1.12; 95% confidence interval, 1.03–1.21; $P < .01$). Receiver operating characteristic curve analysis revealed a postoperative mPAP of 34 mm Hg as the cutoff value for adverse events (area under the curve, 0.90; sensitivity, 0.80; specificity, 0.91).

Finally, the postoperative mPAP cutoff value was used to divide patients into 2 groups. There were no intergroup differences in any preoperative variables, although patients with a postoperative mPAP of at least 34 mm Hg had a trend toward higher preoperative mPAP values than did those with a postoperative mPAP lower than 34 mm Hg (50 ± 10 mm Hg vs 45 ± 10 mm Hg; $P = .10$). Comparison of freedom rates from late adverse events between the groups (Figure 2) revealed that patients with postoperative mPAP values lower than 34 mm Hg had good late outcomes. In that group, 10-year freedoms from disease-specific death and adverse events were 100% and 98%, respectively. In contrast, patients with postoperative mPAP values of at least 34 mm Hg had significant adverse events after PEA, and 10-year freedoms from disease-specific death and adverse events were 80% and 41%, respectively.

DISCUSSION

Medical treatment for CTEPH is palliative and unsatisfactory, but PEA is an effective therapeutic option that results in immediate and substantial improvements in pulmonary hemodynamics. This procedure is technically demanding, however, and requires proper patient selection and careful postoperative management, resulting in such relatively high in-hospital mortalities as 4.4% to 16%.[1,4,6,10,14,15] Although our overall in-hospital mortality of 14% may be relatively high, the rate was reduced to 7.5% in the last 40 cases with increasing surgical experience and refinements in operative and postoperative management.

Several risk factors for increased in-hospital mortality have been identified, including advanced age, severe pulmonary hemodynamic compromise, CTEPH type 3 or 4, distinct medical conditions involving other organ systems, and postoperative PVR greater than 500 dyne · s · cm^{-5}.[1,4,6,13,14,16] In this study, only high PVR was identified as a preoperative risk factor, with a PVR cutoff point of 1052 dyne · s · cm^{-5} for in-hospital death. Although other work has shown that postoperative PVR greater than 500 dyne · s · cm^{-5} may be a significant risk factor for in-hospital death,[4] we did not evaluate postoperative PVR because there were 5 patients who could not be weaned from cardiopulmonary bypass.

TABLE 2. Risk factors for hospital mortality

Variable	Survivors (n = 66)	Nonsurvivors (n = 11)	Univariate *P* value	Multivariate *P* value
Male (no.)	27 (41%)	7 (63%)	.20	—
Duration of illness (mo, mean ± SD)	47 ± 40	62 ± 39	.27	—
Mean pulmonary arterial pressure (mm Hg, mean ± SD)	46 ± 10	54 ± 9	.02	—
Pulmonary vascular resistance (dyne · s · cm^{-5}, mean ± SD)	825 ± 303	1124 ± 303	<.01	<.01
Cardiac index (L/[min · m2], mean ± SD)	2.5 ± 0.7	2.1 ± 0.5	.33	—
Chronic thromboembolic pulmonary hypertension type (no.)				
1	52 (87%)	8 (14%)		
2	12 (100%)	0 (0%)		
3	2 (50%)	2 (50%)	.10*	—

*Versus chronic thromboembolic pulmonary hypertension types 1 and 2.

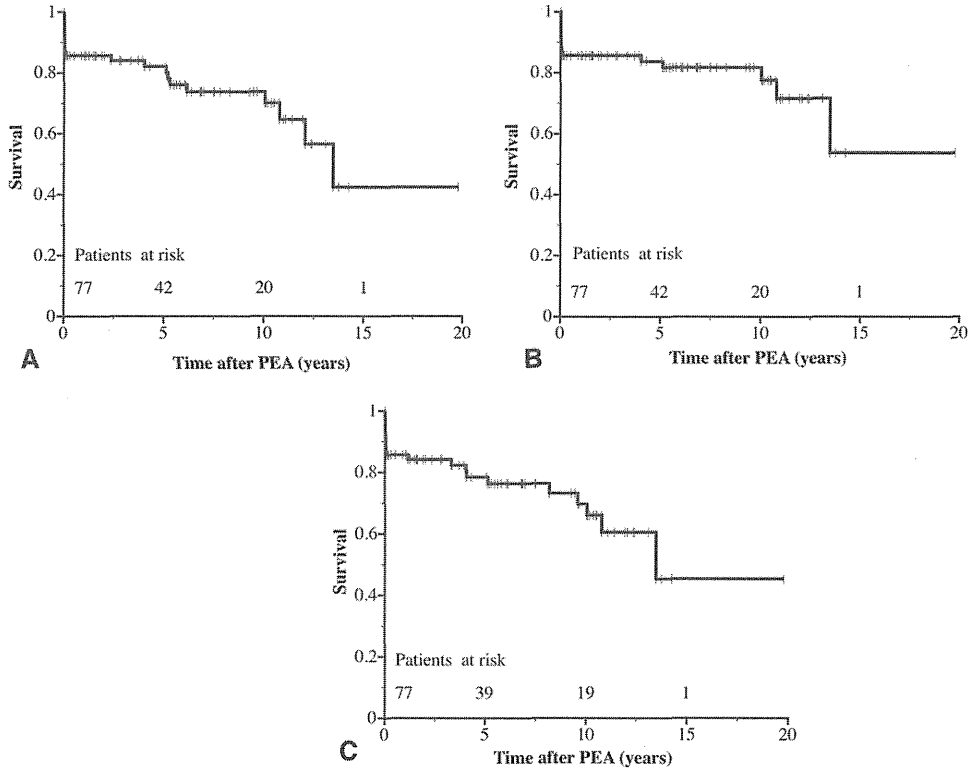

FIGURE 1. Overall Kaplan-Meier survival curves after pulmonary endarterectomy *(PEA)*. A, Freedom from all-cause mortality. B, Freedom from disease-specific mortality. C, Freedom from disease-specific mortality or New York Heart Association functional class III.

A previous study revealed that patients with a PVR greater than 1100 dyne · s · cm^{-5} had 6 times higher operative mortality (37%) than did those with a PVR lower than 1100 dyne · s · cm^{-5}.[16] Another study by Dartevelle and colleagues[1] showed that the mortality was 4% among patients with PVR lower than 900 dyne · s · cm^{-5}; however, this increased to 10% among those with PVR between 900 and 1200 dyne · s · cm^{-5} and to 20% among those with higher PVR. High PVR and CTEPH type 3 have been shown to be associated with persistent pulmonary hypertension, which is among the major complications after PEA and the leading cause of in-hospital death.[1,4] In a study by Thistlethwaite and associates,[13] patients with CTEPH type 3 disease had higher rates of perioperative mortality and morbidity and smaller decreases in PAP and PVR than did those with proximal disease (type 1 or 2). Likewise, Freed and colleagues[17] reported less improvement in pulmonary hemodynamics among patients with CTEPH type 3: 10 of their 17 patients with type 3 disease had postoperative persistent pulmonary hypertension, with mPAP values greater than 30 mm Hg. In the our study, it was not

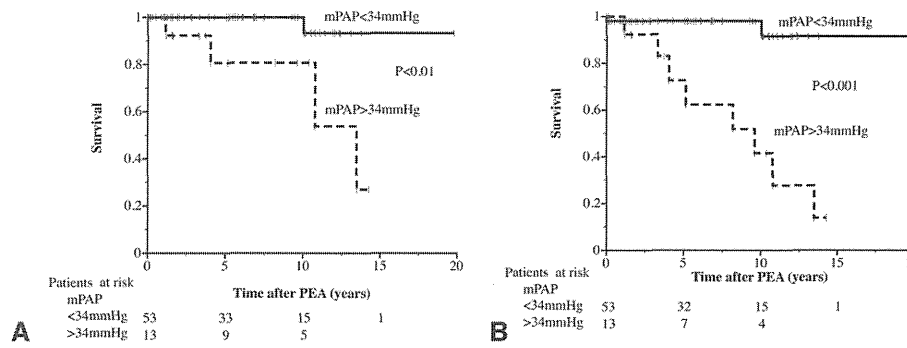

FIGURE 2. Kaplan-Meier survival curves after pulmonary endarterectomy *(PEA)* according to postoperative mean pulmonary arterial pressure *(mPAP)*. A, Freedom from disease-specific mortality. B, Freedom from disease-specific mortality or New York Heart Association functional class III.

conclusive whether CTEPH type 3 was associated with in-hospital mortality, because only a small number of patients had CTEPH type 3. Proper patient selection and careful perioperative management, however, are necessary when treating patients who have a high mPAP as a result of CTEPH type 3.

In addition to immediate improvements in pulmonary hemodynamics, PEA reportedly provides an excellent medium-term survival benefit, with 5-year survivals ranging from 75% to 89%.[6-9] In a long-term follow-up of 308 patients who underwent PEA between 1970 and 1994, a University of California, San Diego, group found that 75% survived beyond 6 years.[9] More recently, Corsico and coworkers[8] reported the late results obtained for 157 patients who underwent PEA between 1994 and 2006. They showed an 84% survival at 5 years, with a 30-day mortality of 11.5%. Our study, which reviewed the 20-year follow-up outcomes of patients who underwent PEA, has confirmed the persistent beneficial effect of PEA, as indicated by our late survival data. Indeed, 10-year survival was as high as 82% (Figure 1, *B*).

Patients with CTEPH are severely clinically compromised as a result of ventilation–perfusion mismatch and decreased cardiac output. In previous studies, more than 90% were in NYHA functional class III or IV, and most required oxygen therapy.[6,8,14] Despite that clinically unpromising background, PEA provided significant and sustained improvements in clinical symptoms, with more than 90% of patients being in NYHA functional class I or II and 2 thirds having been weaned from oxygen therapy at the latest follow-up.[6,8] In addition to prompt pulmonary hemodynamic improvement, PEA gradually improves gas exchange through a period of 6 months to 2 years after a temporal ventilation–perfusion abnormality caused by restrictive pulmonary functional impairment in response to surgical trauma, diffusion limitation in response to pulmonary edema, and a steal of perfusion from high-resistance nonobstructed segment to low-resistance newly perfused segements.[18,19] In our study, which was consistent with the previous results, only 63% of patients could be weaned from oxygen therapy, although 92% of patients were in NYHA functional class I or II. The discrepancy between excellent functional status and frequent oxygen therapy at follow-up can be explained by the fact that 10 of 21 patients with home oxygen therapy had a short follow-up period of less than 2 years.

Although a previous follow-up study found that persistent pulmonary hypertension and recurrent pulmonary embolism were the leading causes of late death,[9] the risk factors for late adverse events after PEA remain to be identified because the data on long-term survival are relatively scarce. In one of the few relevant reports, Bonderman and associates[15] showed that distinct medical conditions causing chronic infection or chronic inflammatory processes were risk factors for persistent pulmonary hypertension after PEA and were associated with both short- and long-term adverse outcomes. Freed and colleagues,[17] who reviewed medium-term follow-up results, showed that although persistent pulmonary hypertension with an mPAP value greater than 30 mm Hg led to impairments in both functional status and exercise capacity, it had no adverse impact on 5-year survival. Our long-term follow-up study showed that persistence and worsening of pulmonary hypertension were associated with late death or impairment of functional status and identified postoperative mPAP as a risk factor for late adverse events, whereas the preoperative risk factors for in-hospital death were not associated with late adverse events.

It has been shown that mPAP determines the prognosis of patients with medically treated CTEPH.[3] Actually, in previous studies, mPAP values greater than 30 mm Hg adversely affected survival in patients with medically treated CTEPH, whereas borderline pulmonary hypertension (20–26 mm Hg) was not associated with a poor prognosis.[3,20] In our study, a postoperative mPAP value of at least 34 mm Hg was found to be the cutoff value for late adverse events. To judge from these results, a high mPAP determines the prognosis of patients with CTEPH, whether the disease is treated surgically or medically, and thus a decrease of mPAP is the most important goal if we hope to achieve good late survival. In contrast to the poor outcomes among patients with persistent pulmonary hypertension, an excellent 10-year event-free survival (98%) was achieved among patients with resolved pulmonary hypertension (Figure 2). Our result indicates that PEA can be a curative and definitive surgical treatment and may be particularly important for patients with CTEPH, who are generally middle-aged (mean age around 52–57 years).[1,4,6,10,14,15]

Persistent pulmonary hypertension develops in 10% to 35% of patients who have undergone PEA, despite removal of sufficient proximal thromboembolic materials.[6,10,14,17] Pathologic examination of lung tissue in patients with CTEPH has shown that small vessel arteriopathy occurs not only in the area served by open proximal arteries but also in the area distal to occluded pulmonary arteries.[1,21,22] This small vessel arteriopathy causes progressive pulmonary hypertension and a symptomatic decline in the course of the CTEPH and is related to the development of persistent pulmonary hypertension after a successful PEA.[1,22] A reliable preoperative diagnostic tool for the involvement of distal arteriopathy has not been established[2,20,22]; however, patients who have a PVR that is disproportionately high with respect to the degree of proximal obstructions seen on pulmonary angiography are likely to have significant distal arteriopathy.[1,2,4] These patients have an elevated risk of persistent pulmonary hypertension and therefore may be selected for PEA only if a 50% reduction in PVR is predicted.[1]