Protective effect of IL-6 on alveolar epithelial cell death induced by hydrogen peroxide

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Diffuse alveolar damage (DAD) is a severe pathological state with a high mortality rate for which there is currently no effective pharmacotherapy. During DAD, alveolar epithelial cell injury, together with capillary endothelial cell injury, is the earliest change (15). Accumulating evidence suggests that alveolar epithelial injury plays a crucial role in the pathogenesis of DAD (4, 10, 13, 40), and it is reasonable to consider the prevention of alveolar epithelial cell death as a new therapeutic target of DAD (5).

IL-6-type cytokines, including IL-6, IL-11, leukemia inhibitory factor, cardiotrophin-1, oncostatin M, and ciliary neurotrophic factor, share gp130 as the signal transducer and activator of transcription (STAT) and the gp130, which is essential for signal transduction. Downstream of gp130 are two distinct signaling pathways, the Janus kinase/signal transducer and activator of transcription (STAT) and the RAS/mitogen-activated protein kinase pathways (34). IL-6 is a multifunctional cytokine. It is a growth factor of hybridoma cells, plasmacytoma cells, and multiple myeloma cells, promotes B cells to differentiate into immunoglobulin-secreting cells, and stimulates the hepatocytes to synthesize acute-phase proteins (46). Although the cytoprotective function is among the more remarkable activities of IL-6 (36, 39), it has never been known whether IL-6 could protect alveolar epithelial cells. The effects of IL-6 on lung epithelial cells that have been reported to date are growth inhibition and stimulation of surfactant protein production (35, 44).

It has been shown that IL-6 expression is increased in lungs with DAD (3, 27), but the function of IL-6 in these lungs is still unknown. On one hand, it has been reported that IL-6 promotes inflammation in DAD (22, 32), yet it has also been reported that IL-6 inhibits inflammation in the lung (42). Recently, it was reported that transgenic mice that overexpress IL-6 in their lungs were more resistant to hyperoxia than wild-type (WT) mice. DNA fragmentation and the leak in the alveolar-capillary barrier, both hyperoxic stress induced, were reduced in the lungs of these transgenic mice (37). There are two possible explanations for this resistance. First, IL-6 could protect lung tissue through the inhibition of injurious inflammation. Second, IL-6 could directly protect alveolar epithelial cells or capillary endothelial cells.

We hypothesized that IL-6 directly protects the lung resident cells, especially alveolar epithelial cells, from oxidative injury. To examine the direct effects of IL-6 on the lung resident cells, we needed a blood-free system to eliminate the indirect effects of circulating inflammatory cells. The organ culture system is blood free (1, 43), and organotypic culture of lung slices is an established method for estimating toxicity (23, 24, 30). Using this method, we examined the cytoprotective effect of IL-6 against oxidative stress.

Materials and Methods

IL-6-deficient mice. The profile of IL-6-deficient (IL-6−/−) mice has been described elsewhere (26). Briefly, IL-6−/− mice (gift from Dr. M. Kopf, Max-Planck-Institut für Immunobiologie, Freiburg, Germany) (16) were backcrossed with DBA/1J mice (Nippon Charles River, Kanagawa, Japan) for eight generations. After the eighth backcrossing with DBA/1J mice, loss of IL-6 mRNA expression of lipopolysaccharide-stimulated splenocytes in IL-6−/− mice was confirmed by RT-PCR. In all experiments, we used male IL-6−/− mice and their WT littermates, which were between 12 and 24 wk of age. Mice were bred under pathogen-free conditions at the Experimental Animal Center of Osaka University Medical School. The animal protocol in this study was approved by the Institutional Laboratory Animal Care and Use Committee of Osaka University.

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Organotypic lung slice culture. Mice were killed by inhalation of sevoflurane (Maruishi Pharmaceutical, Tokyo, Japan) and were blotted through the abdominal aorta. The thorax was opened, and the lungs were perfused via the right ventricle with 20 ml of ice-cold Gey’s balanced salt solution (GBSS) containing 6.5 g/l of glucose. A 24-gauge intravenous catheter (Terumo, Tokyo, Japan) was introduced into the trachea and sutured in place. The lungs were infused with 1.5 ml of 1.2% low-melting-temperature agarose (BioWhittaker Molecular Application, Rockland, ME) solution in organ culture medium (40°C). After the trachea was clamped, the trachea, lungs, and heart were dissected en bloc and cooled at 4°C in GBSS for 10 min to solidify the agarose. Once the lung had cooled, the left lobe was separated, and complete transverse serial sections, 500 μm thick, were cut using a rotator slicer (DK7Y000; Dosaka, Kyoto, Japan). After floating in 4°C GBSS for 1 h, the slices were mounted on transwell chambers (8-μm pore size; Costar, Cambridge, MA). The slices were cultured in Waymouth’s MB751/1 (GIBCO, Rockville, MD) containing 1% heat-inactivated FBS and supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 100 nM hydrocortisone, 8 ng/ml sodium selenite, 100 U/ml penicillin G, and 10 μg/ml streptomycin. The slices were incubated at 37°C in 5% CO2-95% air. H2O2 was added into the culture medium, and the slices were harvested at each time point.

Treatment of anti-IL-6 MAb. Anti-mouse IL-6 MAb and isotype control rat IgG1 were purchased from Genzyme-Techne (Cambridge, MA). WT mice were treated with antibodies (5 μg/body) injected intraperitoneally at 24 and 1 h before slice culture was done. The same protocol was used to abolish the effect of endogenous IL-6 in the mouse model of the pleurisy and lung injury (8). Antibodies were also added to both the agarose solution and culture medium (1 μg/ml).

Detection of DNA fragment. The harvested slices were preserved at −80°C until DNA extraction. For DNA extraction, the slices were suspended in 500 μl of buffer (100 mM Tris-HCl, pH 8.0, 5 mM Na2EDTA, 0.1% SDS, 200 mM NaCl) and digested with 100 μg/ml of proteinase K at 56°C for 12 h. After phenol and phenol/CHCl3 extraction, DNA was precipitated by isopropanol, resolved in 50 μl of Tris-EDTA, and incubated with 40 μg/ml of RNase at 37°C for 2 h. The levels of DNA fragmentation were determined with ApoAlert ligation-mediated (LM)-PCR Ladder Assay kit (Clontech Laboratories, Palo Alto, CA). In brief, 50 ng of purified DNA were used for LM-PCR assay. Dephosphorylated adapters were ligated to 5'-phosphorylated blunt ends with T4 DNA ligase (at 16°C for 14 h). They then served as primers in LM-PCR under the following conditions: hot start (72°C for 8 min), 20 cycles (94°C for 1 min and 72°C for 3 min), and postcycting (72°C for 15 min). Amplified DNA was subjected to electrophoresis on a 1.2% agarose gel containing ethidium bromide. All the bands detected were quantified by FluorChem FC (Alpha Innotech, San Leandro, CA). The levels of DNA fragmentation were quantitated relative to one of the DNA samples, extracted from the slice of IL-6−/− cultured for 8 h with H2O2.

TdT-mediated dUTP nick end labeling staining. The harvested slices were fixed with buffered 10% formalin for 24 h. After being embedded in paraffin, the sections, 4 μm thick, were prepared on slides. TdT-mediated dUTP nick end labeling (TUNEL) staining of tissue sections was performed with the In Situ Cell Death Detection kit (Roche, Mannheim, Germany). In brief, the sections were baked at 60°C for 30 min, dewaxed, and rehydrated. To facilitate access of the reagent to DNA fragments, sections were treated with 20 μg/ml of proteinase K at 37°C for 30 min. End labeling with TdT was subsequently done in TdT buffer containing fluoroscin-dUTP at 37°C for 1 h. Sections were counterstained with Hoechst 33342 (Sigma, St. Louis, MO). In some experiments, immunohistochemical staining for cytokerin was also done after TUNEL staining. Sections were treated with anti-cytokeratin antibody (1:50; Progen, Heidelberg, Germany) and then incubated with Cy3-anti-rabbit IgG (1:200, Jackson ImmunoResearch, West Grove, PA).

Electron microscopy. The harvested slices were fixed in 2.5% glutaraldehyde at 4°C for 2 h. After being washed two times in PBS, the slices were postfixed with 2% OsO4 at 4°C for 2 h. They were then dehydrated with a graded series of alcohol and embedded into Quetol 812. Ultrathin sections were cut with 8800 Ultrotome (LKB) and stained with uranyl acetate and lead nitrate. Electron micrographs were taken with Hitachi H-300.

Analysis of alveolar epithelial cell line (A549 cells). A549 cells, a tumor cell line with properties of type II alveolar epithelial cells, were purchased from Japan Cancer Research Resources (Tokyo, Japan). A549 cells were maintained in DMEM (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated FBS and penicillin/streptomycin in a humidified atmosphere containing 95% air-5% CO2. Confluent A549 cells were detached by 0.05% trypsin and 0.02% EDTA, washed two times with serum-free DMEM, and resuspended in serum-free DMEM (2 × 10⁵/ml). These cells were inoculated into a collagen-coated dish (Asahi Techno Glass, Tokyo, Japan) with 0–20 ng/ml of recombinant human IL-6 (Genzyme/Techne). After 30 h of preincubation, H2O2 (600 μM) was added, and then adhering surviving cells were counted by trypan blue staining after 0–72 h.

RT-PCR. Total RNA was extracted from each organotypic lung slice by the acid guanidinium thiocyanate-phenol-chloroform extraction method with Isogen (Nippon Gene, Tokyo, Japan). One microgram of total RNA was reverse transcribed in the presence of 25 units of SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA), 500 ng oligo(DT)12–18, 0.01 M DTT, and 1 mM dNTP in a total volume of 20 μl at 42°C for 1 h. Each 2 μl of resultant cDNA preparation was used directly for PCR. Amplification was performed in a 50-μl reaction mixture containing 0.4 μM each primer (see below), 250 μM each dNTP, and 2.5 units of TaKaRa Ex Taq (Takara, Kyoto, Japan). The primers used were mouse IL-6 sense primer, 5′-GATGCTACCAACTGGATATAATC-3′; mouse IL-6 antisense primer, 5′-GTTCCCTAGCCACCTCTTCTGTG-3′; mouse β-actin sense primer, 5′-TGTTGGCCGCTTACGACCAAA-3′; and mouse β-actin antisense primer, 5′-CTTTGTTATGTACGCACTTTTGC-3′. Reaction mixtures were incubated in GeneAmp PCR System 9600 (Perkin Elmer) for 35 cycles (denaturation, 30 s at 94°C; annealing, 1 min at 60°C; and extension, 1 min at 72°C). PCR products were subjected to electrophoresis on a 1.2% agarose gel containing ethidium bromide.

Primary culture of mouse lung fibroblast. For each experiment, the lungs from two male C57BL/6J mice (Clea Japan, Tokyo, Japan) were pooled, minced with razor blades into 1-mm squares, washed with PBS, and centrifuged at 800 rpm for 5 min to remove the aqueous bloody layer until the sample was relatively free from blood. After digestion with 5 ml of collagenase S-1 (Nitta Gelatin, Osaka, Japan) solution (4 mg/ml) at 37°C for 1 h, the sample was strained through sterile nylon mesh (Falcon, Bedford, MA), washed twice with 10% FBS-DMEM, and resuspended in 15 ml of culture medium. The cell suspension was plated on a 100-mm plastic culture dish (Sumitomo Bakelite, Tokyo, Japan). After a 1-h incubation, nonadherent cells were removed by gentle washing. Fibroblast monolayers were grown in DMEM supplemented with 10% heat-inactivated FBS and penicillin/streptomycin at 37°C in a 5% CO2 atmosphere. Confluence was usually achieved after 5–7 days, and cells were passaged by detaching with 0.05% trypsin and 0.02% EDTA in PBS and splitting 1:3. All experiments were undertaken with cells of passage 2. These cells are >95% pure fibroblasts based on appearance and vimentin-positive staining. When the cells were confluent, the medium was changed into 1% FBS-DMEM. After a 24-h preincubation, cells were exposed to bleomycin hydrochloride (Nippon Kayaku, Tokyo, Japan) or H2O2.
RNA isolation and Northern blot analysis. Total RNA was extracted with Isogen (Nippon Gene). Twenty micrograms of total RNA was electrophoresed in a 1% agarose gel containing 6% formaldehyde and transferred to Hybond-N+ (Amersham, Piscataway, NJ) for Northern blot analysis by hybridization with radiolabeled mouse IL-6 cDNA probes (EcoRI/BglII-digested fragment, 0.6 kb) (17).

Analysis of intracellular H$_2$O$_2$ production. Intracellular H$_2$O$_2$ production was analyzed by flow cytometry using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma). In brief, cells were incubated with 40 μM DCFH-DA for 5 min, washed with PBS, detached by trypsinization, and analyzed immediately with FACSsort (Becton-Dickinson) equipped with an Argon laser lamp (FL-1: emission, 480 nm; band pass filter, 530 nm) using the data acquisition program Cell Quest (Becton-Dickinson). For each analysis, 10,000 cells were evaluated.

Statistical analysis. Data are expressed as means ± SE. Data were analyzed for statistical significance using Student’s t-test. Differences with P values <0.05 were considered statistically significant.

RESULTS

Reactive oxygen species stimulated IL-6 mRNA production in organotypic lung slices. First, we examined whether reactive oxygen species (ROS) stimulated IL-6 mRNA production in the organotypic lung slices of WT. IL-6 mRNA levels were evaluated by RT-PCR. At 4 h after stimulation by 600 μM H$_2$O$_2$, expression of IL-6 mRNA had already increased (Fig. 1A). The level of IL-6 mRNA expression was increased 4 h after the addition of H$_2$O$_2$, in an H$_2$O$_2$ concentration-dependent manner (Fig. 1B). Because the organotypic lung slices were free from circulating blood, these results showed that ROS stimulated the expression of IL-6 mRNA at least in one of the lung resident cells, which included alveolar macrophages, epithelial cells, endothelial cells, and lung fibroblasts.

ROS simulated IL-6 mRNA production in mouse primary lung fibroblasts. We examined whether the intracellular ROS stimulated IL-6 mRNA production in mouse primary lung fibroblasts, one of the resident cells of the lung. It has been reported that culturing under a reduced serum condition placed the cells under oxidative stress. Bleomycin is a well-known anti-cancer drug that produces ROS within the target cell. We added increasing concentrations of bleomycin to the reduced serum condition (Fig. 2A). The expression of IL-6 mRNA was examined by Northern blot analysis. After the serum concentration was reduced, the expression of IL-6 mRNA increased over 72 h (data not shown). The levels of IL-6 mRNA expression at 72 h were increased in a bleomycin concentration-dependent manner (Fig. 2B). N-acetyl-L-cysteine (NAC, 5 mM), a ROS-scavenger, diminished the IL-6 mRNA production under these conditions (Fig. 2C).

Organotypic lung slices of WT mice were resistant to ROS-induced DNA fragmentation compared with those of IL-6$^{-/-}$ mice. First, we determined to what extent cell death occurred in the lung slice culture system with or without 600 μM H$_2$O$_2$ exposure. The extent of DNA fragmentation in a lung slice...
cultured without H$_2$O$_2$ for 12 h is much lower than that with 600 μM H$_2$O$_2$ (Fig. 3, A and B). TUNEL staining was performed to examine the distribution of cell death in the lung slice without H$_2$O$_2$. For unknown reasons, TUNEL-positive, cell-rich areas were observed in all the cultured lung slices. These areas were distributed among the subpleural narrow spaces surrounding the lung slices. The size of these areas did not change over time or conditions, indicating that it was likely due to this artificial culture system. Therefore, in the TUNEL study, we selected the fields far from pleura in the same extent. In these fields, no TUNEL-positive cells were observed after 12 h of culture without H$_2$O$_2$ (data not shown).

To examine whether endogenous IL-6 is protective against ROS-induced tissue injury, the levels of DNA fragmentation induced by H$_2$O$_2$ in the organotypic lung slices of WT and IL-6$^{-/-}$ mice were compared. Because organotypic lung slices are free from blood circulation, we can estimate the direct cytoprotective effect of endogenous IL-6 without influx or accumulation of circulating inflammatory cells. To estimate the level of DNA fragmentation, LM-PCR was performed. At the start of culture, no DNA fragmentation was detected in the lung slices of either WT or IL-6$^{-/-}$ mice; however, after 8 and 12 h of incubation with 600 μM H$_2$O$_2$, the levels of DNA fragmentation in the lung slices of IL-6$^{-/-}$ mice were higher than those of WT mice (Fig. 3, C and D). TUNEL staining was also examined. In the preliminary studies, we cut serial sections through all the lung slices, compared them with hematoxylin-eosin and TUNEL staining, and confirmed that no significant differences were observed. To avoid sampling bias, we compared the sections cut from the same part of the lung slices by counting the number of sections from the upper surface of the lung slices. Compared with the lung slices of WT mice (Fig. 4A), more TUNEL-positive cells were observed in the lung slices of IL-6$^{-/-}$ mice (Fig. 4B). Counterstaining by Hoechst 33342 showed that the numbers of nuclei in the lung slices of WT (Fig. 4C) and IL-6$^{-/-}$ mice (Fig. 4D) were not different. To examine the distribution of TUNEL-positive cells in the slices of IL-6$^{-/-}$ mice, triple staining by TUNEL, Hoechst 33342, and immunohistochemical staining for cytokeratin was done. At the higher magnifications, it was observed...
that the TUNEL-positive nuclei in the lung slices of IL-6−/− mice existed in the alveolar walls (Fig. 5A). Some of the TUNEL-positive nuclei show typical nuclear condensation (Fig. 5B). With electron microscopy, condensed nuclei were observed in both capillary endothelial cells and alveolar epithelial cells (Fig. 5, C and D).

Anti-IL-6 MAb abrogated the resistance of the organotypic lung slices of WT mice to ROS-induced DNA fragmentation. The organotypic lung slices of WT mice were more resistant to ROS-induced DNA fragmentation compared with those of IL-6−/− mice. Next, the effect of anti-IL-6 antibody on ROS-induced DNA fragmentation in WT mice was examined. Higher levels of DNA fragmentation were observed in the slices treated with anti-IL-6 MAb compared with the slices treated with control IgG (Fig. 6, A and B).

IL-6 protected A549 cells from ROS-induced cell death. To clarify that IL-6 protects alveolar epithelial cells from ROS-induced cell death, A549 cells, a type II alveolar epithelial cell-derived cell-line, were treated with or without IL-6. After 30 h of pretreatment with serum-free medium with or without IL-6 (10 ng/ml), H2O2 (600 μM) was added. The number of surviving cells counted by trypan blue staining decreased in a time-dependent manner. However, the number of survival cells treated with IL-6 was significantly greater than that of the control (Fig. 7A), and the number of survival cells 24 h after H2O2 stimulation increased in an IL-6 concentration-dependent manner (Fig. 7B).

DISCUSSION

In this study, we showed that 1) in the lung resident cells, including fibroblasts, ROS upregulated IL-6 gene expression in a concentration-dependent manner; 2) the organotypic lung slices of WT mice were relatively resistant to ROS-induced DNA fragmentation compared with those of IL-6−/− mice; 3) in the organotypic lung slices of IL-6−/− mice, DNA fragmented cells included alveolar epithelial cells; 4) the resistance of WT mice was abrogated by the treatment with anti-IL-6 antibody; and 5) A549 alveolar epithelial cell death induced by ROS was decreased by IL-6 in a concentration-dependent manner. These results demonstrated that IL-6 has a direct protective effect from ROS on the lung resident cells, especially alveolar epithelial cells.

Ward et al. (37) reported that the transgenic mice that overexpress IL-6 in the lungs are resistant against DAD induced by hyperoxemia. In the lungs of transgenic mice, the leakage of the alveolar-capillary barrier and DNA fragmentation were reduced. There are two possible explanations for this resistance. First, IL-6 could protect lung tissue through the inhibition of injurious inflammation. Second, IL-6 could directly protect alveolar epithelial cells or capillary endothelial
cells. To examine the direct protective effects, we used the model of organotypic culture of lung slices, which has the advantage that circulating inflammatory cells can be eliminated. In a recent report, this system was also applied as the strategy to estimate the direct effect of transforming growth factor-β on lung resident cells without the effect of inflammatory cells (43). Our results demonstrated that IL-6 had a direct protective effect against ROS-induced lung injury. There were two additional advantages in using this organ culture system. Lung toxicity could be estimated in a model similar to the in vivo situation. The differentiated state of each cell is maintained by cell-matrix contact, cell-cell contact, and local soluble factors (19, 31). Cell death can also be influenced by these local environments (28, 33). Because the alveolar structure of the lung slice was kept intact, the cells did not lose their local contacts. The other advantage of using this organ culture system was that it enabled us to examine the autocrine and paracrine factors. Our results showed that the organotypic lung slices of WT mice were relatively resistant to ROS-induced DNA fragmentation and that this resistance was abrogated by the treatment of anti-IL-6 antibody, suggesting that the lung cells were protected by endogenous IL-6 locally produced by the resident cells in an autocrine or paracrine manner.

TUNEL stain revealed the distribution of DNA fragmentation in the organotypic lung slices of IL-6−/− mice compared with those of WT mice, more TUNEL-positive cells were observed. Many of the TUNEL-positive cells were located in the corner of the alveolar walls, as are the type II alveolar epithelial cells. Electron microscopy revealed nuclear condensation in alveolar epithelial cells. IL-6 protected A549 cells of a type II epithelial cell-derived cell line from ROS-induced cell death in a concentration-dependent manner. These results indicated that IL-6 had a protective effect on alveolar epithelial cells. We used the organotypic lung slices of IL-6−/− mice, not of IL-6 transgenic mice. In the lungs of IL-6 transgenic mice, IL-6 is overexpressed during their lifetime, far beyond the physiological levels. Transgenic mice are suitable for examining the effects of exogenous IL-6. On the other hand, through comparison between WT and IL-6−/− mice, we could estimate the physiological effect of endogenous IL-6 in the lung. Our study strongly suggests that endogenous IL-6 had a protective role against H2O2-induced lung injury.

The mechanism by which IL-6 protected alveolar epithelial cells from ROS-induced cell death needs to be examined further. IL-6−/− mice have been deprived of IL-6 effects since their birth so that the effects of IL-6 deficiency in IL-6−/− mice are both acute and chronic. In IL-6−/− mice, IL-6 deficiency is supposed to affect the homeostasis, which is regulated by a variety of genes; therefore, it remains unclear whether the effect of IL-6 deficiency is direct or indirect. Our study also demonstrated that treatment by anti-IL-6 antibody abrogated the resistance of WT mice against ROS-induced lung injury. This result suggested that IL-6 had a relatively acute and direct effect of alveolar epithelial cytoprotection.

In our preliminary data, IL-6 did not reduce the intracellular ROS level of A549 cells (data not shown). Waxman et al. (39) reported that both IL-6 and IL-11 protected pulmonary endothelial cells from ROS-induced cell death in an antioxidant-independent manner. Upregulation of bcl-2 family proteins by STAT pathway was reported to be one of the mechanisms of the cytoprotective effect of IL-6 type cytokines (12, 37). The target genes of IL-6, which are associated with the protection of alveolar epithelial cells, are also to be studied.

In lungs with DAD, alveolar epithelial cell death has properties of both necrosis and apoptosis (2, 15, 18). It remains unknown which mode of cell death is more critical in the pathogenesis of DAD. In the organotypic lung slices stressed by H2O2, cell death was associated with DNA fragmentation and nuclear condensation. These are the characteristic features of apoptosis. In the case of A549 cells, both annexin V (+)-PI (+) and necrotic and annexin V (+)-PI (−) apoptotic cells were increased. It has been reported in several in vitro studies that the same agent induced both necrosis and apoptosis in the same cell population (29). The distinction between these two modes of cell death has been reported to be determined by the energy state of the cell (9) or by the severity of the stress (21).

Regardless of the mode of cell death, our results clearly...
demonstrate that IL-6 protected against H2O2-induced lung epithelial cell death.

It is unknown which stimulus is the most critical for epithelial cell death during DAD, and the question of whether the cytoprotective effect of IL-6 is specifically against ROS or is generally against several cytotoxic stimuli should be studied. An increased level in plasma lipoperoxides and a decreased concentration of circulating endogenous antioxidants were reported among acute respiratory distress syndrome patients (20, 45). In the experimental animal models of DAD, ROS were reported to participate in their pathogenesis (6, 7). Furthermore, a variety of lung toxins, such as O2, anti-cancer drugs, and radiation, exert their cytotoxic effects through the production of ROS (11, 14). Together, ROS could be considered to be the major contributor of alveolar epithelial injury in DAD.

The mortality rate of DAD remains high (38); however, no effective pharmacotherapy has been found. Accumulating evidence shows that alveolar epithelial injury plays a crucial role in the pathogenesis of DAD (4, 10, 13, 40). The protection from alveolar epithelial injury is a possible target of DAD therapy (5). Keratinocyte growth factor (KGF) was reported to be one of the protective factors for alveolar epithelial cells (41). In the transgenic mice that overexpress KGF in an inducible, lung-specific fashion, lung epithelium, not endothelium, was protected from hyperoxia-induced cell death (25). KGF is a candidate for therapeutic agent in DAD. Our study suggests that IL-6, which together with its signaling pathway and its target gene is associated with cytoprotective effects, is another attractive candidate. For clinical application, however, further investigation is required to understand the precise mechanism by which IL-6 protects the alveolar epithelial cells.

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