Interactions of keratinocyte growth factor with a nitrating species after marrow transplantation in mice

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Haddad, Imad Y., Angela Panoskaltis-Mortari, David H. Ingbar, Ernesto R. Resnik, Shuxia Yang, Catherine L. Farrell, David L. Lacey, David N. Cornfield, and Bruce R. Blazar. Interactions of keratinocyte growth factor with a nitrating species after marrow transplantation in mice. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L391–L400, 1999.—We reported that allogenic T cells given to irradiated mice at the time of marrow transplantation stimulated tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and nitric oxide (NO) production in the lung, and the addition of cyclophosphamide (known to stimulate superoxide production) favored the generation of a nitrating species. Although keratinocyte growth factor (KGF) prevents experimental lung injury by promoting epithelial repair, its effects on the production of inflammatory mediators has not been studied. KGF given before transplantation inhibited the T cell-induced increase in bronchoalveolar lavage fluid protein, TNF-α, IFN-γ, and nitrite levels measured on day 7 after transplantation without modifying cellular infiltration or proinflammatory cytokines and inducible NO synthase mRNA. KGF also suppressed NO production by alveolar macrophages obtained from mice injected with T cells. In contrast, the same schedule of KGF failed to prevent permeability edema or suppress TNF-α, IFN-γ, and NO production in mice injected with both T cells and cyclophosphamide. Because only epithelial cells respond to KGF, these data are consistent with the production of an epithelial cell-derived mediator capable of downregulating macrophage function. However, the presence of a nitrating agent impairs KGF-derived responses.

nitric oxide; peroxynitrite; idiopathic pneumonia syndrome; polymerase chain reaction; proinflammatory cytokines

IDIOPATHIC PNEUMONIA SYNDROME (IPS) remains a significant cause of morbidity and mortality and a major factor limiting the success of bone marrow transplantation (BMT). Allogeneity is an established risk factor for the development of IPS (5, 19, 39). Once infiltrating T cells, alloactivated by antigen-presenting cells, encounter pulmonary antigens, immune-mediated damage begins. Major mediators responsible for killing by cytolytic T cells are via release of perforin (cytolysin, a pore-forming protein) or granzymes (10) and a Fas-dependent lytic pathway (23). In addition, T cells can activate alveolar macrophages to release tissue-damaging mediators such as tumor necrosis factor (TNF)-α (3).

Our group (14) recently reported that alveolar macrophage-derived reactive oxygen and nitrogen species such as nitric oxide (·NO), superoxide (O2·−), and ·NO-derived species play a central role in the development of lung injury in a murine IPS model after allogeneic BMT. The specific nature of the reactive species was dependent on the transplantation conditions. Total body-irradiated (TBI) mice given allogeneic donor spleen T cells at the time of BMT (BMS) developed lung dysfunction associated with the induction of TNF-α, interferon (IFN)-γ, and ·NO. However, the combined injection of T cells and a conditioning regimen of cyclophosphamide (Cy), known to enhance O2·− production (4), favored the generation of a nitrating agent, most likely peroxynitrite (ONOO−), formed by the rapid reaction between ·NO and O2·− (18a). The presence of a nitrating agent was associated with the greatest severity of lung dysfunction. Because Cy in the absence of donor T cells did not result in significant lung dysfunction (35), ONOO− formation clarifies the dependence of Cy-facilitated injury and lethality on the presence of allogenic T cells.

Keratinocyte growth factor (KGF), a member of the fibroblast growth factor family, is protective in lethal experimental lung injury models of hyperoxia, acid instillation, radiation, and bleomycin (32, 47, 48). The mechanisms of the protective effects of KGF involve the selective stimulation of alveolar type II pneumocyte proliferation and restoration of normal alveolar structure and function in vivo (42). However, the observation that the preinsult intravenous injection of KGF was as effective as an intratracheal administration despite a significantly less alveolar type II cell proliferative response suggested that the mechanism of protective effects are incompletely understood (13). The effects and mechanisms of KGF on proinflammatory cytokines and ·NO production in the injured lung have not been elucidated.

Nitrating agents such as ONOO− can disable tyrosine phosphorylation (9, 21, 22). KGF binds to epithelial cell surface receptors, which results in receptor autophosphorylation (on tyrosine) and activation of a phosphorylation cascade (18). We therefore hypothesized that conditions favoring the generation of a nitrating species interferes with the biological effects of KGF by nitration of proteins critical for KGF-derived signaling. Our results indicate that the pre-BMT injec-
tion of KGF suppressed T cell-dependent alveolar macrophage activation and the production of inflammatory mediators in the lungs. However, these inhibitory effects of KGF were impaired during conditions associated with the generation of a nitrating species.

METHODS

Mice. Female B10.BR (H2b) and C57BL/6 (H2b) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The mice were housed in microisolator cages in the specific pathogen-free facility of the University of Minnesota (Minneapolis) and cared for according to the Research Animal Resources guidelines of our institution. For BMT, donors were 4–6 wk of age and recipients were used at 8–10 wk of age. Sentinel mice were found to be negative for 15 known murine viruses including those that contribute to pneumocystis (e.g., cytomegalovirus, pneumonia virus of mice, K-virus) by our animal facility during repeated extensive evaluations over the study period.

BMT. BMT was performed as previously described (34, 35). B10.BR mice received phosphate-buffered saline (PBS) or recombinant human KGF (5 mg·kg−1·day−1 subcutaneously; Amgen, Thousand Oaks, CA) on days −6, −5, and −4 pre-BMT. Mice were then segregated into those receiving either PBS or Cy (120 mg·kg−1·day−1; Cytoxan, Bristol-Myers Squibb, Seattle, WA) as a conditioning regimen on days −3 and −2. All mice were lethally irradiated (7.5-Gy total body irradiation by X ray at a dose rate of 0.41 cGy/min) on the day before BMT (1). Donor C57BL/6 BM was T cell depleted with a monoclonal anti-Thy-1.2 antibody (clone 30-H-12, rat IgG2b; kindly provided by Dr. David Sachs, Massachusetts General Hospital, Boston, MA) plus complement (Neffenegger, Woodland, CA). For each experiment, a total of 30–40 recipient mice/treatment group were transplanted via the caudal vein with 2 × 106 natural killer (NK) cell-depleted (PK136, anti-NK1.1 plus complement) spleen cells (BMS) as a source of IPS-causing T cells.

Bronchoalveolar lavage. On day 7 post-BMT, the mice were killed after an intraperitoneal injection of pentobarbital sodium, and the thoracic cavity was partially dissected. The trachea was cannulated with a 19-gauge needle and infused with 1 ml of ice-cold sterile PBS, and the fluid was withdrawn. This was repeated three times, and the return fluids were combined. The bronchoalveolar lavage fluid (BALF) was immediately centrifuged at 500 × g for 10 min at 4°C to pellet the cells. BALF total protein was determined with a hemocytometer, and cell viability was assessed by trypan blue exclusion. Cells (2 × 105/well) were added to 96-well flat-bottom microtiter plates (Costar, Cambridge, MA), and macrophages were allowed to adhere for 1 h at 37°C in 5% CO2 in air, followed by removal of unbound cells. The cells were maintained in culture at 37°C for 48 h in 5% CO2 in air. At the termination of cell culture, the supernatants were aspirated from the individual culture wells for measurement of nitrite with the Greiss method and lactic dehydrogenase (LDH) with the colorimetric CytoTox 96 assay with bovine heart LDH as the standard (Promega, Madison, WI). The cells were washed twice with PBS and lysed with lysis solution (10× Triton X-100; Promega), and cellular LDH release was measured. The percent cytotoxicity during the culture of macrophages obtained from transplanted mice was calculated by dividing the cell-free supernatant LDH by the total cellular plus supernatant LDH of each well and multiplying by 100. Total (supernatant plus cellular) LDH values also were used to correct for possible differences in adherent cell number between groups. Nitrite readings were adjusted accordingly with the BM group at an assigned reference value for 2 × 105 cells (the number of cells originally plated per well).

Immunohistochemistry. In one to two mice per group per experiment, a mixture of 1 ml of optimal cutting temperature medium (Miles Laboratories, Elkhart, IN) and PBS (3:1) was infused into the trachea. The lung was snap-frozen in liquid nitrogen and stored at −80°C. Frozen sections were cut 4 µm thick, mounted onto glass slides, and fixed for 10 min in 3% paraformaldehyde at 4°C. Nonantigenic sites were blocked with 10% normal goat serum (Sigma) for 30 min at 23°C followed by incubation overnight at 4°C with 1) rabbit polyclonal anti-nitrotyrosine antibody (NTAb; 1:50 dilution; Upstate Biotechnology, Lake Placid, NY) or 2) rabbit polyclonal anti-mouse macrophage inducible NO synthase (iNOS) antibody (1:100 dilution; Transduction Laboratories, Lexington, KY). In control measurements, tissues were incubated with NTAb in the presence of 10 mM nitrotyrosine or with nonspecific rabbit IgG (20 µg/ml). All sections were incubated with a secondary antibody, goat anti-rabbit IgG conjugated with horseradish peroxidase (1:500 dilution), for 45 min at 23°C, followed by the addition of 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA) chromogenic substrate. The sections were counterstained with hematoxylin, dehydrated, overlaid with Permount (Sigma), and sealed with coverslips.

Semi quantitative RT-PCR. In some animals, day 7 post-BMT lungs were extracted without lavage and immediately frozen in liquid nitrogen. Total RNA was extracted with the guanidium thiocyanate-phenol-chloroform method (TriReagent, Sigma). RT was performed with a cDNA synthesis kit (First-Strand cDNA Synthesis Kit, Amersham Pharmacia Biotech, Uppsala, Sweden). The RT mixture consisted of 2 µg of RNA; 5 µl of Moloney murine leukemia virus RT containing RNase/DNase-free BSA, dATP, dCTP, dGTP, and dTTP; 1 µl of random hexadeoxynucleotide primer [pd(N)6; 0.2 µg/µl]; and 1 µl of dithiothreitol (200 mM). The RT mixture was incubated for 1 h at 37°C. The products were further amplified by PCR with Klentaq DNA polymerase (Clontech, Palo Alto, CA). The oligonucleotide upstream and downstream primer sequences, annealing temperatures, and cycle numbers were as follows: 1) mouse β-actin, 5′-AAGTGTGACCTGTCATCCGT-3′ and
RESULTS

KGF does not modify total BALF cellularity or composition. Allogeneic T cells with or without Cy conditioning given to TBI mice at the time of BMT increased the total number of inflammatory cells recovered by bronchoalveolar lavage on day 7 post-BMT and shifted the cellular differential toward more lymphocytes. KGF administered subcutaneously on days −6, −5, and −4 pre-BMT did not modify the effects of allogeneic T cells and Cy-conditioning regimen on the total number or differential of cells in the BALF (Table 1).

KGF prevents the increased BALF total protein mediated by T cells (BMS) but not by T cells and Cy (BMS + Cy). The percent recovery of BALF was similar in all groups of mice (>90% of instilled volume). Pre-BMT KGF treatment suppressed the T cell-mediated increase of BALF total protein on day 7 after BMT, suggesting that KGF prevented permeability edema (Fig. 1). In contrast, BALF obtained from mice treated with the same schedule of KGF and injected with both T cells and Cy (BMS + Cy) contained the highest level of protein. To characterize the proteins in the BALF, 10 µl of fluid were subjected to SDS-PAGE with both T cells and Cy (BMS) but not by T cells and Cy (BMS + Cy). Allogeneic T cells increased the BALF levels of the stable by-products of NO, nitrite, and nitrate, and the proinflammatory cytokines TNF-α and IFN-γ as measured on day 7 after transplantation. KGF administered before conditioning suppressed the T cell-mediated generation of NO, TNF-α, and IFN-γ levels (Fig. 2). In contrast, pre-BMT KGF failed to suppress the levels of these inflammatory mediators in the BALF obtained from mice injected with both T cells and Cy (BMS + Cy; Figs. 2 and 3). Although the reason for the enhanced nitrite levels in KGF-treated BMS + Cy recipient mice compared with the BMS + Cy recipients (P < 0.05; Fig. 2) is unclear at this time, it may be the result of KGF-mediated abnormal epithelial cell proliferation in a milieu containing high levels of nitrated proteins.

To begin to understand the mechanisms responsible for the KGF-induced suppression of T cell-mediated induction of NO, TNF-α, and IFN-γ, we determined the effects of KGF on iNOS, TNF-α, and IFN-γ gene expression. As assessed by relative semiquantitative RT-PCR, day 7 post-BMT iNOS, TNF-α, and IFN-γ messages were unaffected by KGF treatment in either BMS or BMS + Cy recipients (Fig. 4). Two approaches ensured semiquantitative conditions. First, the cycle titration experiments described in METHODS showed that the RT-PCRs were terminated during the linear range below the saturation of the PCR products. Second, inclusion of an internal standard (18S rRNA) during the RT-PCRs confirmed our semiquantitative conditions (Fig. 5).

Table 1. Effects of KGF on lavage fluid cellular number and profile on day 7 after BMT

<table>
<thead>
<tr>
<th></th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Cell Number, viable cells x 10⁶/mouse</th>
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<tr>
<td>BM</td>
<td>−KGF</td>
<td>94 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td></td>
<td>1 ± KGF</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>BMS</td>
<td>−KGF</td>
<td>54 ± 5*</td>
<td>46 ± 4*</td>
</tr>
<tr>
<td></td>
<td>1 ± KGF</td>
<td>59 ± 4*</td>
<td>41 ± 4*</td>
</tr>
<tr>
<td>BMS + Cy</td>
<td>−KGF</td>
<td>60 ± 6*</td>
<td>40 ± 5*</td>
</tr>
<tr>
<td></td>
<td>1 ± KGF</td>
<td>58 ± 5*</td>
<td>42 ± 6*</td>
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Values are means ± SE for n = 5–8 mice/group obtained from 2 representative experiments. KGF, keratinocyte growth factor; BM, bone marrow transplantation (BMT) + total body irradiation (TBI); BMS, BMT + TBI + donor spleen T cells; BMS + Cy, BMS + cyclophosphamide; ND, not determined. Lavage fluid cell pellets from each treatment group were combined. Total cell number and differential were determined with a hemocytometer, and cell viability was assessed by trypan blue exclusion. *P < 0.05 compared with BM group.
tors. Although cultured alveolar macrophages obtained from mice injected with both T cells and Cy (BMS + Cy) did not release significantly higher NO (compared with that in the BMS group), they released very high levels of LDH (Fig. 6). This increased cytotoxicity in macrophages obtained from BMS + Cy recipient mice were most likely due to enhanced TNF-α and IFN-γ production and the generation of ONOO⁻. Pre-BMT KGF administration suppressed cytotoxicity and nitrite production in macrophages obtained from mice injected with T cells (BMS) but not in mice injected with Cy (BMS + Cy). *P < 0.05 compared with control (BM). †P < 0.05 compared with effect of KGF in each group.

KGF does not modify iNOS protein and nitrotyrosine expression in lungs of mice injected with T cells and Cy (BMS + Cy). Day 7 post-BMT lung sections taken from

Fig. 1. A: total protein levels in cell-free bronchoalveolar fluid (BALF) of irradiated (7.5-Gy total body-irradiated (TBI)) B10.BR mice 7 days after bone marrow (BM) transplantation (BMT). Values are means ± SE; n = 12-20 mice/group. Allogeneic splenic T cells with (+) and without (−) cyclophosphamide (Cy; 120 mg/kg on days −3 and −2) increased BALF protein levels. Keratinocyte growth factor (KGF) pretreatment restored total protein levels in mice given T cells (BMS) but not in mice given T cells and Cy (BMS + Cy). *P < 0.05 compared with control (BM). †P < 0.05 compared with effect of KGF in each group. B: to characterize proteins in BALF, 10 μl of fluid were subjected to SDS-PAGE and visualized with Coomassie stain. MSA, mouse serum albumin. No. on left, molecular mass.

Fig. 2. Nitrite levels in cell-free BALF of irradiated B10.BR mice 7 days post-BMT. BMS or BMS + Cy mice exhibited increased nitrite levels. BMS + Cy mice did not show increased nitrite levels. Values are means ± SE; n = 12-20 mice/group. KGF pretreatment suppressed nitrite in BMS but not in BMS + Cy recipient mice. *P < 0.05 compared with control (BM). †P < 0.05 compared with effect of KGF in each group.

Fig. 3. Levels of proinflammatory cytokines in cell-free BALF of irradiated B10.BR mice collected 7 days after transplantation. A: interferon (IFN)-γ measured by ELISA. B: tumor necrosis factor (TNF)-α measured by ELISA. Values are means ± SE; n = 7-14 mice/group. BMS and BMS + Cy mice exhibited increased TNF-α and IFN-γ. KGF pretreatment suppressed TNF-α and IFN-γ levels in BMS but not in BMS + Cy recipient mice. *P < 0.05 compared with control (BM). †P < 0.05 compared with effect of KGF in each group.
TBI mice were incubated with polyclonal antibodies to iNOS (Fig. 7). Increased iNOS binding was observed in lung sections obtained from mice given T cells (BMS) and T cells plus Cy (BMS + Cy). Staining was reduced to control levels in lung sections from mice not given T cells. Moreover, background staining with nonimmune IgG was similar in all groups. KGF treatment inhibited iNOS protein expression in BMS but not in BMS + Cy recipients (Fig. 7).

Nitrotyrosine formation is considered a “footprint” for the in vivo generation of a nitrating species (20, 30). High levels of nitrated proteins in the concentrated BALF of recipient mice treated with T cells and Cy were reported (14). To determine whether KGF treatment modified nitrotyrosine formation, day 7 post-BMT lung sections were incubated with NTAbs. Only lung sections from BMS + Cy recipients, but not from BMS or BM recipients, exhibited increased immunostaining with the NTAbs. Antibody binding was specific because it was completely blocked in the presence of excess antigen (10 mM nitrotyrosine). Nitrotyrosine in sections of mice injected with Cy without allogeneic T cells was at background levels (data not shown). Pre-BMT KGF failed to prevent nitrotyrosine formation in the lungs of BMS + Cy recipients (Fig. 8). In addition, the high levels of nitrated proteins in the BALF of recipient mice treated with T cells and Cy were not modified by KGF treatment (data not shown).

DISCUSSION

The two major findings of this study were that 1) KGF given to TBI mice before BMT suppressed T cell-dependent inflammatory events occurring in the...
early peri-BMT period and 2) the combined administration of donor spleen T cells and Cy (BMS+Cy) was associated with an impaired response to KGF treatment. Because Cy-facilitated lung dysfunction was dependent on allogeneic T cells (BMS+Cy recipients) and associated with the presence of nitrated proteins (14, 35), we hypothesized that the generation of a nitrating agent was the most likely reason for the impaired KGF effects observed in BMS+Cy recipient mice. Although we cannot rule out other pathways capable of nitrating reactions in vivo (8, 29), the most likely nitrating species under our conditions is ONOO⁻, formed by the simultaneous generation of ·NO (by T cell-activated epithelial and inflammatory cells) and O₂⁻ [by macrophages after exposure to Cy (4)]. In a cell culture system, Shin et al. (37) reported that growth factors enhanced ONOO⁻-induced apoptosis and suggested a unique interaction between growth factors and ONOO⁻ during the resolution of inflammation and repair processes in vivo.

KGF can prevent lung dysfunction by enhancing proliferation and growth of alveolar type II cells (42, 48), increasing surfactant protein (SP) A mRNA and secretion (45), and facilitating alveolar epithelial cell DNA repair (41, 44). Because KGF did not affect the composition of cells infiltrating the lungs or prevent induction of TNF-α, IFN-γ, or iNOS mRNA expression, the suppression of inflammatory mediators observed in KGF-treated allogeneic T cells recipient mice (BMS) was not only due to a dampened inflammatory response associated with KGF-induced enhanced epithelial repair. Therefore, we hypothesized that KGF prevented epithelial permeability by an additional mechanism: the posttranscriptional suppression of TNF-α, IFN-γ, and iNOS protein expression. This hypothesis is strengthened by the finding that macrophages obtained from KGF-treated mice given T cells spontaneously produced significantly less ·NO (Fig. 6) and TNF-α (data not shown).

How does KGF suppress the production of inflammatory mediators? The inhibitory effects of KGF on the T cell-mediated production of inflammatory mediators were observed 10 days after cessation of KGF treatment, suggesting the involvement of a KGF-derived mediator. Work in progress in our laboratories suggests that the KGF-derived mediator is SP-A. SP-A, a calcium-dependent lectin produced by alveolar type II cells, possesses immunoregulatory functions including inhibi-
tion of TNF-α production from lipopolysaccharide-stimulated alveolar macrophages (25). In addition, our group observed the induction of the T helper 2 (Th2)-type anti-inflammatory cytokine interleukin (IL)-13 in KGF-treated mice starting 4 days after the cessation of KGF administration even in the absence of irradiation or BMT (33). IL-13, normally produced by activated T cells and alveolar macrophages (17, 26), is a potent inhibitor of human and rodent macrophage-derived proinflammatory cytokines and \( \cdot \)NO production (6, 27). Consistent with our data, IL-13 downregulates inflammatory mediators at the posttranscriptional level (2) and has no effect on the chemotactic properties of monocytes (46). Therefore, we speculate that the KGF-derived increase in SP-A levels is responsible for the induction of IL-13 and the subsequent downregulation of macrophage function.

How does nitration of critical proteins impair the protective effects of KGF? There are two pathways by which nitrating agents may interfere with the ability of KGF to suppress the production of inflammatory mediators. First, the nitration of tyrosine residues of critical proteins is known to prevent phosphorylation by tyrosine kinases (21, 22, 24). KGF mediates biological functions by autophosphorylation of tyrosine residues present on the KGF receptor (18). Therefore, one possibility is that nitration of the KGF receptor may interfere with the ability of KGF to suppress \( \cdot \)NO, TNF-α, and IFN-γ production. However, KGF was injected several days before BMT and should have manifested its effects before the generation of a nitrating agent. In addition, IL-13 shares with IL-4 a common receptor subunit, the IL-4 receptor-α, essential for intracellular signaling (38, 50). After binding, IL-4 and IL-13 induce receptor association, receptor tyrosine phosphorylation, and coupling with kinases, which activate each other by tyrosine phosphorylation (43). Therefore, the presence of a nitrating species in mice injected with both T cells and Cy may disable IL-13-mediated signaling. The second pathway by which nitrating agents may impair KGF-derived responses is via the nitration of SP-A. Nitration of tyrosine residues of SP-A may abolish its capability to downregulate macrophage and T-cell activation, as was shown for several other properties of SP-A (16, 49). Elimination of the inhibitory effects of IL-13 on proinflammatory cytokines and \( \cdot \)NO production will lead to a resumption of iNOS, TNF-α, and IFN-γ mRNA translation and

Fig. 8. Immunoperoxidase staining of lung sections taken from indicated treatment groups of irradiated BMT mice and incubated with nitrotyrosine antibody (NTAb) or NTAb in presence of 10 mM nitrotyrosine (Block). Increased staining of epithelium and inflammatory cells was observed only in sections obtained from BMS+Cy mice. Shown is a representative experiment, which was repeated once. NTAb binding was specific because it was completely blocked in presence of excess antigen.
production of high levels of ·NO, TNF-α, and IFN-γ protein. The high levels of proinflammatory cytokines and the generation of ONOO− may explain the tissue injury and enhanced permeability edema observed in KGF-treated mice given both T cells and Cy (BMS + Cy).

However, the presence of a nitrating agent impairs some, but not all, of the biological effects of KGF. For example, KGF pretreatment of mice given allogeneic T cells and Cy (BMS + Cy) 1 attenuated BALF LDH levels, although KGF-mediated suppression of LDH levels did not reach significance (P = 0.08; data not shown); 2) suppressed levels of the costimulatory molecules B7-1 (CD80) and B7-2 (CD86); and 3) decreased the number of cells expressing granzyme B mRNA as assessed by in situ hybridization (33). Suppression of granzyme B may explain why KGF pretreatment favorably shifted the survival curves of BMS + Cy recipient mice, although all mice still died, possibly because of increased epithelial permeability and persistent release of inflammatory mediators in the lungs (34). Taken together, these data suggest that KGF prevents lung dysfunction and prolongs survival by tyrosine phosphorylation-dependent and phosphorylation-independent mechanisms. The presence of a nitrating agent will impair only a KGF-mediated tyrosine phosphorylation-dependent signaling pathway.

There is no longer doubt that human monocytes and alveolar macrophages can express functional iNOS, albeit under different and tighter regulatory control compared with rodent cells (11, 28, 36, 40). In humans, other sources of ·NO and ONOO− include airway and alveolar epithelial cells (12, 15) and inhaled ·NO gas commonly administered to IPS patients to improve oxygenation without consideration to the conditioning regimens received. In addition, other nitrin-dependent reaction pathways may contribute to the in vivo nitration of proteins through the formation of nitryl chloride (NO₂Cl) by reaction of nitrite with hypochlorous acid or myeloperoxidase (7).

Because transplantation is always scheduled, the pre-BMT administration of KGF has great potential to prevent the development of IPS. KGF has been shown to prevent lung injury by mediating alveolar type II cell proliferation. We report herein an additional mechanism by which KGF can prevent allogeneic T cell-mediated enhanced epithelial permeability: downregulation of macrophage activation. This mechanism is disabled in the presence of nitrating agents. Further studies are required to determine the signaling proteins responsible for KGF-derived inhibition of proinflammatory cytokines and ·NO and establish a cause-and-effect relationship between the generation of a nitrating species and impaired response to KGF treatment. Identification of specific nitrotyrosyl polypeptides may provide insight into mechanisms regulating not only ONOO−-induced injury but also signal transduction pathways associated with peri-BMT lung repair. Efforts should be directed toward preventing the formation of a nitrating species without extreme inhibition of ·NO production, an essential molecule in the modulation of immune responses.

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REFERENCES


