Early growth response factor-1 in acute lung injury

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EGR1 and acute lung injury. EGR1 is induced by a variety of stimuli in the lung, including hypoxia (29), hyperoxia, ischemia-reperfusion (28), and high tidal volume (4). Exposure to low oxygen concentrations (6–20%) resulted in EGR1 mRNA induction that was greater with lower O2 concentrations and longer exposure times (29). Upregulation of EGR1 DNA binding secondary to hypoxia was also shown to activate tissue factor in mononuclear macrophages, and its absence (i.e., in EGR1 null mice) was associated with reduced tissue factor activation and fibrin deposition in the lungs following exposure to hypoxia (30). Supporting these findings is a recent study in which EGR1 protein upregulation greatly augmented the induction of serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (SERPINE1) in mouse macrophages following exposure to hypoxia (17). This may be especially important as SERPINE1 plays a key role in suppressing fibrinolysis, and its upregulation could therefore be a necessary element in the development of hypoxia-induced thrombosis (17).

Ischemia-reperfusion reflects processes that are central to many acute illness states. In murine pulmonary ischemia-reperfusion, EGR1 mRNA upregulation was evident and localized to lung macrophages and smooth muscle cells (28). Moreover, EGR1 knockout mice had longer survival and lower levels of circulating inflammatory mediators, as well as reduced fibrin and leukocyte accumulation compared with wild-type mice, following exposure to pulmonary ischemia-reperfusion (28). The same inflammatory mediators examined in the ischemia-reperfusion model stated above were strongly activated by LPS (i.e., endotoxin) administration, a model of sepsis, in the knockout mice. This study implicates EGR1 activation in the pathogenesis of ischemic lung tissue damage and delineates specific downstream inflammatory mediators involved, including ICAM1, IL-1β, coagulation factor III (thromboplastin, tissue factor) (F3), SERPINE1, and VEGF (28).

We have previously found, using gene array techniques, that high tidal volume ventilation in rats increased the expression of several genes, including EGR1 (4). This upregulation was evident 30 min after the initiation of injurious ventilation and well before lung injury was demonstrable by histological assessment (4). These findings were further substantiated in fetal lung epithelial cells subjected to in vitro stretch (5).

EGR1 and consequences of lung injury. Fibrosis and apoptosis are key consequences of many lung injury states (16), and use of EGR1 knockout mice and null cells has demonstrated that EGR1 is potentially important in these processes (20). EGR1 knockout mice demonstrated lesser degrees of transforming growth factor (TGFI)-β1-induced pulmonary apoptosis and fibrosis (15). Thus EGR1 may play a role in augmenting multiple aspects of the pathogenesis and pathophysiology of lung injury.

EGR1 regulation. EGR1 belongs to a family of early response genes that also include EGR2, EGR3, EGR4, and the Wilm’s tumor product. It is an 80-kDa nuclear phosphoprotein and contains three zinc-finger DNA-binding domains (11). The promoter region of the EGR1 gene contains genetic elements that link other signaling pathways with enhanced transcription of the EGR1 gene (Fig. 1). This region contains five serum response elements (SREs), nuclear factor of kappa light polypeptide gene enhancer in B cells (p105) (NF-κB1) and specificity protein 1 (Sp1) transcription factor binding sites, and a cAMP response element (CRE) (25). These are sites to which the corresponding transcription factors would bind to and activate transcription of mRNA. The interaction between EGR1, Sp1, and CRE have been shown to be critical for activation of effector genes in several studies (23). At the SRE binding site, additional transcription factors such as serum response factor (SRF) and the ternary complex factor (TCF) are required for SRE-mediated activity. Proteins ELK1, member of ETS oncogene family, ELK4, ETS-domain protein (SRF accessory protein 1), ELK3, and ETS-domain protein (SRF accessory protein 2) are examples of TCFS. Such TCFS need to bind to both the SRF and the DNA binding site to activate transcription. TCFS also need to be first phosphorylated to enhance DNA-binding activity. For example, ELK1 is phosphorylated by c-Jun NH2-terminal protein kinase and extracel-
lular signal-regulated protein kinase (ERK) (27), a group of kinases known as MAPKs. Thus, transcription of EGR1 is activated, and the EGR1 protein moves into the nucleus to bind to promoter regions on genes that have EGR1 binding sites. This results in transcription of these genes.

Inhibition of EGR1 activity occurs through several pathways. NGFI-A binding proteins 1 and 2 (NAB1, NAB2) are transcription cofactors that bind to the inhibitory domain of the EGR1 gene, and these proteins serve to block its biological activity (24). The EGR1 protein also binds to its own gene via the Ets binding sequence (EBS) 5' -CGCCCCCGC-3' to downregulate its own activity (3). EGR1 also controls the expression of NAB2, providing another negative feedback loop for controlling its biological activity (8). The regulation of EGR1 in the cell is dependent on multiple activation pathways and the binding of cofactors (Fig. 1). Therefore, the interaction of these pathways and cofactors with EGR1 are responsible for triggering injurious effects in the cell.

**EGR1-mediated injury.** EGR1 may mediate injury through regulating the transcription of a wide array of downstream genes involved in inflammation, matrix formation, thrombosis, and apoptosis. EGR1 preferentially binds to the GC-rich sequence 5' -GCGGGGGCG-3' (3). This sequence has been found on many target genes, the most prominent being platelet-derived growth factors and β-polypeptides (fibrosis), fibroblast growth factor 2 (fibrosis) (13), VEGF (vascular permeability), CD44 (extracellular matrix turnover and resolution of inflammation), tissue factor (fibrin deposition), TGF-β1 (apoptosis, inflammation, and fibrosis) (14), fibronectin (extracellular matrix), matrix metalloproteinases (chemotaxis and tissue destruction), and SERPINE1 (fibrinolysis) (14). Many of these molecules are effector molecules in acute lung injury. Following upregulation by injurious stimuli, EGR1 may intensify lung injury through thrombosis, edema formation, fibrosis, and apoptosis.

EGR1 has been shown to be a potent stimulator of cellular apoptosis in in vitro studies, such as through its effects on TNFα, PTEN, p53 (26), and retinoblastoma 1 (RB1) (7). All of these genes have EGR1 binding sites. EGR1 directly upregulates synthesis of p53 (19) and PTEN (26). The gene products are all proapoptotic, and EGR1 modulates RB1-mediated apoptotic effects (6). The effects of EGR1 on apoptosis are dependant on both the type of cell and the nature of the stimulus. Indeed, some studies show that EGR1 confers resistance to apoptosis (12), whereas others demonstrate that EGR1 synthesis is increased in dying neuronal cells, but without proof of a cause-effect relationship (2).

**Current gaps in knowledge.** It is clear that EGR1 is an early response gene that has immediate, and, in some cases, a pervasive effect on the immune system in response to certain forms of injury. Recent research efforts in EGR1 have been focused on delineating the mechanisms through which these effects cause injury. However, neither the exact pathways through which EGR1 is activated nor its specific downstream effects have been fully mapped out. Although different injury states activate EGR1, the effects of activation appear to be stimulus and tissue specific. More studies are needed to dissect the interacting pathways of other transcription factors with EGR1 as well as the specific molecules involved in EGR1-triggered injury.

**EGR1-directed therapy.** Attempts to blunt the EGR1 response to injury in experimental models have given insight into novel therapeutic options that may be useful in the clinical setting. Direct methods through the use of EGR1 antisense oligodeoxynucleotides (ODN) and small interfering RNAs have been studied (1, 20, 21). ODN administered to the donor lung reduced postransplant thrombosis, leukostasis, and inflammation and improved survival and graft function in a rat model (21). ODN also reduces pulmonary artery adventitial fibroblast proliferation associated with EGR1 upregulation as well as attenuates hypoxia-induced cell proliferation ex vivo (1).

Several anti-inflammatory or immunosuppressive agents have also been tried to mitigate the EGR1 response to injury. In a rat model of pulmonary ischemia-reperfusion injury,
mycophenolate mofetil has been shown to reduce lung vascular permeability, myeloperoxidase content, and alveolar leukocyte counts through a reduction in EGR1 production (9). The potent and commonly used immunosuppressant cyclosporine administered in pulmonary endothelial cells reduced ERK activation, EGR1 upregulation, and proinflammatory mediator secretion in hypoxia-reoxygenation (10).

Carbon monoxide in a rat model of lung ischemia-reperfusion injury suppressed ERK activation and EGR1 expression (18), resulting in diminished leukostasis and fibrin deposition as well as improved gas exchange and survival. If EGR1 is involved, these interventions could have an important role in conditions in which lung injury can be anticipated, such as lung transplantation or ventilator-induced lung injury.

Conclusion. There is a growing body of evidence implicating EGR1 in the pathogenesis of acute lung injury. Improved survival after exposure to injurious stimuli as well as reduced tissue injury have been reported in EGR1 knockout mice and in other models where expression of EGR1 has been inhibited. Future studies may demonstrate that inhibition of the effects of EGR1 constitute a useful therapeutic approach for attenuating lung injury.

REFERENCES