Signal Transduction Pathways of Apoptosis and Inflammation Induced by the Tumor Necrosis Factor Receptor Family

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A apoptosis has been implicated as a physiologic cell death program critical for homeostasis. Dysregulation of apoptosis may be involved in human diseases such as cancer, AIDS, degenerative and autoimmune diseases, and infectious diseases. A apoptosis may also play important roles in lung diseases in two different ways. First, failure to clear unwanted cells by apoptosis will prolong the inflammation because of the release of their toxic contents. Repair after an acute lung injury requires the elimination of proliferating mesenchymal and inflammatory cells from the alveolar air space or alveolar wall (1). Second, excessive apoptosis may cause disease. An intratracheal injection of agonistic anti-Fas antibody into adult mice causes epithelial cell apoptosis and lung inflammation, which subsequently leads to pulmonary fibrosis (2). DNA damage and apoptosis in lung epithelial cells have been reported in acute lung injury (3), diffuse alveolar damage (4), and idiopathic pulmonary fibrosis (IPF) (5). Therefore, epithelial cell injury is the common manifestation of lung injury, and apoptosis contributes to such injury of epithelial cells.

Tumor necrosis factor (TNF)-α is a proinflammatory cytokine, which can induce a broad spectrum of biologic effects and is associated with inflammatory lung disease. TNF causes inflammation by damaging tissues and by inducing the expression of adhesion molecules and cytokines in epithelial and endothelial cells, as well as in inflammatory cells. The cellular effects of TNF are mediated by two distinct cell surface receptors termed TNF-receptor 1 (TNFR 1) and TNF-receptor 2 (TNFR 2) (6). Most cytotoxic effects of TNF are mediated by TNFR 1 through interaction of its death domain with the TNF-associated death domain protein (TRADD) (7). TRADD interacts with Fas-associated death domain protein (FADD) (8) to activate caspase-8, thereby initiating the apoptosis pathway. Death domain is the sequence in TNFR 1, TRADD, and FADD. The death domain is a protein–protein interdomain, and adopter molecules FADD and TRADD use these domains to interact with other death domain-containing molecules and trigger the apoptosis-signaling pathway. A new well-known death receptor, Fas, also transduces apoptosis signal through FADD and shares the same signaling machinery downstream of FADD with TNFR (Figure 1). Since the Fas-mediated apoptosis-signaling pathway is relatively short and straight compared with that of TNFR, Fas-ligation takes hours to kill target cells, whereas TNF takes a day or more. Furthermore, TNF does not usually kill most type of cells without metabolic inhibitors, which is different from Fas-ligation.

Although TNFR mediates apoptotic signal transduction, it can transduce intracellular signals that activate transcription factor nuclear factor κB (NF-κB) by proteolytic breakdown of the inhibitor of κB (IκB). TNF-associated factor-2 (TRAF2) and receptor interacting protein (RIP) (9) indirectly bind to TNFR 1 through TRADD or directly to TNFR 2 and activate the NF-κB-inducing kinase (NIK) (10), which in turn activates the inhibitor of IκB kinase (IKK) complex (11–14). IKK phosphorylates IκB, which leads to IκB degradation and allows NF-κB to translocate to the nucleus and activate transcription (Figure 1). TNF or agonistic anti-Fas antibody administration can lead to production of interleukin-8 (IL-8) by colon epithelial cells (15) or by bronchial epithelial cells, in addition to inducing apoptosis in vitro (16). A TNF activates the IL-8 promoter transcriptionally via NF-κB activation, IL-8 secretion induced by Fas ligation also seems to be regulated via NF-κB activation (16). It has been reported that the kinase activity of NIK is part of the signaling cascade that leads to NF-κB activation and that this signaling pathway is common to TNFR and Fas (10). Disruption of the NF-κB pathway with the dominant-negative TRAF2 enhances the cytolytic effects of TNF (17). NF-κB subunit RelA−/− mouse fibroblasts and macrophages with TNF result in a significant reduction in viability, whereas RelA+/+ cells were unaffected (18). Therefore, death receptor activation induces NF-κB activation, which triggers inflammation and also plays an important role in regulating apoptosis.

Cellular proteins homologous to baculovirus inhibitors of apoptosis (IAPs) block cell death. TRAF1, TRAF2, XIAP, c-IAP1, and c-IAP2 were identified as gene targets of NF-κB transcriptional activity (19). The caspases are a family of cysteine proteases, and it is now thought that many forms of cell death are ultimately dependent on caspase activation (Figure 1). XIAP, c-IAP1, and c-IAP2 are direct

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inhibitors of caspase-3 and caspase-7. These proteins also indirectly prevent the activation of caspase-3, caspase-6, and caspase-7 by blocking the cytochrome c–induced activation of procaspase-9 (20, 21). Furthermore, the recruitment of TRAF1 and TRAF2 to the TNFR complex through interactions with TRAF1 or TRAF2 inhibits the activation of the initiator caspase, caspase-8 (22). NF-κB–controlled expression of TRAF1 and TRAF2, along with c-IAP1 and c-IAP2, serves as a primary mechanism to protect cells against TNFR-mediated apoptosis. Unlike Fas activation, the fact that most cells are resistant against apoptosis induced by TNFR activation implicates that a defense mechanism through the activation of NF-κB is efficiently induced by TNFR activation.

Modulation of apoptosis could be a new strategy used against lung inflammation and injury. In this issue of the *Journal*, TNF-induced expression of anti-apoptotic genes TRAF1 and c-IAP2 in lung epithelial cells is reported (23). Pryhuber and colleagues demonstrated that TRAF1, TRAF2, XIAP, c-IAP1, and c-IAP2 mRNA (mRNA) were expressed in lung epithelial cell lines. They also demonstrated that TRAF1 mRNA, TRAF1 protein, and c-IAP2 mRNA expression were upregulated in lung epithelial cell lines by the administration of TNF. Pryhuber and associates also demonstrated that TRAF1 expression was increased in the lungs of infants dying from pneumonia or bronchopulmonary dysplasia. These results offer a possibility that the TRAF1 and c-IAP2 may be involved in inflammatory lung disease. Although the precise functions and regulation of TNF signaling molecules remain to be addressed, understanding and manipulating those molecules could provide new therapeutic strategies against inflammatory lung disease.

Although many factors are known to promote growth, differentiation, or survival, only a few cytokines, including TNF and Fas ligand (FasL), have been found to induce apoptosis. The administration of bleomycin has been used extensively to induce apoptosis in vitro and in vivo. The acute pulmonary toxicity induced by bleomycin in vivo is DNA damage (24), which is known to induce apoptosis in vitro (25). It is known that TNF mediates bleomycin-induced pulmonary fibrosis (26) and that the expression of TNF transgene in murine lung causes lymphocytic and fibrosing alveolitis (27). Ortiz and coworkers demonstrated that the normal murine lung constitutively expresses both TNFR1 and TNFR2 mRNA and that the exposure to either silica or bleomycin results in upregulation of TNFR1 but not TNFR2 mRNA in lung tissue (28). They also demonstrated that both TNFR1- and TNFR2-gene–deleted mice demonstrate an enhanced expression of TNF mRNA but did not develop lung injury and pulmonary fibrosis. These data suggest that TNFR is fundamental to the development of bleomycin-induced pulmonary fibrosis. As well as TNF, the role of the Fas-FasL pathway has been studied.
in bleomycin-induced pulmonary fibrosis. It has been reported that excessive apoptosis of lung epithelial cells is induced by the Fas–FasL pathway is essential in the development of this model (29, 30). The neutralization of FasL by Fas-immunoglobulin (Ig) fusion protein or anti-FasL antibody could prevent the development of this model, and Fas- or FasL-deficient mice are resistant to the induction of this model (30). The involvement of the Fas–FasL pathway in fibrosing lung disease was also demonstrated (31). These results implicate that the damage and loss of lung epithelial cells induced by death receptors are critical in lung injury and pulmonary fibrosis.

As well as death receptors/ligand, death signals such as reactive oxygen, nitrogen species, proinflammatory cytokines, chemokines, and others are involved in inflammatory lung disease. In animal models of lung injury or human diseases such as acute respiratory distress syndrome (ARDS) and IPF, various inflammatory mediators and death factors induce epithelial cell damage and apoptosis. Therefore, it is unlikely that a single treatment is sufficiently effective in severe lung injury (32, 33). The survival of Fas–FasL transgenic mice is not the only indicator of death receptor–mediated pathway, and another pathway is also essential in the development of this model (29, 30). The neutralization of FasL by Fas–immunoglobulin (Ig) fusion protein or anti-FasL antibody is essential in the development of this model (29, 30). The neutralization of FasL by Fas–immunoglobulin (Ig) fusion protein or anti-FasL antibody could prevent the development of this model, and Fas- or FasL-deficient mice are resistant to the induction of this model (30). The involvement of the Fas–FasL pathway in fibrosing lung disease was also demonstrated (31). These results implicate that the damage and loss of lung epithelial cells induced by death receptors are critical in lung injury and pulmonary fibrosis.

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