

# Study on the Critical Roles of Apoptosis in Asthma Disease

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**Abstract-** Inflammation, and remodeling in airways are the two crucial characteristics of asthma, a common respiratory disease. In asthma pathophysiology, the recruitment of granulocytes finally results in inflammation, leading to lung damage. In this regard, failure to clear inflammatory cells by programmed cell death, apoptosis will cause the prolongation of inflammation. On the other hand, in airway epithelial cells, apoptosis may occur, resulting in airway remodeling. Hence, dysregulation of apoptosis has been suggested to contribute to the development of asthma. Importantly, knowledge of the factors related to apoptotic cascade seems vital to explore various pharmacological interventions for the treatment of asthma. In this review, we highlight several important apoptotic and anti-apoptotic factors contributing either to inflammatory cells or airway epithelial cells involved in asthma pathogenesis.

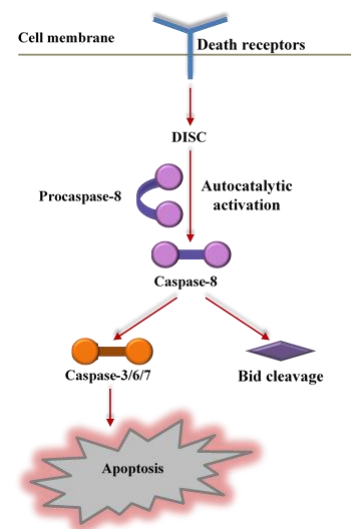
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**Keywords:** Inflammation; Airway remodeling; Programmed cell death; Apoptosis; Asthma

## Introduction

Apoptosis, commonly described as programmed cell death, is a complex process in which a cell dies due to survival factor withdrawal or exposure to pro-apoptotic signals (1-3). It is believed that apoptosis preserves the process of normal cell turnover in almost every organ of the body. Apoptosis is also called a non-inflammatory phenomenon because the cell membrane retains its integrity throughout the process while the destructive content is intracellularly maintained (4). There are two principle signaling pathways of apoptosis, namely, extrinsic and intrinsic routes (5). The extrinsic pathway is triggered by ligation of the death receptor Fas/Cluster of differentiation (CD) 95, leading to the creation of a multi-protein complex called the death-inducing signaling complex (DISC). DISC regulates initiator caspase-8 activation which may either stimulate effector caspases that result in apoptosis or cleave BH3-interacting-domain death agonist (Bid), causing the activation of an additional mitochondrial loop (Figure 1) (5,6).



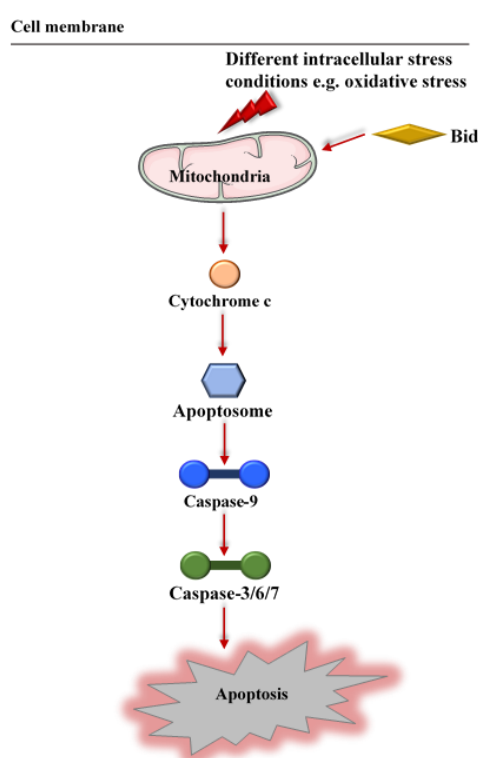
**Figure 1.** The summary of the extrinsic pathway of apoptosis. When death receptors are activated, DISC is formed. Then, DISC activates caspase-8, which may activate caspase-3 and -7, resulting in apoptosis, or cleaves Bid causing activation of an additional mitochondrial loop. Bid: BH3-interacting-domain death agonist, DISC: Death-inducing signaling complex

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The intrinsic pathway can be triggered by many stimuli, such as infectious factors and oxidative stress. The B-cell lymphoma 2 (Bcl-2) family members monitor intracellular damage and stimulate the pore-forming Bax activation and consequently mitochondrial membrane permeabilization (MMP), an important occurrence in apoptosis. MMP causes loss of mitochondrial membrane potential ( $\Delta\Psi_m$ ), stops the synthesis of mitochondrial ATP, and releases pro-apoptotic proteins including cytochrome c to the cytoplasm. Cytochrome c stimulates the creation of the apoptosome, a platform for caspase-9 activation. Then, caspase-9 triggers effector caspases, caspase-3, -7 and -9, leading to the degradation of cellular components and apoptosis (Figure 2) (5,6).



**Figure 2.** The summary of intrinsic pathway of apoptosis. Various intracellular stress conditions e.g. oxidative stress and bid produced from the extrinsic pathway cause cytochrome c release from mitochondria. Then, cytochrome c stimulates the formation of the apoptosome that activates caspase-9 which itself subsequently activates caspases-3, -6 and -7 resulting in apoptosis. Bid: BH3-interacting-domain death agonist

## Materials and Methods

Various databases (e.g. Google Scholar, PubMed, Medline and ISI Web of Knowledge) were searched for the terms such as "asthma", "programmed cell death," "apoptosis," "clinical trial," and "animal studies" to

identify studies on the critical role of apoptosis in asthma disease and factors involved in this process.

### Apoptosis in asthma disease

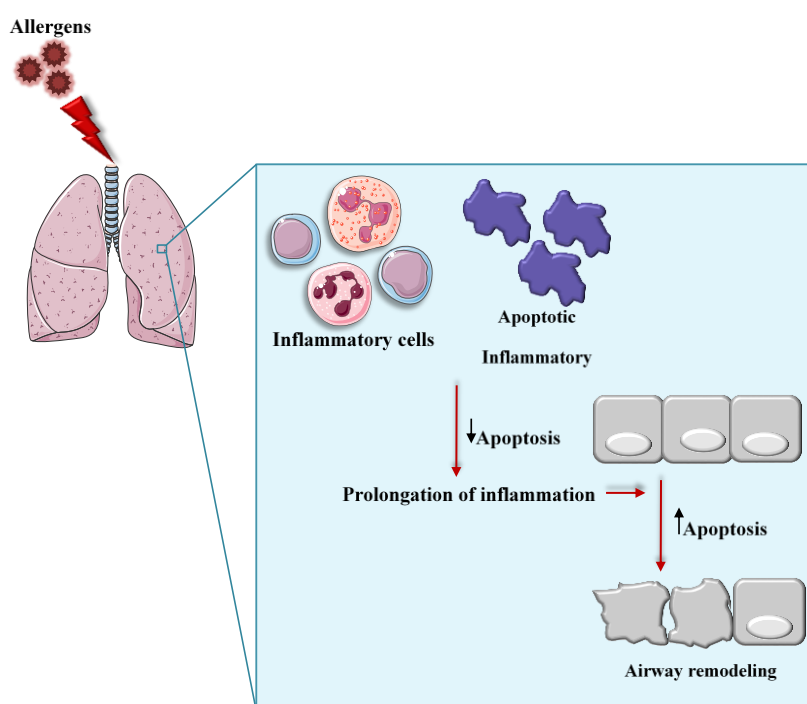
The inflammatory process consists of interrelated cellular and biochemical events that remove infectious factors and repair damaged tissues. However, an ineffective inflammatory response causes cellular dysfunction and tissue damage, resulting in various chronic inflammatory diseases (7). Asthma is a common chronic respiratory disorder which is pathologically associated with airway inflammation induced by overexpression of T helper (Th) 2 lymphocyte-related cytokines (e.g., interleukin (IL)-13 and IL-4)) and infiltration of inflammatory cells (e.g., eosinophils) in the airway, causing a variety of clinical symptoms with airway hyper-reactivity and damage to structural cells including epithelial cells (i.e., airway remodeling) (8-10). In physiological conditions, granulocytes play a vital role in host defense by migrating to the site of injury. However, proteolytic enzymes, reactive oxygen species (ROS), and inflammatory mediators released by these cells lead to extensive lung tissue injury in asthma (11). Inflammatory cells also show increased viability in vivo and in vitro studies. For example, prolonged survival and higher viability of Th2 cells and eosinophils have been reported in asthma (12). One possible explanation is that over-expression of an anti-apoptotic factor, Bcl-2, in hematopoietic cells sustains the viability of inflammatory cells and inhibits the resolution of inflammation (13).

Another major characteristic of asthma pathogenesis is damage to airway epithelial cells (14,15). Physiologically, the epithelium is a dynamic tissue that undergoes renewal continuously. As the first physical barrier, when airway epithelial cells are exposed to environmentally-induced damage, apoptosis is induced in these cells. Then, non-professional phagocytes, as well as professional phagocytes (e.g. macrophages and dendritic cells), engulf apoptotic cells (16). One study has added more complexity to the function of airway epithelial cells as it revealed that viable airway epithelial cells engulf apoptotic epithelial cells, thus suppressing immune cell activation (17). Airway epithelial cells are also active in the elimination of apoptotic eosinophils and are vital in the resolution of eosinophilic inflammation in the asthmatic lungs (18). Therefore, this report suggests that cell clearance in the airway can prevent inflammatory responses. Nevertheless, in asthma disease, airway epithelial cells are damaged, and thus, their functions are disturbed. Moreover, it is

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known that the airway epithelium seems to be proliferating in this disorder. This abnormal proliferative pattern in airway epithelial cells is uncontrolled, leading to epithelial hyperplasia and thickening, which are manifestations of airway remodeling. What causes this proliferative epithelial remodeling process? It can be induced by persistent airway inflammation (19). Interestingly, some authors have demonstrated the occurrence of apoptosis in airway epithelial cells characterized by loss of normal airway pseudostratified epithelium and maintenance of a few basal cells on a thickened basement membrane (20). The exact reasons for this apoptotic process are not fully understood. However, it may relate to the

abnormally increased thickness of epithelial cells, which causes a loss of epithelial cell contact with basement membrane matrix and extracellular matrix interactions, resulting in apoptosis (19). In addition, more attention is now given to possible roles for Th2 cells and eosinophils in the apoptotic process of airway epithelial cells in asthma through secreting cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Figure 3) (21). According to the above mentioned, the removal of inflammatory cells and survival of airway epithelial cells seem to be the important steps in the prognosis of asthmatic patients. In the following section, we describe several apoptotic and anti-apoptotic factors which affect either inflammatory cells or airway epithelial cells.



**Figure 3.** Exposure of the lungs to allergens causes the recruitment of inflammatory cells. Unfortunately, in some conditions, failure to clear inflammatory cells by apoptosis will prolong the course of inflammation. On the other hand, inflammatory cells are able to trigger apoptosis in airway epithelial cells leading to airway remodeling. All contribute to the development of asthma

## Bcl-2 family

Bcl-2 family includes more than twenty members that are categorized into three sub-categories according to their function and the number of Bcl-2 homology domains which they contain: the multi-domain pro-apoptotic members, the Bcl-2 homology 3 (BH3)-only domain pro-apoptotic members, and the anti-apoptotic family. In eosinophils and neutrophils, the multi-domain pro-apoptotic family members Bcl-2-associated X protein (Bax), Bcl-2-associated death promoter (Bad), Bcl-2

homologous antagonist/killer (Bak) and BH3-only domain pro-apoptotic members BH3-interacting domain death agonist (Bid) are expressed, which are important components of the apoptotic cascade (22). As indicated earlier, eosinophil viability is increased in asthma disease that may be through mechanisms including delayed Bid cleavage, blockade of Bax translocation to mitochondria, and inhibition of caspase activity (23). In neutrophils, the pro-apoptotic BH3-only domain proteins Bcl-2 interacting mediator of cell death (Bim) and p53 upregulated

modulator of apoptosis (PUMA) are also expressed. It is known that the pro-apoptotic Bcl-2 family proteins function through translocation to mitochondria where they effect on MMP or through downregulation of pro-survival proteins. In granulocytes, in addition to pro-apoptotic proteins, there are anti-apoptotic members of the Bcl-2 family. The Bcl-2 homologue A1, anti-apoptotic proteins myeloid cell leukaemia-1 (Mcl-1), Bcl-XL and X-linked mammalian IAP (XIAP) are all expressed in neutrophils (24,25).

On the other hand, lower Bcl-2 expression and higher Bax expression in airway epithelial cells of asthmatic patients have been reported. A balance between proteins of pro-survival Bcl2 and pro-apoptotic Bax determines cell survival or cell death. In individuals with asthma, an increased Bax/Bcl2 transcript ratio in airway epithelial cells promotes cytochrome c release from mitochondria, leading to activation of apoptosis cascade. Moreover, apoptosis of airway epithelial cells is associated with the activation of caspase-3 which is believed to be terminal regulator of apoptosis (26-28).

#### **Fas ligand**

Fas-mediated apoptotic response is vital for the removal of inflammatory cells as decreased FasL expression may predispose to increased inflammation, resulting in conditions of chronic inflammation including asthma. Fas ligand (FasL), a protein ligand, is suggested to induce apoptosis through binding to the FAS cell surface receptors or the adenine phosphoribosyl transferase 1 (APT1) family of receptors including the TNF receptors (29). In asthma, resistance of CD4+ T lymphocytes to FAS-dependent apoptosis occurs, leading to reduced removal of activated T cells and enhanced T-cell recruitment (30,31). In addition, Fas receptor expression on CD3+ lymphocytes have been reported to be down-regulated in asthmatic patients, resulting in resistance of these cells to Fas-mediated cell death (30-32).

In case of airway epithelial cells, the presence of Fas and FasL is approved by flowcytometry and immunohistochemistry technics (29). However, some authors demonstrated that apoptosis induced in airway epithelial cells by T cells is not affected by Fas or FasL, indicating that the Fas-FasL signaling pathway has a less vital role in the death of airway epithelial cells. Therefore, what are the roles of Fas and FasL in these cells? The expression of FasL in airway epithelial cells may control inflammatory cell numbers by triggering cell death. Support for the anti-inflammatory role of FasL is shown from mouse studies and from studies indicating that airway epithelial cells have the ability to control inflammatory cell

numbers and decrease inflammatory response when the insult is removed (33).

#### **Interferon-gamma**

Interferon-gamma (IFN- $\gamma$ ) is a pro-inflammatory cytokine which vitally contributes to regulate apoptosis of T lymphocytes (34,35). It has been having reported that IFN- $\gamma$  induces apoptosis through several mechanisms such as stimulating surface expression of Fas and FasL, up-regulating Bax expression, down-regulating Bcl-2 expression, and activating caspases (36). In asthma disease, a deficiency in the production of IFN- $\gamma$  may be one of the reasons for decreased apoptosis of T lymphocytes activated by allergen (37). One study also found the importance of IFN- $\gamma$  in protection against asthma as Japanese children with polymorphisms in the gene encoding for IFN- $\gamma$  have a genetic susceptibility to this disorder (38).

#### **Mitogen-activated protein kinase pathways**

The mitogen-activated protein kinase (MAPK) signaling cascade is demonstrated to be involved in different aspects of inflammatory responses such as cell recruitment, activation, and apoptosis. The three main MAPK sub-groups activated by MAPK kinases (MEKs) 1-7 are extracellular-signal-related kinase (ERK), c-Jun N-terminal kinases (JNKs), and p38 MAPK, which are involved in regulating granulocyte apoptosis (39-41). It has been suggested that the MAPK signaling pathway usually initiates the survival and proliferation of granulocytes (42-43). Moreover, in the presence of transforming growth factor-beta (TGF- $\beta$ ), the p38 MAPK pathway initiates apoptosis in asthmatic airways (44,45).

#### **Nitric oxide**

It has been indicated that endogenous nitric oxide (NO) regulates eosinophilic inflammation in asthma and is able to inhibit the activation of ERK MAPK, resulting in apoptosis of eosinophils (46,47).

#### **Phosphoinositide-3-kinase pathway**

The phosphoinositide-3-kinase (PI3K) pathway is a key cell-surface-receptor-controlled signal transduction pathway which catalyzes the phosphorylation of the 30-OH position of the inositol ring of phosphoinositides, leading to 30-phosphatidylinositol lipids formation. PI3K regulates major events of leukocytes such as growth, proliferation, recruitment, activation, and survival (40-44). From the point of view of granulocyte survival, PI3K mediates anti-apoptotic proinflammatory signals. It generates the phosphatidylinositol (3,4,5)-trisphosphate (PtdIns (3,4,5) P3) that activates Akt and affects on nuclear factor 'kappa

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light-chain-enhancer' of activated B-cells (NF- $\kappa$ B) and cyclic adenosine monophosphate (cAMP)-response-element binding protein (CREB) to produce anti-apoptotic signals in granulocytes (45-48).

### Peroxisome proliferator-activated receptor $\gamma$

Peroxisome proliferator activated receptor  $\gamma$  (PPAR $\gamma$ ) is a nuclear hormone receptor that plays vital roles in cell proliferation, differentiation and apoptosis (49). In asthma disease, PPAR $\gamma$  is overexpressed in airway epithelium, airway submucosa and smooth muscle cells. The expression of PPAR $\gamma$  on the epithelium correlates with the thickness of the subepithelial membrane (50).

### Sialic acid-binding immunoglobulin-like lectins

It has been currently suggested that sialic acid-binding immunoglobulin-like lectins (Siglecs) may induce apoptosis. Siglecs are single pass trans-membrane receptors and belong to the immunoglobulin (Ig) superfamily that are characterized by the N-terminal V-set domain which binds to sialic acids in glycoproteins and glycolipids. They are predominantly expressed on leukocytes (51). For example, there are Siglec-8 and Siglec-F in human and mouse eosinophils, respectively. Evidence shows that activation of Siglec-8 on blood eosinophils of asthmatic patients triggers cleavage of caspase-3, caspase-8 and caspase-9, leading to mitochondria-dependent apoptosis (52,53). In agreement to this, selective caspase-8 and/or caspase-9 inhibitors inhibit Siglec-8-induced apoptosis. Moreover, application of diphenyleneiodonium, a ROS inhibitor, suppresses this form of cell death, indicating that Siglec-8-induced eosinophil apoptosis is dependent on ROS production. However, mouse Siglec-F causes eosinophil apoptosis by regulation of caspase activity but not ROS production (54).

### TGF- $\beta$

The inflammatory cells (e.g., eosinophils and lymphocytes), fibroblast cells and structural cells in the airways secrete cytokines including transforming growth factor-beta (TGF- $\beta$ ) that regulate the process of airway remodeling (55-60). TGF- $\beta$  is secreted in an inactive form, and when a latency-associated peptide-1 (LAP-1) is removed, an active peptide is released (61). It is suggested that TGF- $\beta$  triggers either apoptotic or anti-apoptotic effects in airway epithelial cells. The apoptotic effects result in the development of asthma associated with enhanced damage of airway epithelial cells, while the anti-apoptotic effects cause epithelial cell hypertrophy. Both effects of TGF- $\beta$  may promote airway remodeling. TGF- $\beta$  exerts its anti-apoptotic effects by the Smad 2/3 pathway.

When Smad7 is overexpressed, anti-apoptotic signaling of Smad 2/3 is inhibited, and Smad 7 leads to the apoptotic effects partially via the p38 MAPK signaling pathway in airway epithelial cells (62-64). TGF- $\beta$  also triggers apoptosis in airway epithelial cells by caspase-3 activation, Bcl-2 downregulation as well as ERK 1/2 and JNK phosphorylation (65,66). These apoptotic effects of TGF- $\beta$  can contribute to the damage of the epithelial layer in asthmatic airways. However, surprisingly, one report revealed that TGF- $\beta$  protected airway epithelial cells from apoptosis in the range of 10-15% (43). TGF- $\beta$  has been reported to have the ability to elicit anti-apoptotic effects in fibroblast cells (67-68). This cytokine also causes differentiation of airway fibroblast cells to a myofibroblast phenotype via the JNK signaling pathway (69,70). The differentiated myofibroblasts have an enhanced capability to express contractile proteins and deposit collagen (71-73). Moreover, TGF- $\beta$  can increase the survival of airway smooth muscle cells indirectly through the production of extracellular matrix proteins or the expression of growth factors, resulting in the development of smooth muscle bulk (74,75).

Based on extensive researches, it is well known that apoptosis has a critical role in asthma pathogenesis. In this regard, apoptosis of inflammatory cells is reduced, leading to the prolongation of inflammation. In addition, some authors have suggested that inflammatory cells may cause apoptosis in airway epithelial cells. Nevertheless, its exact mechanisms need to clarify. Following investigations, also, a variety of factors involved in apoptosis has been determined. But, as mentioned, the precise role of some of these factors, such as Fas and FasL or TGF- $\beta$ , has not been fully elucidated. Thus, much more studies are still required to evaluate this issue, and a deeper understanding of their mechanisms will surely be beneficial to the treatment of asthma disease. Moreover, as survival of inflammatory cells and apoptosis of airway epithelial cells occurs in asthma, disposal of inflammatory cells and survival of airway epithelial cells are vital goals for therapeutic strategies. Therefore, to develop novel treatments for asthma, different impacts of apoptosis on these cells should also be considered.

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