

T LYMPHOCYTE APOPTOSIS IN ASTHMA

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Abstract

The paper describes the possible role of apoptosis of T lymphocytes in asthma pathogenesis. The authors focused on resistance against Fas-mediated programmed cell death and the role of Bcl-2 protein in impaired programmed cell death process. The reports from the literature regarding the imbalance of Th1 and Th2, caused by impaired apoptosis of T cells, in asthma pathogenesis are reviewed.

Key words: apoptosis, asthma, T-lymphocytes

INTRODUCTION

Asthma is characterized by a reversible airway obstruction, airway inflammation and bronchial hyper-responsiveness (BHR) in response to both non-specific irritants and specific triggers such as allergens [1, 2]. Airway inflammation leads to tissue destruction and airway wall remodeling, involving epithelial disruption, smooth muscle and microvascular proliferation, basement membrane thickening, and smooth muscle hypertrophy [2]. Despite advances in the treatment of asthma, the overall mortality has not decreased significantly, while the prevalence and the severity of the disease are increasing. The prevalence of allergic asthma among children has risen drastically during the last decades, especially in Western societies [3, 4].

THE ROLE OF T LYMPHOCYTES IN ASTHMA

Allergic diseases are a result of disordered immunoregulation [5]. The CD4⁺ T lymphocytes play a major role in the pathogenesis of asthma. They cause recruitment and activation of airway eosinophils and an increased IgE production by plasma cells [1, 2, 5]. Patients with acute severe asthma present with a significant increase in three surface proteins associated with T lymphocyte activation: IL-2R, class II histocompatibility antigen (HLA-DR), and 'very late activation' antigen, VLA-1 [5]. Recent findings in asthmatics and animal models point to a crucial role of T cells in mediating many manifestations of asthma, including bronchial hyperresponsiveness. The T cell hypothesis of asthma is based on the concept that the disease is driven and maintained by the persistence of a specialized subset of chronically activated T memory cells sensitized against an array of allergenic, occupational or viral antigens which home in on the lung after ap-

propriate antigen exposure or viral infection [5, 6]. The allergens induce a CD4⁺ T helper (Th) cell response, whereas viruses recognize CD8⁺ cytotoxic (Tc) cells. In the asthmatic airways, there appears to be both CD4⁺ and CD8⁺ cells with a type 2 cytokine phenotype (i.e., Th2 and Tc2 type). These cells produce: interleukin IL-5, IL-3, and granulocyte macrophage colony-stimulating factor, which recruit, mobilize, and activate eosinophils for subsequent mucosal tissue damage, and IL-4, an essential co-factor for local or generalized IgE production [6, 7]. This, in turn, leads to eosinophilic desquamative bronchitis, with epithelial shedding, mucus hypersecretion and bronchial smooth muscle contraction. Although eosinophils are largely responsible for airway symptoms, their function appears to be under T cell control [1, 6].

Overactivation of CD4⁺ T cells in the peripheral blood and airway tissues is an inherent feature of asthma; therefore, a potent mechanism for augmenting the number of activated T cells in this disease would be resistance to the normally programmed pathway for cell death [7-10]. There are three different mechanisms by which a cell commits suicide by apoptosis. One generated by signals arising within the cell, the second triggered by death activators binding to receptors at the cell surface: TNF-alpha, lymphotoxin, Fas ligand (FasL), and the third that may be triggered by reactive oxygen species [4, 11]. Cell surface death receptors can transmit apoptosis signals in response to external stimuli, such as death ligands, growth factor withdrawal, or drugs. Death receptors belong to the tumor necrosis factor (TNF) receptor family and have a characteristic cysteine-rich extracellular domain and a homologous cytoplasmic 'death domain' that initiates apoptotic signaling inside the cell. These receptors can induce apoptotic cell death after ligand binding and may exert their apoptogenic effects differentially in diverse cell types, depending on downstream signaling [11].

TCR (T-cell receptor) is a protein found exclusively on T cell's surface. Its binding by a ligand is necessary either to activate the cell or to induce programmed cell death. TCR interacts with peptides which belong to major histocompatibility complexes (MHC) exposed on antigen presenting cells and deliver major activation signal. The cell, however, cannot be completely activated without co-stimulation mediated by CD28 antigen. It should be pointed out that CD28 antigen

needs interaction with its ligands, CD80 or CD86. Otherwise, the cell does not receive sufficient signal for activation and may turn to anergy (the state of unresponsiveness) and apoptosis [12].

FAS-MEDIATED PATHWAY OF APOPTOSIS

Peripheral T cells' death requires the action of death receptor/ligand systems, particularly Fas (CD95) and Fas-ligand interaction. Pro-apoptotic signal leads to recruitment of pro-caspase-8 to the receptor, forming the death-inducing signaling complex [11, 13]. Upon triggering of CD95, Fas-associated death domain-containing protein and pro-caspase-8 are recruited to the receptor, forming the death-inducing signaling complex. Once activated, caspase-8 can initiate apoptosis of cells. The death signaling function is ensured by the presence of a cytoplasmic protein-protein interaction motif termed death domain. Binding of pro-caspase-8 leads to its autoproteolytic activation, upon which the active enzyme is released into the cytosol [11].

Helper T lymphocytes play a key role in atopic diseases. Majority of Th0 cells in healthy individuals differentiate to Th1, whereas in atopic individuals majority of Th0 differentiate to Th2 subpopulation. Disturbed T helper balance is explained by impaired apoptosis of T cells. It is suggested that T lymphocytes in asthmatic patients are partially unable to get activated in antigen-mediated pathway [5, 14]. It is also postulated that mainly Fas-triggered pathway is impaired. Apoptosis induced by γ -irradiation, mitogens, or ceramid action is not weakened [15]. The role of the number of Fas receptors on the cell surface is still under discussion. Yajaraman et al. [15] described a similar expression of Fas receptors on lymphocytes from healthy and asthmatic individuals, whereas others observed a significant decrease of Fas receptors on T cells' surface from asthmatic patients [16, 17]. Spinozzi et al. [17] observed hypoexpression of Fas mRNA and surface Fas receptors on pulmonary CD3+ T lymphocytes, which may explain the persistence of inflammatory cellular infiltrates in allergic bronchial asthma. The data show a selective resistance of activated T cells to undergo Fas-induced apoptosis in asthmatic in comparison with non-asthmatic subjects; thereby providing initial evidence for a defect in programmed cell death in the pathogenesis of asthma [15].

Because apoptosis of mature T cells is a powerful mechanism for deleting T cells, it raises the possibility that unequal apoptosis of different effector cells may lead to preferential deletion of one subset over another [14, 18]. Apoptosis of circulating memory/effector T cells confined to the selected population of cells may explain allergen-specific immune response in asthmatic individuals. Varadhachary et al. [19] pointed to Th2 apoptosis resistance. It is also postulated that the level of caspase-8 cleavage within Th2 subpopulation is decreased in comparison with the Th1 subpopulation. Increased expression of Fas-associated phosphatase (FAP-1), which inhibits caspase cleavage, might play a key role [19]. On the other hand, Akkoc et al. [20] suggests that Th1 lymphocytes are more sensitive to Fas-mediated programmed cell death than Th2 cells. The same suggestion appears in a paper by

Zhang et al. [16]. The mechanism which might be engaged in impaired apoptosis process is an increased expression of Fas and Fas ligand within Th1 lymphocytes in comparison with Th2 subpopulation. It is proposed that IFN- γ produced by Th1 cells influences the expression of caspases and sensitizes cells to apoptosis. To confirm that hypothesis Akkoc et al. [20] showed that the absence of IFN- γ protects Th1 lymphocytes against Fas-mediated apoptosis. De Rose et al. [21] investigated the effects of recombinant IFN- γ on proliferation, Fas/Fas ligand (FasL) expression, and apoptosis in allergen-stimulated peripheral blood mononuclear cells obtained from atopic, asthmatic patients and non-atopic, control subjects. The addition of IFN- γ at the start of cultures markedly inhibited the proliferative response to a specific allergen in cells from all asthmatic patients, whereas no change was observed in cells from nonatopic, control subjects. IFN- γ induced an increase in the expression of Fas and FasL by allergen-stimulated CD4+ T cells from asthmatic patients and caused the apoptosis of these cells. A Fas-blocking monoclonal antibody prevented the inhibitory effect of IFN- γ on allergen-induced proliferation. These results suggest that IFN- γ inhibits the proliferation of allergen-stimulated CD4+ T cells from atopic, asthmatic patients by inducing the surface expression of Fas and FasL, which, in turn, triggers their apoptotic course [21]. The defect in IFN- γ production involved in the allergic, immune response may, therefore, be responsible for a decrease in apoptosis of allergen-activated T lymphocytes in the airways of atopic, asthmatic patients [20, 21].

BCL-2/BAX PATHWAY OF APOPTOSIS

The Bcl-2 (B cell lymphoma) family of genes is associated with apoptosis. The Bcl-2 protein is a suppressor of programmed cell death that homodimerizes with itself and forms heterodimers with a homologous protein Bax, a promoter of cell death. The two proteins have highly similar aminoacid sequences, but are functionally opposed. The ratio of anti-apoptotic vs. pro-apoptotic dimers is important in determining the resistance of a cell to apoptosis [11]. Bcl-2 is an integral membrane protein located in the membranes of the endoplasmic reticulum (ER), nuclear envelope, and in the outer membranes of the mitochondria. This enhancer is very active in B cells. The Bcl-2 protein suppresses apoptosis by preventing the activation of caspases that carry out the process [7, 11]. In a normal cell, the outer membranes of its mitochondria express the Bcl-2 protein on their surface. Bcl-2 is bound to a molecule of the Apaf-1 protein (apoptotic protease activating factor-1). Internal damage to the cell (e.g., from reactive oxygen species) down-regulates Bcl-2, leading to release of Apaf-1 that penetrates mitochondrial membranes, causing cytochrome c to leak out. The released cytochrome c and Apaf-1 bind to molecules of caspase-9 [22]. The early steps in apoptosis are reversible. Enhanced expression of Bcl-2 inhibits both apoptotic and, in some cases, necrotic cell death in many cell types, and in response to a wide variety of inducers. Also, recent analyses of cells expressing various levels of Bcl-2 and Bax have revealed that the de-

gree of protection against apoptosis correlates with the amount of Bcl-2 that is free of Bax, rather than the total amount of Bcl-2. Bcl-2/Bax apoptosis pathway is an important mechanism limiting an inflammatory response [23, 24].

The amount of the Bcl-2 protein in lymphocytes obtained from asthmatic patients is a controversial issue. Hamzaoui et al. [10] reported that lymphocytes from sputum of asthmatic patients contain more Bcl-2 protein [10], but Ying et al. [24], on the other hand, reported a decreased expression of Bcl-2 on lymphocytes isolated from bronchoalveolar lavage fluid from asthmatic patients, compared with healthy adults. A study by Abdulmir et al. [25] attempts to reconcile the controversy by indicating that the percentage of lymphocytes with Bcl-2 expression depends on asthma severity. However, Bcl-2 expression on T lymphocytes from patients with both severe and mild asthma increases with the advancement of the disease. In general, too, Bcl-2 is found in a greater amount on lymphocytes from asthmatic patients, compared with healthy subjects, regardless of disease severity. Opposite, there is a reverse correlation between the expression of Bax protein and disease severity [25].

SUMMARY

The literature on the role of apoptosis of the immune cells in asthma is scarce. It is unknown why the inflammatory response cannot be turned off in atopic persons. One important mechanism in lymphocyte control is programmed cell death (apoptosis), which occurs in every immune response. This mechanism seems to be impaired in asthmatic subjects. Taken together, the findings suggest that a selective resistance to Fas-dependent apoptosis reflects altered Ag-driven, accessory cell-dependent signaling, and that ineffective activation of Fas signal transduction may contribute to T cell-dependent inflammation in asthma. In addition, a defect in IFN- γ formation which results in a T helper cell type 2-dominated response may be responsible for a decrease in the apoptosis of allergen-activated T cells in asthma. Further investigations are necessary to fully explain observed abnormalities of T cells apoptosis in asthma.

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