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REVIEW ARTICLE

APOPTOSIS AND ASTHMA

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INTRODUCTION

WHAT IS APOPTOSIS?

The term "apoptosis" was first used by Kerr in 1972,[1] when he imagined the shape of the cells undergoing apoptosis as the falling leaf, for which Hippocrates used the word "apoptosis" in Greek. Because it requires gene expression mediated by cell stimulation, apoptosis has been described as programmed cell death or activation-induced cell death.[2]

They have characteristic findings, such as cell shrinkage, chromatin condensation, nuclear segmentation, cellular disintegration, and formation of an apoptotic body.[3]

REGULATION OF APOPTOSIS

The pathway of apoptosis is controlled by various factors classified as inducers, enhancers, effectors, and inhibitors. The representative inducer is the Fas-Fas ligand system, the enhancer is Bax, the effector is p53, and the inhibitor is Bcl-2, which will be mentioned later in this review. Cancer cells irradiated at a lower dose will undergo apoptosis by means of p53, and those at a higher dose will be necrotic. Apoptosis is also induced in the T cells stimulated with a sensitized antigen through T-cell receptors by means of nur-77, erg-1, and c -myc and in those treated with corticosteroids by means of RP-2 and RP-8.[4]

A wide variety of cells are known to become apoptotic after engagement of a certain kind of cell surface receptors, the so-called death receptors, such as TNF receptor 1, Fas/apoptosis-inducing protein 1, and IL-1beta-converting enzyme.[5] Both TNF receptor 1 and Fas are members of the TNFR superfamily and are activated by TNF and Fas ligand (FasL), respectively. Apoptosis induced by the Fas-FasL system is regulated by the rate of expression of FasL, and anti-Fas antibodies can mimic FasL and therefore induce apoptosis efficiently with Fas on the cell surface. An inhibitor, Bcl-2, downregulates apoptosis mediated by p53 and c – myc.[6] An enhancer, Bax, upregulates the cell death by binding to Bcl-2 to suppress the inhibitory effect of Bcl-2 on apoptosis.[7] More genes or gene products seem to be involved in regulating apoptosis, but further investigation is required to clarify the whole scheme of the regulatory system of apoptosis.

MORPHOLOGIC AND BIOCHEMICAL FEATURES OF APOPTOSIS

Apoptotic cell death can be triggered by a variety of extrinsic and intrinsic signals.[8] The physiologic control of apoptosis provides a mechanism for the elimination of cells that have been produced in excess, develop improperly, or sustained genetic damage. The hallmark of apoptosis is controlled autodigestion of the dying ell. Cell death appears to be carried out through the activation of endogenous proteases.[9] As a result of activation of these proteases, the integrity of the cytoskeleton is disrupted and the cell round up and begins to shrink in volume. In response to the contraction in cytoplasmic volume, the membrane begins to bleb and there is loss of the normal asymmetry of plasma membrane lipids.[10] Apoptosis also involves characteristic changes within the nucleus. Endonucleases are activated and begin to danger nuclear DNA. The nucleus becomes then shrunken and pyknotic. The function of other organelles is also disrupted.[11]

Current evidence suggests that the decision to undergo apoptosis occurs within the cytoplasm. Cytoplasts devoid of nuclei respond to apoptotic stimuli by shrinking in volume, losing plasma membrane asymmetry and initiating the degradation of intracellular organelles.[12] The loss of mitochondrial function has been suggested to be an early irreversible event that occurs during apoptotic cell death.[13] Both the redistribution of cytochrome c from the mitochondrial intermembrane space and the loss of mitochondrial membrane potential have been observed during the initial stages of apoptotic cell death.[14]

Phagocytosis of apoptotic cells is not associated with upregulation of activation markers or cytokines by the phagocyte. Apoptotic cells that are not immediately phagocytosed remain intact, breaking down into smaller membrane-bound fragments, called apoptotic bodies. By maintaining plasma membrane integrity, apoptotic death promotes the elimination of the dying cells without the induction of an inflammatory response.[15]

In contrast to apoptotic cell death, both necrotic cell death and traumatic cell death are pathologic forms of cell death resulting from acute cellular injury. Both are characterized by rapid cell swelling and lysis, which result in leakage of cytoplasmic contents and the induction of an inflammatory response.[16]

APOPTOSIS MUST BE SUPPRESSED IF A CELL IS TO MAINTAIN VIABILITY

Cell survival in vivo appears to be dependent on the constant supply of survival signals provided by neighboring cells, the extracellular matrix, or growth factors. This suggests that all cells within multi-cellular organisms are programmed to commit suicide if survival signals are not received from their environment either constantly or at regular intervals.[17]

CELLS CAN BE INSTRUCTED TO INITIATE APOPTOSIS

Apoptosis can be initiated through an instructive mechanism. Specific cell surface receptors have been identified that can transduce signals that result in apoptosis in response to ligand binding.[18] The best characterized cell death receptors are members of the tumor necrosis factor (TNF) receptor family such as Fas. Initially the expression of instructive cell death through surface receptors was thought to be limited to cells that underwent cyclic expansions and contractions as a result of their normal physiologic roles such as lymphocytes or the cells of reproductive organs. Recently, it has become apparent that most cells can be induced to express cell death receptors or their ligands as a result of the activation of various stress response pathways.[19]

APOPTOSIS INITIATION

In addition to the extrinsic regulation of cell survival by growth factors and cell death receptors, intrinsic cellular events can also initiate

apoptosis. DNA damage has been shown to induce apoptosis. A major pathway through which DNA damage initiates apoptosis is initiated by p53.[20] Because resistance to programmed cell death can contribute to carcinogenesis, this may account for why the p53 gene is found mutated in so many human cancers.[21] Virtually any cellular metabolic or cell cycle disturbances can also induce apoptosis. In a wide variety of cellular systems, elevation in intracellular calcium, decrease in cellular pH, and alteration in cellular redox potential have been shown to increase the propensity of the cell to undergo programmed cell death.[22]

Although all mammalian cells appear to be dependent on constant signaling for survival, how a loss of signaling initiates cell death remains undefined. This form of apoptosis has been widely studied using growth factor-dependent cell lines that immediately initiate programmed cell death after growth factor withdrawal. Growth factor withdrawal commonly results in an acute disruption in cellular metabolism and the induction of cell cycle arrest.[23]

CASPASES

Caspases are intracellular cysteine proteases that have the novel ability to carry out protein cleavage after aspartic acid residues, thus the name caspase.[24] To date 10 mammalian caspases have been identified. All cell lines express one or more of such caspases. In viable cells, caspases are expressed as proenzymes that only become activated upon proteolytic cleavage.[25] Once activated, most caspases have the ability to catalyze the activation of multiple other members of the caspase family, resulting in positively amplifying cascade of proteolysis. If physiologically unchecked in a cell, caspase activation has been shown to be sufficient to initiate all the morphologic changes associated with apoptosis.[26]

BCL-2 PROTEINS

The rate at which apoptotic signaling events initiate or amplify caspase activity can be regulated by proteins of the Bcl-2 family.[27] Current evidence suggests that Bcl-2 does not work to inhibit a specific step in programmed cell death but rather alters the so called apoptotic threshold of a cell.[28] Bcl-2 overexpressing cells can still carry out programmed cell death in response to a wide variety of apoptotic initiators. However, the dose of the initiator necessary to induce programmed ell death in the presence of Bcl-2 is significantly greater than in the absence of Bcl-2.[29]

A number of Bcl-2 related proteins have been identified Table 1. Paradoxically, some of these family members have been shown to promote survival, whereas others have been shown to enhance the sensitivity of a cell to programmed cell death.[30]

Table 1. Bcl-2 related proteins. The Bcl-2 related proteins can be divided into 3 subfamilies based on overall sequence similarity.

Anti-apoptotic proteins	Pro-apoptotic proteins.	Proteins with only BH3 Homology.
Bcl-2	Bax	Bad
Bcl-xL	Bak	Bid
Bcl-w	Bcl-xS	Bik
Mcl-1		Hrk
NR-13		

The ratio of Bcl-2 to Bax in the cell correlates with the apoptotic threshold. An excess of Bcl-2 promotes ell survival, whereas an excess of Bax promotes cell death.[31]

The □-helical region in Bak and Bax has been termed the Bcl-2 homology domain 3 (BH3). BH3 domains have been reported in a number of additional molecules capable of forming heterodimers with Bcl-xL, including Bad, Bik, Hrk and Bid. This subgroup of proteins can promote apoptosis by inactivating the antiapoptotic function of Bcl-xL.[32]

THE ROLE OF APOPTOSIS IN THE PATHOGENESIS AND TREATMENT OF DISEASES

The recent interest in cell death mechanisms has revealed that we have been using induction or inhibition of apoptosis for a long time for the treatment of some diseases without knowing it. For example, chemotherapeutic drugs induce apoptosis of tumor cells and haematopoietic growth factors act by preventing apoptosis of haematopoietic progenitors. A better understanding of the mechanisms that regulate cell death pathways might make it possible to improve the current therapeutic strategies and to define new strategies in several different diseases.[33]

TO LIVE OR TO DIE?

In adult multicellular organisms, different cell types vary widely in the mechanisms by which they maintain themselves throughout life. Red blood cells undergo constant renewal from haematopoietic progenitor cells, whereas neural cells have no or limited capacity for self-renewal. Between these extremes, lymphocytes and cells from the reproductive organs undergo cyclic expansions and contractions as they participate in host defence and reproduction, respectively. Within all of these cell lineage, the control of cell number is determined by a balance between cell proliferation and cell death. Recent studies suggest that the mechanisms that control cell proliferation and cell death could share several factors. Growth factors can either stimulate cell growth or inhibit cell death.[34]

TO DIE OR NOT TO DIE?

Beyond different signaling pathways that ultimately converge to activate a cell death signal, apoptosis appears to involve a common final pathway that has been at least partially, conserved throughout animal evolution. In Human studies performed on the role of cysteine proteases and Bcl-2 related proteins suggest that these two components define two checkpoints in the final common pathway that decides whether or not a cell should die.[35]

TO DIE!

When the final common pathway has allowed the execution of the cell suicide programme, characteristic morphological changes of the dying cell precede the production of apoptotic bodies, membrane-enclosed particles containing intracellular material. These particles are rapidly engulfed and digested by neighboring cells and phagocytes to prevent any release of intracellular material that would otherwise trigger an inflammatory response. Apoptosis is usually associated with the activation of one or several nucleases that degrade DNA first into large and subsequently into small fragments.[36]

One of the consequences of DNA fragmentation is the irreversible degradation of viral or mutated cell DNA. Proteolytic cleavage of several nuclear proteins by an activity similar but distinct from ICE is another biochemical marker of apoptotic cell death.[37]

APOPTOSIS AND THE PATHOGENESIS OF DISEASES

DISEASES DUE TO DEFECTIVE CELL DEATH

The failure of cells to undergo apoptosis might be involved in the pathogenesis of several human proliferative disorders characterized by the accumulation of cells, including cancer, autoimmune diseases and certain viral infections (Fig. 1).[38]

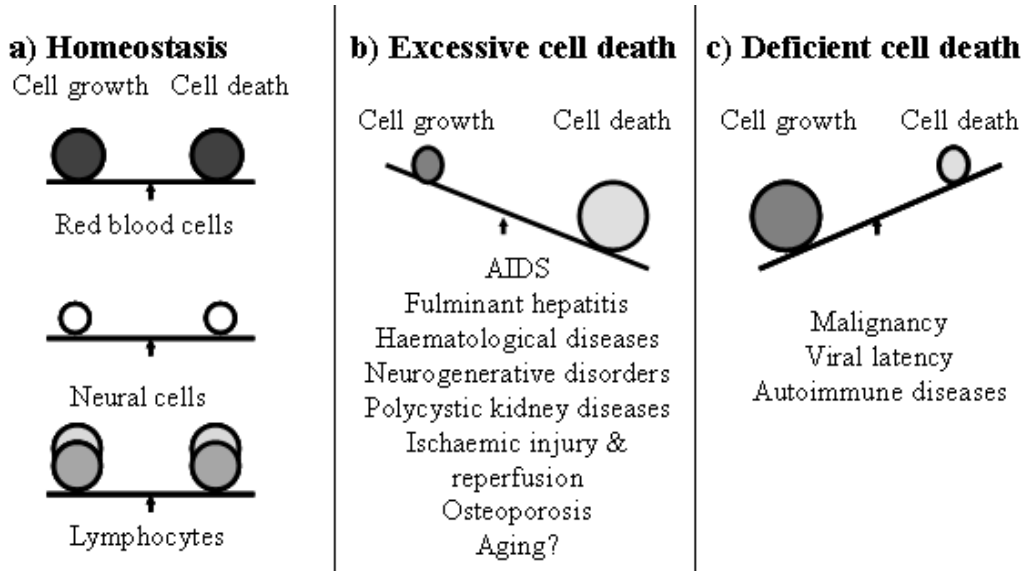

Fig. 1 a) The level of cell death and cell growth varies widely from one cell lineage to another. b) Excessive or c) deficient apoptosis are suspected to be involved in the pathogenesis of various diseases (38).

APOPTOSIS IN ASTHMA

Since the development of bronchoscopy, it has become possible to perform bronchial biopsies in asthmatic patients. Histopathologic studies have disclosed that the asthmatic airway is characterized by chronic airway inflammation, consisting of desquamation of epithelial cells, thickening of the site adjacent to the basement membrane, and accumulation of various inflammatory cells at the subepithelial region, even in subjects with mild asthma.[39] Among the accumulated cells, eosinophils and lymphocytes have been found to play pivotal roles in the development of airway inflammation in asthma.[40] Corticosteroids are the most potent anti-inflammatory agent used for treating bronchial asthma and are known to induce apoptosis in eosinophils, as well as in lymphocytes. Theophylline has been used as a bronchodilator in the treatment of asthma for more than 50 years. However, it has been recently recognized that theophylline can inhibit the functions of various inflammatory cells and act as an anti-inflammatory agent.[41] Because apoptosis, a form of natural or physiologic cell death distinct from necrosis, can remove somatic cells calmly without causing an inflammatory response,[42] one of the appropriate ways to eliminate inflammatory cells from the lesion (e.g. the asthmatic airway) will be the induction of apoptosis.

BIOLOGY OF AIRWAY REMODELING

THE EPITHELIUM-MESENCHYMAL INTERACTIONS

In asthma patients, the bronchial epithelium is highly abnormal, with structural changes involving the separation of columnar cells from their basal attachments, and functional changes including increased expression and release of proinflammatory cytokines, growth factors, and mediator-generating enzymes. Beneath this damaged structure, there is an increased number of subepithelial myofibroblasts that deposit interstitial collagens, causing thickening and increased density of the subepithelial basement membrane. A failure in the injury-repair cycle of damaged epithelial cells seems to play an important role in the development of abnormal epithelium-mesenchymal interactions. Some data(110) show that in asthma patients residual epithelial cells that have not undergone desquamation do not show a greater propensity to

undergo apoptosis than epithelial cells undergoing apoptosis, a feature that may ensure the development of the repair-injury cycle.

Interestingly, the epithelial cells of asthmatic patients also are characterized by a high immunoreactivity for Bcl-2 and Hsp27, two molecules that are characterized by both protective and antiapoptotic properties, suggesting that bronchial epithelial cells of asthmatic subjects have the ability to protect themselves against inflammatory stimuli.[43] In addition, it appears that the bronchial epithelium of untreated asthmatic subjects expresses low levels of proliferating markers, such as proliferating cell nuclear antigen, despite extensive damage,[44,45] revealing a potential failure in the epithelial injury-repair cycle in response to local inflammatory and inhaled agents. Moreover, injury of the epithelium in asthma patients results in a localized and persistent increase in epidermal growth factor (EGF) receptor immunostaining at the wound edge, a mechanism that may cause the epithelium to be locked in a repair phenotype.[46] It is known that during repair, cells produce a wide range of substances, such as growth factors, that are capable of modulating the wound repair process. Thus, the blockage of the epithelium in a repair phenotype with a low proliferative rate establishes a vicious circle that is sustained by the continuous release of growth factors and other profibrotic mediators (such as TGF-beta;, fibroblast growth factor, endothelin, and EGF) that directly regulate the phenotypic and functional features of mesenchymal cells that are located underneath the basement membrane, such as fibroblasts and myofibroblasts. The increased expression of insulin-like growth factor-1, EGF, and TGF-beta; in biopsy specimens or BAL fluid samples has been reported.[47] TGF-beta; expression was found to correlate with the degree of subepithelial fibrosis[48] and to be significantly increased in patients with severe asthma who had a rich eosinophilic infiltration of the airways.[49]

Under the effects of epithelial-derived growth factors, mesenchymal cells produce collagen, reticular and elastic fibers, as well as proteoglycans and glycoproteins of the amorphous ECM, all of which likely contribute to the thickened airway wall of asthmatic subjects. These consequences also are demonstrated by the enhanced proteoglycan deposition in the subepithelial layer of the airway wall of asthmatic subjects.[50] It is therefore likely that ECM production and deposition is under the control of the epithelial-mesenchymal unit, leading to the development of structural alterations that are topographically distributed in the inner layer (i.e. the tissue between the luminal surface and the smooth muscle layer). This process may have dramatic functional consequences as the degree of luminal effacement in response to a given stimulus for smooth muscle contraction is augmented by an increase in the volume of the inner airway wall.

In addition to growth factors, remodeling processes occurring at the level of the inner airway wall are regulated by some cytokines such as interleukin (IL)-11. IL-11 is a pleiotropic cytokine, the expression of which has been found[51] to be increased in the bronchial epithelial cells of asthmatic subjects. Targeted overexpression of this cytokine in mice has resulted in the remodeling of the airways and in the development of airway hyperresponsiveness and airway obstruction. In addition, when compared with control animals, IL-11 transgenic mice develop an enhanced inner wall thickness that is associated with airways obstruction and increased airway responses to methacholine.[52]

EOSINOPHILS AND LYMPHOCYTES IN ALLERGIC INFLAMMATION

One of the characteristic features of allergic inflammation is the accumulation of activated eosinophils at the site of inflammation.[53] The eosinophil accumulation could be induced by upregulation of the growth and differentiation of eosinophils in the bone marrow and their migration to the site of inflammation. Because the life span of eosinophils is as short as 4 days, prolonged survival at the sites where the

eosinophils have migrated is essential to exert their function sufficiently. Through this whole process toward eosinophilic inflammation, 3 cytokines targeting eosinophils (i.e. IL-5, IL-3, and GM-CSF) may play a pivotal role.[54] The 3 factors share the beta-chain of their receptors, which is termed the common IL-3 receptor beta-chain,[55] and act on eosinophils to prolong their life span[56] and to augment their functions, such as migration and degranulation.[57] Among the 3 cytokines, IL-5 has the most potent effect on eosinophil survival.[58] Importantly, activated T cells, which produce the eosinophil survival cytokines (i.e. IL-5, IL-3, and GM-CSF), have been found at the site of allergic inflammation with eosinophilia in patients with bronchial asthma[59] and atopic dermatitis.[60] Therefore it has been hypothesized that T cells, namely TH2 cells, are first activated by some stimuli such as antigens and start producing various cytokines, including IL-5, IL-3, and GM-CSF. With these cytokines, eosinophils are activated not only to survive but also to release their cationic granular proteins, oxygen radicals, and lipid mediators (e.g. leukotriene C4) and to cause tissue damage as a part of allergic inflammation. Therefore the induction of apoptosis in eosinophils, T lymphocytes, or both could resolve allergic inflammation and improve the impaired condition. In other words one of the therapeutic strategies for allergic diseases is to eliminate the activated eosinophils, lymphocytes, or both with a drug capable of promoting apoptosis in these inflammatory cells.

APOPTOSIS OF EOSINOPHILS WITH CORTICOSTEROIDS AND THEOPHYLLINE IN VITRO

Human eosinophils cultured in the presence of IL-5 can mostly stay alive at day 4, whereas 75% are dead by means of apoptosis without IL-5.[61,62] Among the therapeutic drugs for bronchial asthma, glucocorticoids have been reported to inhibit the effects of IL-5 and GM-CSF on eosinophil survival.[63] The steroids can directly act on eosinophils by means of their receptors to accelerate apoptosis, although they will inhibit apoptosis in neutrophils.[64] The acceleration of apoptosis with steroids is overcome by increased amounts of the cytokines, which prolong eosinophil survival, suggesting that the steroids and cytokines are antagonistic to each other. The other possible mechanism by which glucocorticoids induce apoptosis in eosinophils is to decrease the production of cytokines supporting eosinophil viability (i.e. IL-5, GM-CSF, and IL-3) through the regulatory system of cytokine production and/or reduction of cytokine-producing cells, such as T cells, by means of apoptosis.

One study evaluated whether theophylline, a kind of classical and useful agent in treating asthma, has some effect on the prolonged survival of eosinophils with IL-5.[62] It was found that theophylline inhibits IL-5-dependent eosinophil survival by means of apoptosis in a dose-dependent manner. What should be emphasized is that the significant inhibition was observed at 10-4 mol/L (approximately equal to 18 mug/mL), which can be reached clinically.

APOPTOSIS OF LYMPHOCYTES WITH CORTICOSTEROIDS AND THEOPHYLLINE IN VITRO

T lymphocytes can survive in the presence of IL-2, as in the case of eosinophils in the presence of IL-5.[4] Corticosteroids inhibit the survival of T lymphocytes directly by inducing apoptosis[65] and indirectly by suppressing IL-2 formation.[66] The inhibitory effect of the steroids on T-cell survival could be attenuated by adding a combination of IL-1, IL-6, and IFN-gamma to the culture.[67] Interestingly, T cells obtained from patients with glucocorticoid-resistant asthma (or steroid-insensitive asthma) maintain their proliferation and cytokine production in the presence of corticosteroids at least in part because of a decreased affinity of the glucocorticoid receptor caused by either a primary defect of the receptor or increased secretion of certain cytokines, such as IL-2 and IL-4.[68] B cells are also sensitive to glucocorticoids. B cells in patients with chronic

lymphocytic leukemia are reported to become apoptotic with the introduction of steroids, and unexpectedly, the apoptotic effect of the steroids are antagonized by IL-2, which is known to act mainly on T cells.[69]

Only a few studies dealing with apoptosis of lymphocytes with theophylline have been reported so far. Survival of B cells from patients with chronic lymphocytic leukemia was significantly inhibited with theophylline in a dose-dependent manner,[70] and at the same time, the expression of Bcl-2 protein, one of the factors inhibiting apoptosis, was downregulated, whereas the c -myc gene was overexpressed.[71] The changes of Bcl-2 and c -myc are directed toward induction of apoptosis in the cells,[6] and these changes could be involved also in the case of eosinophils. [72] Moreover, the elevation of intracellular cAMP brought about by forskolin, as well as by theophylline and PDE inhibitors, has been reported to induce apoptosis in lymphocytes in a similar fashion as that reported in eosinophils, [73] suggesting that eosinophils and lymphocytes may share the same pathway to apoptosis. Within the T-cell lineage, mature T cells in the peripheral blood appear to be less sensitive than CD4+ CD8+ (double positive) thymocytes to the induction of apoptosis by cAMP. [74] In fact, it was observed that induction of apoptosis in IL-2-dependent T-cell blasts required higher concentrations of theophylline than those found in eosinophils stimulated with IL-5.

CLINICAL RELEVANCE OF APOPTOSIS IN EOSINOPHILS AND LYMPHOCYTES

In as much as the apoptotic cells are eliminated calmly without releasing the harmful cellular contents, the induction of apoptosis in the inflammatory cells could terminate allergic inflammation. In fact, Tsuyuki et al[75] demonstrated that anti-Fas mAb given intranasally in mice completely suppressed eosinophilic inflammation in the airway. Importantly, the antibody was found to induce apoptosis in vitro in eosinophils obtained from the mice with eosinophilic inflammation, suggesting that the induction of apoptosis is associated with the resolution of eosinophilic inflammation. Woolly et al[76] reported more convincing evidence that the treatment of asthmatic patients during exacerbations with corticosteroids for 2 weeks resulted in a decrease of activated EG-2+ eosinophils and an increase of apoptotic eosinophils in their induced sputum samples as their asthmatic symptoms improved. Interestingly, macrophages recovered in the sputum samples contained eosinophil products, indicating that alveolar macrophages are scavenging apoptotic bodies of eosinophils and that induction of apoptosis is clinically relevant to asthma treatment. These results strongly suggest that induction of apoptosis in eosinophils is beneficial in the suppression of allergic inflammation and that one of the modes of action of corticosteroids in the treatment of asthma and atopic diseases is to induce apoptosis in eosinophils and, presumably, in lymphocytes.

LIFE AND DEATH OF EOSINOPHILS IN TISSUES

Several papers focused on eosinophil survival and apoptosis pathways. Sexton et al[77] showed that human alveolar epithelial cells can engulf apoptotic eosinophils by using structures previously implicated in engulfment of apoptotic cells by macrophages, such as alpha integrins and the phosphatidylserine receptor.

Bureau et al[78] explored mechanisms by which engagement of CD40 enhances eosinophil survival. CD40 is a member of the TNF receptor superfamily. Its binding partner is CD40 ligand (CD154) and is primarily found on T-cells. CD40 is not expressed on normal eosinophils but appears on eosinophils in tissue sites or after culture. These authors report that CD40 ligation delays eosinophil apoptosis and that this is likely because of one member of the inhibitor of apoptosis protein (IAP) family, namely c-IAP2, inside the

cell. These data suggest a possible role for c-IAP2 in prolonging eosinophil survival in vivo.

THE AIRWAY EPITHELIUM UNDERGOES APOPTOSIS WHEN STIMULATED TO DO SO

The airway epithelium has death receptors and can undergo apoptosis when these receptors are stimulated. The expression of the death receptor Fas and its ligand, FasL was demonstrated in primary human bronchial epithelial cell in culture, and airway tissue sections from healthy human subjects.[79] While both Fas and FasL are expressed in subjects with fatal asthma and severe, nonfatal asthma, there are no significant differences in either group compared to those with non asthmatic subjects. This suggests that mechanisms other than Fas ligation may be different in subjects with asthma and may be activated during allergen challenge or environmental insult. In support of this idea, house dust mite cysteine proteinases can induce airway epithelial cell apoptosis.[80] Oxidant-induced apoptosis may be substantially greater in epithelial cells collected from asthmatic subjects compared to cells collected from healthy subjects. This suggests the presence of at least quantitative, and perhaps qualitative, differences between the normal and asthmatic epithelium.

The asthmatic airway epithelium also may be apoptotic, although the examination of this may depend, in part, on the disease state and its duration. A series of subjects who died of asthma and subjects with severe asthma who died of other causes were recently reviewed. Terminal deoxyuridine nick-end labeling (TUNEL) staining of the airway epithelium demonstrates the presence of a substantial proportion of apoptotic cells compared to the presence of very few such cells in the epithelium of non asthmatic subjects. In contrast, one recent article[81] suggested that in patients with mild asthma few apoptotic epithelial cells can be demonstrated to be present, regardless of the CS treatment. These divergent data suggest that, at least in some asthmatic patients, the ongoing damage to the epithelium may be due to an apoptotic loss of cells. The contribution of CSs may be to reduce inflammation, which may reduce damage.

CSS ELICIT APOPTOSIS OF THE AIRWAY EPITHELIUM

With this in mind, A study was recently conducted[82] to examine CS-induced apoptosis in the airway epithelium. It was originally hypothesized that CSs would protect the airway epithelium from apoptotic stimuli. This hypothesis fit the available data, which suggested that CS therapy would aid in the restitution of a damaged airway epithelium[83] without eliciting damage itself.[84] To the surprise, CS treatment induced apoptosis in central airway epithelial cells. In both primary human airway epithelial cells and in the central airway epithelial cell line, treatment with dexamethasone, budesonide, triamcinolone, or beclomethasone elicited concentration-dependent and time-dependent apoptosis. Little necrosis was present after CS treatment, as demonstrated by a lack of lactate dehydrogenase activity in culture medium after 48 h. Apoptosis occurred with CS concentrations that are at the high end of the range generally used to treat patients with asthma.

MAST CELL

Mast cell play an effector role in the inflammatory process in bronchial asthma in both early and late phase reaction. One study demonstrated delayed apoptosis of mast cell in either early or late phase reaction associated with recruitment of other cells to the airways.[85]

REFERENCES

1. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implication in tissue kinetics. Br J Cancer. 1972;26:239-57.

2. Cohen JJ. Apoptosis. Immunol Today. 1993;14:126-30.

3. Wyllie AH, Kerr JF, Currie AR. Cell death. The significance of apoptosis. Int Rev Cytol. 1980;68:251-306.

4. Nieto MA, Lopez-Rivas A. IL-2 protects T-lymphocytes from glucocorticoid-induced DNA fragmentation and cell death. J Immunol. 1989;143:4166-70.

5. Nagata S, Golstein P. The Fas death factor. Science.

6. Ryan JJ, Prochownik E, Gottlieb CA, Apel IJ, Merino R, Nunez G, c-myc and bcl-2 modulate p53 function by altering p53 subcellular trafficking during the cell cycle. Proc Natl Acad Sci USA. 1994;91:5878-82.

7. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell. 1993;369:609 -19.

8. Mimori K, Kiyokawa N, Taguchi T, Suzuki T, Sekino T, Nakajima H, Saito M, Katagiri YU, Isoyama K, Yamada K, Matsuo Y, Fujimoto J. Costimulatory signals distinctively affect CD20- and B-cell-antigen-receptor-mediated apoptosis in Burkitt's lymphoma/leukemia cells. Leukemia. 2003;17:1164-74.

9. Inohara N, Nunez G. Cell death and immunity: NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol. 2003;3:371-82.

10. Khanna N, Sen S, Sharma H, Singh N. S29 ribosomal protein induces apoptosis in H520 cells and sensitizes them to chemotherapy. Biochem Biophys Res Commun. 2003;304:26-35.

11. Krieser RJ, MacLea KS, Longnecker DS, Fields JL, Fiering S, Eastman A. Deoxyribonuclease IIalpha is required during the phagocytic phase of apoptosis and its loss causes perinatal lethality. Cell Death Differ. 2002;9:956-62.

12. Tempestini A, Schiavone N, Papucci L, Witort E, Lapucci A, Cutri M, Donnini M, Capaccioli S. The mechanisms of apoptosis in biology and medicine: a new focus for ophthalmology. Eur J Ophthalmol. 2003;13:S11-8.

13. van Gurp M, Festjens N, van Loo G, Saelens X, Vandenabeele P. Mitochondrial intermembrane proteins in cell death. Biochem Biophys Res Commun. 2003;304:487-97.

14. Ganju N, Eastman A. Zinc inhibits Bax and Bak activation and cytochrome c release induced by chemical inducers of apoptosis but not by death-receptor-initiated pathways. Cell Death Differ. 2003;10:652-61.

15. Kaminski M, Karbowski M, Miyazaki Y, Kedzior J, Spodnik JH, Gil A, Wozniak M, Wakabayashi T. Co-existence of apoptotic and necrotic features within one single cell as a result of menadione treatment. Folia Morphol (Warsz). 2002;61:217-20.

16. Dartsch DC, Schaefer A, Boldt S, Kolch W, Marquardt H. Comparison of anthracycline-induced death of human leukemia cells: Programmed cell death versus necrosis. Apoptosis. 2002;7:537-48.

17. Gaumer S, Guenal I, Brun S, Theodore L, Mignotte B. Bcl-2 and Bax mammalian regulators of apoptosis are functional in Drosophila. Cell Death Differ. 2000;7:804-14.

18. Samel D, Muller D, Gerspach J, Assohou-Luty C, Saas G, Tiegs G, Pfizenmaier K, Wajant H. Generation of a FasL based proapoptotic fusion protein devoid of systemic toxicity due to cell surface antigen-restricted activation. J Biol Chem. 2003 [epub ahead of print].

19. Kimura M, Haisa M, Uetsuka H, Takaoka M, Ohkawa T, Kawashima R, Yamatsuji T,Gunduz M, Kaneda Y, Tanaka N, Naomoto Y. TNF combined with IFN-alpha accelerates NF-kappaB-mediated apoptosis through enhancement of Fas expression in colon cancer cells. Cell Death Differ. 2003;10:718-28.

20. Baek KH, Shin HJ, Yoo JK, Cho JH, Choi YH, Sung YC, McKeon F, Lee CW. p53 deficiency and defective mitotic checkpoint in proliferating T lymphocytes increase chromosomal instability through aberrant exit from mitotic arrest. J Leukoc Biol. 2003;73:850-61.

21. Kralj M, Husnjak K, Korbler T, Pavelic J. Endogenous p21(WAF1/CIP1) status predicts the response of human tumor cells to wild-type p53 and p21(WAF1/CIP1) overexpression. Cancer Gene Ther. 2003;10:457-67.

22. Mansfield K, Pucci B, Adams CS, Shapiro IM. Induction of Apoptosis in Skeletal Tissues: Phosphate-Mediated Chick Chondrocyte Apoptosis is Calcium Dependent. Calcif Tissue Int. 2003 [epub ahead of print].

23. Ottaviani E, Barbieri D, Franchini A, Kletsas D. PDGF and TGF-beta partially prevent 2-deoxy-D-ribose-induced apoptosis in the fat body cell line IPLB-LdFB from the insect Lymantria dispar. J Insect Physiol. 2000;46:81-7.

24. Philchenkov AA. Caspases as regulators of apoptosis and other cell functions. Biochemistry (Mosc). 2003;68:365-76. PMID: 12765517 [PubMed - in process].

25. Jaattela M, Tschopp J. Caspase-independent cell death in T lymphocytes. Nat Immunol. 2003;4:416-23. Review.

26. Newton K, Strasser A. Caspases signal not only apoptosis but also antigen-induced activation in cells of the immune system. Genes Dev. 2003;17:819-25. Review. No abstract available.

27. Zhang HM, Cheung P, Yanagawa B, McManus BM, Yang DC. BNips: A group of pro-apoptotic proteins in the Bcl-2 family. Apoptosis. 2003;8:229-36.

28. Humlova Z. Protooncogene bcl-2 in process of apoptosis. Review article.

29. Sb Lek. 2002;103:419-25. Review.

30. Coultas L, Strasser A. The role of the Bcl-2 protein family in cancer. Semin Cancer Biol. 2003;13:115-23.

31. Tsujimoto Y. Cell death regulation by the Bcl-2 protein family in the mitochondria. J Cell Physiol. 2003;195:158-67. Review.

32. Germain M, Shore GC. Cellular distribution of Bcl-2 family proteins. Sci STKE. 2003;173:10. Review.

33. Tolomeo M, Simoni D. Drug resistance and apoptosis in cancer treatment: development of new apoptosis-inducing agents active in drug resistant malignancies. Curr Med Chem Anti-Canc Agents. 2002;2:387-401. Review.

34. Adams JM, Cory S. Life-or-death decisions by the Bcl-2 protein family. Trends Biochem Sci. 2001;26:61-6. Review.

35. Mirkes PE. 2001 Warkany lecture: to die or not to die, the role of apoptosis in normal and abnormal mammalian development. Teratology. 2002;65:228-39.

36. Palmer E. Cell death and immunity: Negative selection - clearing out the bad apples from the T-cell repertoire. Nat Rev Immunol. 2003;3:383-91.

37. Jewell AP. Role of apoptosis in the pathogenesis of B-cell chronic lymphocytic leukaemia. Br J Biomed Sci. 2002;59:235-8. Review.

38. Solary E, Dubrez L, Eymin B. The role of apoptosis in the pathogenesis and treatment of diseases. Eur Respir J. 1996;9:1293-1305. Review.

39. Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. Am Rev Respir Dis. 1993;147:697-704.

40. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. N Engl J Med. 1990;323:1033-9.

41. Rabe KF, Dent G. Theophylline and airway inflammation. Clin Exp Allergy. 1998;28:35-41.

42. Raff MC. Social controls on cell survival and cell death. Nature. 1992;356:397-400.

43. Vignola AM, Chiappara G, Siena L, et al. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. J Allergy Clin Immunol. 2001;108:738-46.

44. Bousquet J, Jeffery PK, Busse WW, et al. Asthma: from bronchoconstriction to airways inflammation and remodeling. J Allergy Clin Immunol. 2000;105:1720-45.

45. Holgate ST, Davies DE, Lackie PM, et al. Epithelial-mesenchymal interactions in the pathogenesis of asthma. J Allergy Clin Immunol. 2000;105:193–204.

46. Puddicombe SM, Polosa R, Richter A, et al. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. FASEB J. 2000;14:1362-74.

47. Vignola AM, Kips J, Bousquet J. Tissue remodeling as a feature of persistent asthma. J Allergy Clin Immunol. 2000;105:1041-53.

48. Vignola AM, Chanez P, Chiappara G, et al. Transforming growth factor-beta; expression in mucosal biopsies in asthma and chronic bronchitis. Am J Respir Crit Care Med. 1997;156:591-99.

49. Wenzel SE, Schwartz LB, Langmack EL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med. 1999;160:1001-8. Abstract.

50. Huang J, Olivenstein R, Taha R, et al. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. Am J Respir Crit Care Med. 1999;160:725-29.

51. Minshall E, Chakir J, Laviolette M, et al. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. J Allergy Clin Immunol. 2000;105:232-8.

52. Kuhn CR, Homer RJ, Zhu Z, et al. Airway hyperresponsiveness and airway obstruction in transgenic mice: morphologic correlates in mice overexpressing interleukin (IL)-11 and IL-6 in the lung. Am J Respir Cell Mol Biol. 2000;22:289-95.

53. Bousquet J, Chanez P, Lacoste JY, Arneon G, Ghavanion N, Enander I, et al. Eosinophilic inflammation in asthma. N Engl J Med. 1990;323:1033-9.

54. Kay AB, Barata L, Meng Q, Durham SR, Ying S. Eosinophils and eosinophil-associated cytokines in allergic inflammation. Int Arch Allergy Immunol. 1997;113:196-9.

55. Kitamura T, Miyajima A. Functional reconstitution of the human interleukin-3 receptor. Blood. 1992;80:84-90.

56. Tai P-C, Sun L, Spry CFJ. Effects of IL-5, GM-CSF and IL-3 on survival of human blood eosinophils in vitro. Clin Exp Immunol. 1992;85:312-6.

57. Her E, Frazer J, Austin KF, Owen WF. Eosinophil hematopoietins antagonize the programmed cell death of eosinophils. J Clin Invest. 1991;88:1982-7.

58. Yamaguchi Y, Suda T, Ohata S, Tominaga K, Miura Y, Kasahara T. Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. Blood. 1991;78:2542-7.

59. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med. 1992;326:298-304.

60. Kay AB, Ying S, Varney V, Gaga M, Durham SR, Moqbel R, et al. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med. 1991;173:775-8.

61. Yamaguchi Y, Sudo T, Ohata S, Tominaga K, Miura Y, Kasa T. Analysis of the survival of mature human eosinophils: Interleukin-5 prevents apoptosis in mature human eosinophils. Blood. 1991;78:2542-7.

62. Ohta K, Sawamoto S, Nakajima M, Kubota S, Tanaka Y, Miyasaka T, et al. The prolonged survival of human eosinophils with interleukin-5 and its inhibition by theophylline via apoptosis. Clin Exp Allergy. 1996;26:10-5.

63. Hallsworth MP, Litchfield TM, Lee TH. Glucocorticoids inhibit granulocyte-macrophage colony-stimulating factor-1 and interleukin-5 enhanced in vitro survival of human eosinophils. Immunology. 1992;75:382-5.

64. Meagher LC, Cousin JM, Seckl JR, Haslett C. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. J Immunol. 1996;156:4422-8.

65. Neito MA, Lopez-Rivas A. Glucocorticoids activate a suicide program in mature T lymphocytes: protective action of interleukin-2. Ann NY Acad Sci. 1992;650:115-20.

66. Northrop JP, Crabtree GR, Mattila PS. Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. J Exp Med. 1992;175:1235-45.

67. Almawi WY, Lipman ML, Stevens AC, Zanker B, Hadro ET, Strom TB. Abrogation of glucocorticoid-mediated inhibition of T cell proliferation by the synergistic action of IL-1, IL-6, and IFN-g. Immunology. 1991;146:3523-7.

68. Leung DYM, de Castro M, Szefler SJ, Chrousos GP. Mechanisms of glucocorticoid-resistant asthma. Ann NY Acad Sci. 1998;840:735-46.

69. Huang RW, Tsuda H, Takatsuki K. Interleukin-2 prevents programmed cell death in chronic lymphocytic leukemia cells. Int J Hematol. 1993;58:83-92.

70. Mentz F, Merle-Beral H, Ouaaz F, Binet JL. Theophylline, a new inducer of apoptosis in B-CLL: role of cyclic nucleotide. Br J Haematol. 1995;90:957-9.

71. Mentz F, Mossalayi MD, Ouaaz F, Baudet S, Issaly F, Ktorza S, et al. Theophylline synergizes with chlorambucil in inducing apoptosis of B-chronic lymphocytic leukemia cells. Blood. 1996;88:2172-82.

72. Yasui K, Hu B, Nakazawa T, Agematsu K, Komiyama A. Theophylline accelerates human granulocyte apoptosis not via phosphodiesterase inhibition. J Clin Invest. 1997;100:1677-84.

73. Lomo J, Blomhoff HK, Beiske K, Stokke T, Smeland EB. TGF-b1 and cyclic AMP promote apoptosis in resting human B lymphocytes. J Immunol. 1995;154:1634-43.

74. Kim DH, Lerner A. Type 4 cyclic adenosine monophosphate phosphodiesterase as a therapeutic target in chronic lymphocytic leukemia. Blood. 1998;92:2484-94.

75. Tsuyuki S, Bertrand C, Erard F, Trifilieff A, Tsuyuki J, Wesp M, et al. Activation of the Fas receptor on lung eosinophils leads to apoptosis and the resolution of eosinophilic inflammation of the airways. J Clin Invest. 1995;96:2924-31.

76. Woolley KL, Gibson PG, Carty K, Wilson AJ, Twaddell SH, Woolley MJ. Eosinophil apoptosis and the resolution of airway inflammation in asthma. Am J Respir Crit Care Med. 1996;154:237-43.

77. Sexton DW, Blaylock MG, Walsh GM. Human alveolar epithelial cells engulf apoptotic eosinophils by means of integrin- and phosphatidylserine receptor-dependent mechanisms: a process upregulated by dexa-methasone. J Allergy Clin Immunol. 2001;108:962-9.

78. Bureau F, Seumois G, Jaspar F, Vanderplasschen A, Detry B, Pastoret P-P, et al. CD40 engagement enhances eosinophil survival through induction of cellular inhibitor of apoptosis protein 2 expression: possible involvement in allergic inflammation. J Allergy Clin Immunol. 2002;110:443-9.

79. Hamann KJ, Dorscheid DR, Ko FD, et al. Expression of Fas (CD95), and FasL (CD95 L) in human airway epithelium. Am J Respir Cell Mol Biol. 1999;19:537-42.

80. Winton HL, Wan H, Cannell MB, et al. Class specific inhibition of house dust mite proteinases which cleave cell adhesion, induce cell death and which increase the permeability of lung epithelium. Br J Pharmacol. 1999;124:1048-59.

81. Vignola AM, Chiappara G, Siena L, et al. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. J Allergy Clin Immunol. 2001;108:738-46.

82. Dorscheid DR, Wojcik KR, Sun S, et al. Apoptosis of airway epithelial cells induced by corticosteroids. Am J Respir Crit Care Med. 2001;164:1939-47.

83. Laitinen LA, Laitinen A. Inhaled corticosteroid treatment and extracellular matrix in the airways in asthma. Int Arch Allergy Immunol. 1995;107:215-16.

84. Wanner A. Clinical aspects of mucociliary transport. Am Rev Respir Dis. 1977;116:73-125.

85. Abo Shehata ME, El-adrawy MKF, Abbas ASN, Sayed Ahmad AA Mast cell apoptosis in bronchial asthma. MSc Thesis, Mansoura University. 2002.