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Apoptosis and airway inflammation in asthma

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Asthma is a disease characterized by a chronic inflammation of the airways and by structural alterations of bronchial tissues, often referred to as airway remodelling. The development of chronic airway inflammation in asthma depends upon the continuous recruitment of inflammatory cells from the bloodstream towards the bronchial mucosa and by their subsequent activation. It is however increasingly accepted that mechanisms involved in the regulation of the survival and apoptosis of inflammatory cells may play a central role in the persistent inflammatory process characterizing this disease. Increased cellular recruitment and activation, enhanced cell survival and cell:cell interactions are therefore the key steps in the development of chronic airway inflammation in asthma, and represent the major causes for tissue damge, repair and remodelling.

Keywords: apoptosis; asthma; inflammation.

Introduction

Apoptosis is generally defined as a genetic program that eliminates unneeded, senescent, or damaged cells. It has attracted tremendous interest from biologists at large for its essential role in development, organ differentiation, and the constant monitoring of homeostasis in the adult organism.[1] Cell death/viability pathways exhibit a fascinating complexity. This involves intertwined gene families of stimulators and inhibitors of cell death; biochemical control of mitochondrial homeostasis; and cascade activation of executioner cysteine proteases, caspases.[2] It is known that deregulation of apoptosis causes, or at least contributes to, the pathogenesis of human diseases.[3]

Airways inflammation has been widely demonstrated in all forms of asthma and an association between the extent of inflammation and the clinical severity of asthma has been demonstrated[4,5] but the mechanisms underlying its persistence are still poorly understood. During the last years an increasing number of evidence have lend support to the concept that a dysregulation of cell apoptosis may play a central role in the development of airway inflammatory associated with asthma.[6,7] That inflammation in asthma might be the consequence of a dys-regulation of apoptosis was hypothesised in 1995 by Simon and coworkers who speculated that an inhibition of programmed eosinophil death may represent a key pathogenic event underlying the eosinophilic inflammation characterising the disease.[8] Since then, more evidence have been accumulated showing that changes in programmed cell death mechanisms of both mobile and resident cells of the airways may directly contribute to the development of asthma as well as on its clinical severity. This review will concentrate on the mechanisms which may be involved in apoptosis dysregulation of inflammatory cells involved in the pathogenesis of bronchial asthma.

Apoptosis and inflammatory cells recruitment into the airways from bone-marrow to peripheral blood

Inflammatory cells mature and are released by bone marrow and traffic in the circulation before being recruited into the airway wall. Asthma is associated with increased levels of hemopoietic progenitor cells both in bone marrow and peripheral blood. The increased amount of differentiating progenitor cells within the bone marrow of asthmatic subjects depends upon the effects of several cytokines and chemokines, such as IL-5 and GM-CSF.[10–12] It is likely that these mediators can substantially influence the survival and proliferation of progenitor cells leading to an increased influx of cells from the bone marrow to the blood. Recently it has been shown that Stat 5, which is activated by multiple receptors of hematopoietic cytokines, can play an important role in rescuing differentiating cells

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from apoptosis. This indicates that transdunctional and transcriptional pathways are highly sensible targets, and their activation may play a fundamental role in promoting either survival or apoptosis of differentiating myeloid progenitor cells during inflammation of the airways.[13]

Leukocyte-endothelium interactions and tissue infiltration

Another important step in the recruitment of inflammatory cells into the airways is represented by complex interactions occurring between leukocytes and endothelial cells. The activation of endothelium is important in recruiting neutrophils and other inflammatory cells to sites of inflammation and in modulating their function and survival. In asthma, endothelium activation is the result of both paracrine and autocrine mechanisms. The former are due to the release by activated epithelial cells, mast cells and T-lymphocytes of several mediators, such as histamine[14,15], cytokines (TNF-a)[16], and chemokines[17] which can induce the expression on endothelial cells of cell surface adhesion molecules[15], such as ICAM-1 and VCAM-1[18].

The development of leukocyte-endothelium interactions are also dependent upon the release of several signals that are capable of influencing the survival of both these cell types in an autocrine fashion. Activated endothelial cells acts to significantly delay the constitutive apoptosis of neutrophils, resulting in their enhanced survival and increased phagocytic function. The antiapoptotic activity is, in part, attributable to granulocyte/macrophage colony-stimulating factor (GM-CSF) secreted by activated endothelial cells,[19] and may serve as mechanism theleologically addressed at potentiating the host response against external noxious agents. It is of interest that also neutrophils can regulate endothelial cell survival. For example, neutrophil-borne heparin-binding protein (HBP) is a multifunctional protein involved in the progression of inflammation. It has been shown that a significant fraction of proteoglycan-bound HBP is taken up by the endothelial cells, and internalised within mitochondria. Internalised HBP markedly protected endothelial cells from apoptosis sustaining viability of endothelial cells in the context of locally activated neutrophils.[20] In addition, interactions of circulating leukocytes with endothelium during the course of an inflammatory reaction has been shown to provide survival signals to endothelial cells stimulating the expression of the Bcl-2 family member in endothelial cells and conferring protection against apoptosis.[21] Thus, it appears that as soon as leukocyte-endothelial cells interactions are established, under the effects of a highly dynamic autocrine loops, endothelial cells actively promote the recruitment and survival of activated leukocytes at the site of inflammation, and this is facilitated by proinflammatory changes occurring in endothelial cells, which respond to cytokines and inflammatory mediators by upregulating leukocyte adhesion-promoting molecules and transcribing their own chemotactic/inflammatory genes.[22] The relevance of all these mechanism in asthma are still not completely elucidated, but several lines of evidence suggest that they may potentially be involved in the development of the inflammatory process. Indeed, GM-CSF, IL-5[23] and other Th-2-type cytokines[24] are released in a greater amount in the airways of asthmatic subjects. Interestingly, the bronchial endothelium of asthmatic subjects is an important source of both Th-2 cytokines and chemokines, including RANTES, eotaxin and monocyte chemoattractant proteins (MCPs),[25] suggesting that the above described autocrine and paracrine loops may also occur in asthmatic subjects.

The problem is that some of the cytokines that elicit these responses (*i.e.*, TNF-a) are also powerful inducers of apoptosis, and an increased TNF-a α release has been observed in asthma.[24] Thus, the preservation of endothelial cell viability is one of the clearest examples of how flexible apoptotic mechanisms must be to preserve homeostasis. It seems that the way the endothelium protect itself from committing suicide every time it participates in inflammation is by upregulating several protective, antiapoptotic genes through TNF-/NFκB activation[26]. Therefore, it appears that the same transcriptional mechanisms mediating inflammation also prevent cytokine-induced cell death, blunting caspase activity and opposing further NFkB activation.[26]

Cell survival in airway tissues

Recruitment of cells into the airways wall is associated with their priming and activation[27,28] and is also dependent on cytokines such as IL-5[29,30] and GM-CSF acting to enhance eosinophil recruitment, terminal maturation[31] and expression of their adhesion molecules.[27,32,33] Chemokines such as RANTES[34,35] and the newly described eotaxin[36,37] also act on eosinophils to enhance markedly their recruitment and possibly their activation. IL-16, a lymphocyte chemoattractant factor, and macrophage inflammatory protein 1α (MIP-1α) are found in BAL fluid of antigen-challenged asthmatics.[38] All these cytokines have also the ability to modulate the survival of leukocytes, and in particular of eosinophils, and can therefore regulate the persistence of the cells in the inflamed airways.

Indeed, the regulation of eosinophil apoptosis contributes to the control of the 'tissue load' of cells at inflamed sites, and tends to limit inflammatory tissue injury and to promote resolution rather than progression of inflammation.[39,40] Since the initiation of apoptosis serves to terminate the inflammatory process by reducing the

number of inflammatory cells within the bronchial mucosa, the persistence of inflammation may be due to abnormalities in the regulation of cell apoptosis leading to a chronic and self-perpetuating inflammatory cell survival and accumulation. Compelling evidence have been accumulated showing that a decreased or suppressed apoptosis of immune-effector cells in inflamed tissues is crucial to the chronic evolution of an inflammatory process in different organs.[6,41–43]. In rheumatoid arthritis, the active suppression of T-cell death by the synovial microenvironment has been shown to be an important mechanism for the persistence of T cell infiltrates in chronically inflamed rheumatoid arthritis joints.[41] More recently, the delayed eosinophil apoptosis in nasal polyps has been indicated as a novel mechanism by which eosinophils specifically accumulate in human tissues.[42]

Apoptosis and eosinophilic inflammation in asthma

The number of non apoptotic eosinophils has been found to be significantly higher in asthma than in chronic bronchitis or normal subjects, suggesting that these cells can survive longer in the airways of asthmatic subjects.[7] In addition, it has been suggested that the persistence of these cells may have important clinical consequences as demonstrated by the significant correlation between the number of non-apoptotic eosinophils and macrophages and the severity of asthma.[7] In a recent study it has been shown that patients with severe or life threatening asthma have more bcl-2 positive eosinophils and a higher ECP level in the sputum than those with mild to moderate asthma. bcl-2 positive eosinophils has also been found to directly correlate with ECP levels whereas a significant negative correlation was found between bcl-2+ eosinophils and FEV1/FVC. The increased expression of bcl-2 in eosinophils from sputum of subjects with severe asthma suggests that bcl-2 may contribute to prolong survival and decrease apoptosis of airway eosinophils in asthma.[44] Thus, it is conceivable that the chronic accumulation of eosinophils in asthma may be due to an increased recruitment of these cells in the airways as well as by an inhibition of their programmed cell death. This hypothesis is also supported by the evidence that, although the number of CD45 positive cells is similar in asthma and chronic bronchitis subjects, the ratio between apoptotic cells / CD45 positive cells has been found to be significantly lower in asthma than in chronic bronchitis.[7]

Modulation eosinophil apoptosis by inflammatory mediators

It is likely that the reduced apoptosis of eosinophils is due to the effects of pro-inflammatory cytokines overexpressed in the airways of asthmatic subjects. Several cytokines and chemokines may also promote cell survival. Among them, GM-CSF, IL-3, IL-4, IL-5 and RANTES which are overexpressed in asthmatic airways[45–50] are able to enhance cell survival and in particular that of eosinophils[31,51,52] by blocking apoptosis.[53]

Among the possible eosinophil and macrophage survival factors, an important role is played by GM-CSF[53,54] which via the activation of JAK2, but not of PI 3-kinase/Akt and MAP kinase pathways, is able to transmit anti-apoptotic signals in human eosinophils.[55] Targeting GM-CSF transgene to the airway cells in a mouse model of ovoalbumin (OVA)-induced allergic airways inflammation, a model in which there is marked induction of endogenous IL-5 and IL-4 but not GM-CSF, results in a much greater and sustained accumulation of various inflammatory cell types, most noticeably eosinophils, both in BALF and airway tissues post-OVA aerosol challenge. The hypothesis raised by these animal studies is that GM-CSF can significantly contribute to the development of allergic airways inflammation by potentiating and prolonging inflammatory infiltration induced by cytokines such as IL-5 and IL-4. The direct role played by GM-CSF in apoptosis modulation of inflammatory cells is also demonstrated by the evidence that the use of GM-CSF analogues can induce apoptosis of normal and activated eosinophils and can regulate their numbers and activities.[56]

Another cytokine which is critically involved in the modulation of eosinophil activation and survival is IL-5. IL-5 is indeed a potent eosinophil viability-enhancing factor that has been strongly implicated in the pathogenesis of IgE-mediated inflammation *in vivo.* Recently published data have suggested that IL-5 (and related cytokines) may act by altering the expression of the anti-apoptotic regulator Bcl-2 or its homologues.[44,57] An additional mechanism seems to be the IL-5 ability to regulate activation of the caspase cell death cascade. In particular, the (upstream) caspase 8 and (downstream) caspase 3 proenzymes, which are detectable in eosinophils at baseline, are completely blocked by IL-5 both in spontaneous and dexamethasone-induced cell death, or significantly slowed during Fas ligation.[57]

Cytokines, cellular signalling and eosinophil apoptosis

It is therefore clear that several pro-inflammatory cytokines released during the inflammation associated with bronchial asthma can contribute to the increased survival of granulocytes and to their persistence in the inflamed airways. It is well know that most of these cytokines, such as IL-3, IL-5, GM-CSF exert their effects by activating intracellular signalling pathways, which are then able to modulate the cellular response by activating the transcription of specific genes. The receptors for IL-3, IL-5 and GM-CSF share a common ß subunit, which does not

contain an intrinsic tyrosine kinase activity, but is nevertheless essential for signal transduction.[58,59] It has been shown that activation of the IL-3/IL-5/GM-CSF receptor ß subunit leads to the phosphorilation of Lyn and Syk proteins which seems to be essential for anti-apoptotic signalling.[60] Aerosolized Syk antisense has been shown to suppress Syk expression, the mediator release from macrophages, and pulmonary inflammation, suggesting that the blockage of this specific pathway may be effective for the resolution of airway inflammation.[61]

NFκB activation has also been indicated as an important pathway in apoptosis regulation. This transcription factor plays a central role in the modulation of many cellular responses regulating the expression of a large number of inducible genes, such as inflammatory cytokines, growth factors, and adhesion molecules.[62] The induction of apoptosis in granulocytes can be abolished by the inhibition of NFkB, indicating that this transcription factor plays a crucial role in regulating the physiological cell death pathway in granulocytes.[63] NFkB is also involved in mediating the effects of tumor necrosis factor (TNF)-α, another proinflammatory cytokine, which can induce a broad spectrum of biologic effects and is associated with inflammatory lung disease. The cellular effects of TNF are mediated by two distinct cell surface receptors termed TNF-receptor 1(TNFR1) and TNF-receptor 2(TNFR2).[64] Most cytotoxic effects of TNF are mediated by TNFR1 through interaction of its death domain with the TNFR-associated death domain protein (TRADD).[65] TRADD interacts with Fas-associated death domain protein (FADD)[66] to activate caspase-8, thereby initiating the apoptosis pathway. Death domain is the sequence in TNFR1, TRADD, and FADD. The death domain is a protein-protein interaction domain, and adopter molecules FADD and TRADD use these domains to interact with other death domain-containing molecules and trigger the apoptosis-signalling pathway. Another well known death receptor, Fas, also transduces apoptosis signal through FADD and shares the same signalling machinery downstream of FADD with TNFR. Since the Fas-mediated apoptosis-signalling 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 NFkB by proteolytic breakdown of the inhibitor of kB (IkB). TNFR-associated factor-2 (TRAF2) and receptor interacting protein (RIP)[67] indirectly bind to TNFR1 through TRADD or directly bind to TNFR2 and activate the NFκB-inducing kinase (NIK)[68] which in turn activates the inhibitor of IB kinase (IKK) complex. IKK phosphorylates IB, which leads to IkB degradation and allows NFκB to translocate to the nucleus and activate transcription. TNF or agonistic anti-Fas antibody administration can lead to production of interleukin-8 (IL-8) by colon epithelial cells[69] or by bronchial epithelial cells, in addition to inducing apoptosis *in vitro*.[70] As TNF activates the IL-8 promoter transcriptionally via NFkB activation, IL-8 secretion induced by Fas ligation also seems to be regulated via NFkB activation.[70] Therefore, death receptor activation induces NFkB activation, which triggers inflammation and also plays an important role in regulating apoptosis.[71]

In addition to NFκB, also p38 MAP-kinase seems to be involved in the regulation of eosinophil apoptosis. In this regard, the ability of TNF-α to increase eosinophil survival is mediated in part via activation of p38 MAP kinase, indicating that this pathway is involved in the regulation of eosinophil survival and, thus, might be important for the development of allergic eosinophil-rich inflammation.[72] Indeed, the use of p38 MAP-Kinase has been shown to inhibit eosinophil recruitment, in addition to enhancing apoptosis of these cells.[73]

Modulation of eosinophil apoptosis via surface membrane markers

Programmed cell death in eosinophils can also be regulated via the many cell surface molecules expressed by these cells. Among these molecules an interesting role seems to be played by CD45 and by FcgammaRII. The common leukocyte antigen CD45 and the isoforms CD45RA, are highly expressed by freshly isolated eosinophils. Incubation with mAb against CD45 or CD45RA can result in significant enhancement of eosinophil constitutive rate of apoptosis, indicating that ligation of CD45 or CD45RA may represent a novel pathway for the induction of apoptosis in human eosinophils.[74]

The low-affinity IgG Fc receptor, FcgammaRII (CD32), mediates various effector functions of lymphoid and myeloid cells and is the major IgG Fc receptor expressed by human eosinophils. In addition, allergen-specific IgG1 and IgG3 have been shown to induce eosinophil degranulation through Fc gamma RII activation, suggesting that this receptor may play a crucial role in cellular activation following allergene challenge.[75] When cultured *in vitro* without growth factors, most eosinophils undergo apoptosis within 96 h. Ligation of FcgammaRII by anti-CD32 mAb in solution is able to inhibit eosinophil apoptosis and prolong survival in the absence of growth factors. Cross-linking of human IgG bound to FcgammaRII by anti-human IgG Ab or of unoccupied FcgammaRII by aggregated human IgG also prolong eosinophil survival. It has been shown that the enhanced survival with anti-CD32 mAb is sustained by an autocrine production of GM-CSF by eosinophils mediated survival. In fact, mRNA for GM-CSF was detected in eosinophils cultured

with anti-CD32 mAb. In contrast to mAb or ligands in solution, anti-CD32 mAb or human IgG, when immobilized onto tissue culture plates, have been shown to facilitate eosinophil cell death even in the presence of IL-5. Cell death induced by these immobilised ligands id inhibited when eosinophil ß2 integrin is blocked by anti-CD18 mAb, suggesting that beta2 integrins play a key role in initiating eosinophil apoptosis. Thus, FcgammaRII may pivotally regulate both survival and death of eosinophils, depending on the manner of receptor ligation and beta2 integrin involvement.[76]

Apoptosis and neutrophilic inflammation in asthma

The role of neutrophils in stable asthma remains unclear. Although they can be recovered in the sputum of asthmatics,[77] this cell type is usually found in low numbers in BAL[78,79] and in especially low numbers in bronchial biopsies[79–81] from asthmatic subjects. IL-8, a chemokine involved in neutrophil recruitment and neutrophil-derived mediators such as myeloperoxidase were not found increased in BAL of asthmatics.[82,83] Neutrophils have been implicated in many lung diseases including emphysema, fibrosing alveolitis and respiratory distress syndrome.[84] However, neutrophils have also been found in increased numbers in the airways during the late phase reaction after an allergen challenge,[85,86] in some patients who died within hours after an asthma exacerbation,[87,88] in nocturnal asthma,[89] in some patients with long-standing asthma[90] or in patients with corticosteroid-dependent asthma.[91]

Recent reports have shown that an increased neutrophilic inflammation can be observed in patients suffering from severe corticosteroid-dependent asthma. These patients present with an ongoing inflammation of the airways usually characterised by an increased number of neutrophils[92,93] or activated T lymphocytes.[94] In addition, an increased immunoreactivity for GM-CSF, which can also act as a neutrophil-activating cytokine, has been found in bronchi of these patients.[95] Despite these evidence, the mechanisms underlying neutrophilic infiltration of the airways are not completely understood. It is likely that a persistent increased release of chemotactic cytokines for neutrophils, such as IL-8 and GM-CSF, may play a central role in the pathogenesis of this phenomenon, and data recently obtained by our group (unpublished data) are consistent with this hypothesis.

Modulation neutrophil apoptosis by inflammatory mediators

It is conceivable that both IL-8 and GM-CSF contribute to an increased recruitment of neutrophils from the bloodstream towards the bronchial mucosa. However it is also possible that these cytokines may play a role in the dysregulation of neutrophil apoptosis and in the persistent neutrophilic inflammation of the airways in severe asthmatic subjects. G-CSF and GM-CSF have been found to be present in BALF from patients with ARDS, a disease characterized by a massive neutrophilic inflammation. Interestingly, the concentrations of these chemokines are increased in ARDS BALF, supporting the concept that they can modulate the life-span of neutrophil in the air spaces contributing to the pathogenesis of this disease.[96]

The notion that GM-CSF can play a crucial role also in regulating the neutrophil-mediated inflammatory response, is also supported by the evidence that this growth factor is a proliferative stimulus for bone marrow neutrophil stem cell precursors and has at least 3 important roles in regulating neutrophil-mediated immunity: (a) a direct effect on the proliferation and development of neutrophil progenitors; (b) synergistic activity with other haemopoietic growth factors; (c) stimulation of the functional activity of mature neutrophils. The production of GM-CSF may be triggered directly by exogenous factors such as antigens and endotoxins, or indirectly through the release of cytokines by a variety of cells including lymphocytes, activated macrophages and endothelial cells exposed to products of mononuclear phagocytes. Such production of GM-CSF may serve to quickly release mature neutrophils from the bone marrow in response to inflammatory stimuli. Moreover, enhancement of the function of mature neutrophils may also augment their ability to migrate to sites of inflammation and then phagocytose and kill pathogens. Increased expression of CD11b/CD18 may play a fundamental role in this mechanism because this receptor is essential for the adhesion of neutrophils to the endothelium. A further level of neutrophil upregulation occurs by increasing the functional life span of neutrophils by GM-CSF. Thus, by delaying neutrophil apoptosis, GM-CSF greatly extends the time over which neutrophils may function at inflammatory sites. GM-CSF can thus exert a variety of important regulatory controls of neutrophil function during airway inflammation. Both the number and the functional status of neutrophils is highly regulated by GM-CSF. It is also possible that GM-CSF produced within localised sites of acute inflammation or infection may attract, trap and then activate neutrophils within this site.[97]

GM-CSF, slows neutrophil apoptosis through various mechanisms. There are evidence that GM-CSF is able to modulate neutrophil apoptosis by modulating the expression of Mcl-1 an antiapoptotic protein whose levels correlate with neutrophil survival.[98,99] In addition, GM-CSF has been shown to reduce Bax expression in neutrophils, a pro-apoptotic member of the Bcl-2 family, contributing to a delayed apoptosis of these cells.[100] Moreover, GM-CSF can activate the phosphoinositide 3-kinase (PI 3-kinase/Akt) pathway as well as the

extracellular signal-regulated kinase (ERK) , a pathway mediating IL-8 stimulation.[101] Thus, it is likely that both IL-8 and GM-CSF act to delay neutrophil apoptosis by stimulating PI 3-kinase and ERK-dependent pathways.

IL-8 is also able to interfere with extracellular death receptor signalling or intracellular caspase activation to suppress neutrophil apoptosis. Indeed, caspase 3 activity has been shown to be associated with a marked suppression at 24 h by the inclusion of IL-8 as well as with an increased superoxide anion production and phagocytic activity. *In vitro* studies have shown that the effect of IL-8 does not depend on activation of the Fas, TNFR55, or R75 receptor pathways but involves suppression of caspase 3 activity.[102] Caspase 3 activity in neutrophils are also induced by Fas engagement by an agonistic anti-Fas antibody. This process is also associated with an increased mitochondrial permeability. Studies with pharmacological inhibitors of caspase activity showed that activation of caspase 8 occurred before, and activation of caspase 3 occurred after mitochondrial disruption. The stabilisation of mitochondria is therefore thought to be an important step for neutrophil apoptosis to be delayed. Interestingly, LPS, GM-CSF and increased glutathione which are able to stabilise the mitochondria are also capable of inhibiting caspase 3. Thus, inhibition of Fas antibody induced apoptosis by inflammatory proteins appears to be an important mechanism associated with augmented mitochondrial stability and reduced caspase 3.[98,103] Although, most of these mechanisms have not yet been described in asthma ,they are likely to paly a role in the development of neutrophilic inflammation associated with nocturnal asthma,[89] long-standing asthma[90] or with corticosteroid-dependent asthma.[91]

In addition to G-CSF, GM-CSF and IL-8, there are evidence showing that nitric oxide may play a role in the regulation of neutrophil apoptosis. NO is an intercellular transmitter, both in the central and in the peripheral nervous system. In addition to nerve cells, NO is also produced by epithelial cells and by the endothelium. NO plays a key role as a vasodilator, neurotransmitter, and inflammatory mediator in the airways and is produced in increased concentrations in asthma.[104] It may be the major bronchodilator of airways normally.[105] However, NO may have deleterious effects on the airways as a vasodilator, by increasing plasma exudation, and may also amplify the asthmatic inflammatory response. Proinflammatory cytokines and oxidants increase the expression of an inducible form of NO synthase (iNOS) in airway epithelial cells in asthma,[106] and this may be the explanation for the increased levels of NO found in exhaled air of asthmatic patients.[107] Co-culture of human neutrophils with the NO donors causes a dramatic and concentration-dependent induction of apoptosis. It has been suggested that NO-mediated apoptosis, although caspase-dependent, is also mediated by a cGMP-independent mechanism and involves the concurrent generation of oxygen free radicals and, potentially, peroxynitrite.[108]

Modulation of neutrophil apoptosis via surface membrane markers

Neutrophil apoptosis can be regulated by a number of other immunologic and non-immunologic mechanisms. The interactions between neutrophils and extracellular matrix components represents an additional pathway influencing neutrophil apoptosis.[109] These interactions are mediated by several adhesion molecules (most of which belong to the integrin family) expressed on the surface of activated neutrophils. The interactions between adhesion molecules and extracellular matrix components activate intracellular signalling pathways which are involved in the regulation of cell apoptosis. Human neutrophil apoptosis has also been shown to be modulated by immune complexes (IC).[110] Precipitating IC (pIC) and Ab-coated erythrocytes (E-IgG) trigger a marked stimulation of apoptosis, while heat-aggregated IgG and soluble IC, significantly delayed spontaneous apoptosis. Blocking Abs directed to Fcgamma receptor type II (FcgammaRII), but not to FcgammaRIII, markedly diminished the acceleration of apoptosis triggered by either pIC or E-IgG, supporting a critical role for FcgammaRII in apoptosis stimulation. This phenomenon, on the other hand, does not appear to involve IC phagocytosis or the participation of CR3. Acceleration of neutrophil apoptosis triggered by either pIC or E-IgG seems to require the activation of the respiratory burst, as suggested by (1) the ability of catalase to prevent apoptosis stimulation; (2) the effect of azide, an heme enzyme inhibitor, which dramatically enhanced apoptosis induced by pIC or E-IgG; and (3) the inability of pIC or E-IgG to accelerate apoptosis of neutrophils isolated from CGD patients. Therefore, IC are able not only to affect the course of inflammation by inducing the release of inflammatory cytokines, proteolytic enzymes, oxidative agents, and other toxic molecules, but also the course of inflammation by virtue of their ability to modulate neutrophil apoptosis. In asthma immune complexes can play an important role as initiators of the immune response. IgE immune complexes have been shown to be more potent inducers than antigen alone of airway inflammation in a murine model.[111] In addition, with signs of complement activation, the presence of rheumatoid factor, and circulating immune complexes have been found in patients with aspirin-induced asthma,[112] a disease state in which an increased neutrophilic activity has been described.[113]

Epithelial cells and apoptosis

Bronchial epithelial cells play an active role in the development of airway inflammation and remodelling in

asthma.[114] Bronchial epithelial injury initiates a complex series of repair mechanisms, one of which is the re-epithelialization of a denuded lumenal surface. Regenerative changes are sometimes observed as demonstrated by varying stages of ciliogenesis in the non-ciliated "metaplastic" surface epithelium.[115] It is likely that bronchial epithelial cells initially affected by bronchial injury may be able to initiate repair of an injured area by producing chemotactic factors for epithelial cells. Epithelial cells in asthma express several membrane markers, including adhesion molecules,[5] and release a wide spectrum of molecules participating in airway repair including fibronectin,[116] growth factors,[114,117,118] cytokines including IL-9, IL-16[119] and IL-18[120] or chemokines such as GM-CSF,[45,121], and eotaxin.[122,123]

A major histologic feature in asthma is the fragilization and shedding of the bronchial epithelium. Although this structural change has been documented in many studies, the underlying pathogenetic mechanisms are still a matter of debate. Recently, it has been postulated that epithelial cell loss might be due to apoptosis. However, the data are still conflicting and not leading to a unifying hypothesis. The reason for this may be due to the fact that the fate of columnar cells, which are usually involved in the desquamation process, may be different from that of basal cells, which are usually not affected by the desquamation process and, by contrast, may be involved in the promotion of the injury-repair cycle following inflammation. Fas and FasL expression has been recently demonstrated in normal human central airway, suggesting that alterations in the function of one of these molecules may represent a mechanism responsible for the loss of the epithelium barrier integrity.[124] In another recent study, an increased Fas expression has been shown in the airway epithelium of severe asthmatics,[125] but this finding was not found to be associated with cell apoptosis. By contrast it has been hypothesised the Fas or FasL expression by bronchial epithelial cells may have some immune regulatory functions, being able to control inflammatory cell infiltration and survival in the submucosa and in within the epithelium layer. Indeed, interactions between Fas-L positive epithelial cells and Fas expressing leukocytes may lead to the resolution, or at least the modulation, of the inflammatory process.[126]

T lymphocytes

T-cells are play a role in controlling the chronic inflammation of asthma. The number of T lymphocytes infiltrating the bronchial airways is significantly increased in asthma and they are characterized by a Th2-phenotype.[127–131] It is accepted that the increased numbers of mucosal T-lymphocytes is the result of an increased recruitment of these cells within the airways. However, it is also likely that T-lymphocyte accumulation in the airways of asthmatic subjects depend upon an increased survival of these cells. This hypothesis is supported by several lines of evidence. In bronchial biopsies taken from asthmatic subjects most of T-lymphocytes appear not be apoptotic and to express Bcl-2 suggesting that these cells may have the ability to live longer in the inflamed airways.[7] This is also confirmed by results obtained using allergen-induced cutaneous late-phase response,[132] an experimentally reproduce the *in vivo* inflammation characterising bronchial asthma. Finally, apoptosis in induced sputum mononuclear cells was found decreased in patients with asthma compared to COPD patients and healthy controls. In addition, Bcl-2 expression was found to be increased in sputum mononuclear cells from patients with asthma, compared to healthy controls and patients with COPD[133] All together these evidence lend support to the concept that apoptosis dysregulation of T-lymphocytes may occur in asthma, and that such a mechanism may play an important role in the persistent inflammatory process associated with the disease. Because of this, induction of cell apoptosis in activated T-lymphocyte may represent an important future therapeutic target.

Clearance of apoptotic cells

Another important step regulating the fate of apoptotic granulocytes is their removal from the inflamed tissues. *In vivo*, apoptotic cells are efficiently removed by professional or non-professional phagocytes, a process thought to be essential for tissue remodeling and resolution of inflammation. Macrophages recognize apoptotic cells by several mechanisms, including recognition of exposed phosphatidylserine on apoptotic cells. This mechanism is mainly mediated by CD36 which is able not only to recognize and interact with alpha(v)beta3 integrin expressing cells but also phosphatidylserine.[134] In a recent study we evaluated in bronchial biopsies the number of cells expressing CD36, and found that it was very low and not statistically different between normal and asthmatic subjects, suggesting a similar clearance rate of apoptotic cells in normal and inflamed airways.[7]

It is also known that CD14, another surface marker expressed by macrophage serves an additional unexpected function, namely as a receptor involved in the recognition and phagocytosis of cells undergoing apoptosis. In contrast to its role in eliciting pro-inflammatory responses following binding of microbial ligands, macrophage CD14 mediates clearance of apoptotic cells without inciting inflammation.[135]

The removal of apoptotic cells can be regulated by mediators released in the microenvironment. In this regard, lipoxins, lipoxygenase-derived eicosanoids generated during inflammation, are known to inhibit polymorphonuclear neutrophil chemotaxis and adhesion and are putative braking signals for neutrophil-mediated tissue injury.

It is has been reported that lipoxin-A4 (L × A4) promotes another important step in the resolution phase of inflammation, namely, phagocytosis of apoptotic neutrophils by monocyte-derived macrophages, suggesting that LXA4 is an endogenous stimulus for neutrophil clearance during inflammation.[136] That phagocytosis of apoptotic cells is theleologically addressed at the resolution of the inflammatory process is also demonstrated by the evidence that phagocytosis of apoptotic cells inhibits the release of inflammatory cytokines by human macrophages. Interestingly, human monocyte/macrophages taking up apoptotic neutrophils have been shown to be able to release FasL and to induce apoptosis of bystander leukocytes, suggesting the existence of redundant apoptosis-related mechanisms promoting the resolution of inflammation.[137]

In addition to macrophages also human small airway epithelial cells have been shown to recognize and ingest apoptotic cells, and in particular human eosinophils. Interestingly, the ability of airway epithelial cells to ingest apoptotic eosinophils is enhanced by interleukin-1ß or TNFalpha and is mediated via lectin- and integrin-dependent mechanisms. Therefore it is likely that human bronchial epithelial cells represent an additional important mechanism in the resolution of eosinophil-induced asthmatic inflammation.[138]

Effects of anti-asthma treatments

Anti-asthmatic treatments may induce the resolution of inflammation by apoptosis.[139] Corticosteroids are the most potent anti-inflammatory agent used for treating asthma. They inhibit the prolonged survival of eosinophils and lymphocytes directly by inducing apoptosis and indirectly by suppressing the release of cytokines supporting their survival.[6,140–142] Wooley and colleagues[6] showed that the clinical amelioration of asthmatic patients treated with glucocorticosteroids was associated with a significant increase of apoptotic eosinophils recovered from induced sputum samples indicating that eosinophil apoptosis is clinically relevant in asthma. In addition to induce apoptosis in eosinophils, corticosteroids have also been shown to potentiate nonphlogistic clearance of apoptotic leukocytes by phagocytes,[143] suggesting a multimodal activity of steroids in the resolution of inflammation . In addition to mobile cells, corticosteroids appear to modulate apoptosis in structural cells. Interestingly, dexamethasone exerts a potent inhibition of IFN-gamma- and IFN-gamma plus anti-Fas-induced apoptosis in epithelial cells. Although the consequences of this effect need to be fully elucidated, it is likely that through this mechanism corticosteroids can play an important role in the restoration of the epithelium layer following inflammation.

As for epithelial cells, also for neutrophils steroids appear to promote survival rather than death.[144–148] Although it is still controversial as to whether this specific effect may influence the evolution of the inflammatory response, it is likely that the reduced ability of steroids to induce neutrophil apoptosis may play an important role in the accumulation of these cells in the bronchi of severe asthmatic and COPD patients.

Theophylline, a classical bronchodilator, has been reported to have anti-inflammatory effects. Theophylline has also been shown to[149,150] reduce the survival of inflammatory cells including eosinophils via a mechanisms which seems to be independent from phosphodiesterase inhibition.[151]

Finally, anti-leukotrienes might be able to exert a pro-apoptotic effect. Interruption of leukotriene synthesis by inhibition of 5-LO or FLAP, and blockade of neutrophil-derived LTB4 activity with receptor antagonism, have been shown to reverse LPS-, GM-CSF-, and dexamethasone-induced neutrophil survival. LTB4 receptor blockade alone also reduced unstimulated basal neutrophil survival. In addition, the capacity of LTB4 receptor antagonism and leukotriene synthesis inhibition to reverse neutrophil survival responses was also demonstrated in cells from patients with COPD.[152]

Conclusions

There are now compelling evidence showing that apoptosis dys-function can play en important role in the pathogenesis of airway inflammation associated with bronchial asthma. The identification of the cellular and molecular mechanisms underlying the reduction of inflammatory cell apoptosis must be considered as a fundamental step for new and effective therapeutic strategies.

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