Review Article

# Apoptosis and eosinophils

## *Regulation and clinical relevance*

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## ABSTRACT

يعتبر الموت الخلوي الفسيولوجي (Apoptosis) من أهم العمليات ذات العلاقة بالجهاز المناعي. وقد أصبح من المؤكد أن موت الخلايا الحامضية هو من الخطوات الأساسية والأولية لإزالة الخلايا الحامضية من الرئتين بواسطة الخلايا البلعمية (Phagocytes)، ونظراً لأهمية هذا الموضوع فإن هذه البحث يناقش العلاقة بين الموت الخلوي الفسيولوجي والخلايا الحامضية ويلخص أهم التطورات في هذا الحقل.

Apoptosis, or program cell death, is a process of fundamental biological importance, and eosinophil apoptosis is believed to be the primary mechanism for removing eosinophils from the lung followed by their recognition and phagocytosis by macrophages or resident bronchial epithelial cells. There is, therefore, an increased interest in the fundamental role of the signals and intracellular signaling molecules that initiate and control apoptosis in human eosinophils though much remains to be established. This article reviews briefly the cross talks between apoptosis and eosinophils and summarizes the recent developments in this field.


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***1. Eosinophil.*** In 1846, it wass described as 'course granule cells' in the blood of various species, including humans. This represented the first description of the eosinophil and was further expanded upon in 1865 and detailed some of the eosinophil's morphological features and considered it a distinct cell type.[1] The term 'eosinophil' was first used in 1879 and acquired its named because of its affinity to the acid aniline dye eosin. Since 1879 an understanding of their role in a number of diseases has slowly evolved. Increased eosinophil numbers in the blood (eosinophilia) have been associated with a number of diseases including parasitic infections (such as helminthic infections), cancer, asthma and other allergic diseases.[2] Initial assumptions of the protective role of eosinophils in parasite immunity have been proved correct. However, an equally protective role was ascribed to the eosinophil in asthma and other allergic diseases though evidence continues to mount on the damage caused by these cells in these disease states when their toxic potential is realized.[3]

Eosinophils are non-dividing, bone marrow-derived, fully differentiated end cells. They are approximately 10-15 μm in diameter and are morphologically distinguishable by their bilobed nucleus and distinct granules of varying sizes. Eosinophil half-life in the circulation is 13-18 hours. In healthy individuals, the majority of eosinophils are found in tissues, primarily in the gut, where they survive for several days to weeks. Their presence in the skin or the airway in significant numbers is usually associated with disease processes.[3-5] Eosinophils are produced in the bone marrow. As with basophils, they are derived from $CD34^+$ pluripotent progenitor cells through the influences of Th2 cytokine interlukin (IL)-3, IL-5, and granulocye-monocyte colony stimulating factor (GM-CSF). Interlukin-3 and GM-CSF seems to act on early precursors, whilst IL-5 acts later, during differentiation.[6] Interlukin-5 has a very cell-specific effects on differentiation and, in terms of eosinophil production, it acts acutely to release large

numbers of eosinophils into the circulation from the bone marrow.

***2. Characteristics of eosinophils.*** Eosinophils are principally secretory cells. They feature membrane-bound, arginine-rich specific granules stain a striking reddish-pink glow with basic aniline dyes such as eosin and consists of a crystalline core composed of major basic protein (MBP), which surrounded by a matrix of eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil protein X/eosinophil-derived neurotoxin (EPX/EDN).[7,8] In addition to their granule-derived performed mediators (namely platelet activating factor [PAF]), eosinophils have been shown to have the capacity to generate cytokines (namely IL-2, IL-6), chemokines (namely monocyte chemotactic protein 1 [MCP-1]), and reactive oxygen species.

***3. Eosinophil granule proteins.*** *3.1. Major basic protein (MBP).* Major basic protein is an arginine and cysteine-rich polypeptide (13.8-kDa) composed of 117 amino acids.[9,10] Studies using guinea pigs identified this protein as the major (-50%) protein in eosinophil granules.[11,12] The protein is not, however, restricted to eosinophils as both basophils and placental cells possess the protein[2] though at much lower concentrations. Major basic protein cytotoxicity is capable of killing a variety of mammalian and parasitic cells, including human pneumocytes.[13] Its other functions include stimulating degranulation of mast cells and basophils, contraction of airway smooth muscle, inhibition of airway mucous production and stimulation of EDN and IL-8 secretion by eosinophils.[13] In addition, MBP can, like EPO, act as an allosteric antagonist against the muscarinic M2 receptors on the airway epithelium.[14] The function of the eosinophil as a cytotoxic, pro-inflammatory cell in asthma is in part a consequence of the function of its secreted granule proteins.

*3.2. Eosinophil cationic protein (ECP).* Eosinophil cationic protein is a variably glycosylated, zinc and arginine-rich single chain peptide with a molecular mass of 16-21.4 kDa.[15] It is also termed RNase 3 due to its homologies with both human and other vertebrate RNases[16] and also shares a 70% amino acid sequence homology with EDN. Eosinophil cationic protein is cytotoxic to mammalian cells and microorganisms killing cells by creating pores in their surface membrane. These pores are large enough to allow water to freely enter the cell, which dies by osmotic lysis.[16] Eosinophil cationic protein has been shown to stimulate mucous hypersecretion and macromolecular transport in the microvasculature, which are important contributors to the pathological appearance of the asthmatic airway.[17] Eosinophil cationic protein has also been shown to activate mast cells and induce secretion of histamine and trypase, further evidence of its pro-inflammatory capacity.[3] The stored and secreted forms of ECP differ structurally and antigenically, allowing the secreted form of ECP to be detected as an eosinophil activation marker by immunostaining with the monoclonal antibody (mAb) EG2.[3] Eosinophil cationic protein can also be measured by radioimmunoassay. There is a closed relationship between asthma severity and levels of ECP and EG2 stained esopnophils.[18-20]

*3.3. Eosinophil peroxidase (EPO).* Eosinophil peroxidase is a dimeric haem-containing protein with a total molecular mass of 67kDa, and is composed of a light (15kDa) and heavy (52kDa) chain.[21,22] It catalyses the peroxidative oxidation of halides and pseudohalides and, as such, is a member of the wider family of haloperoxidases. It is exclusively localized to the matrix of the secondary granules[23,24] where, in humans, it accounts for 15% (-15pg/cell) of the total granule protein.[21] Its toxic effects are more potent when combined with hydrogen peroxide and halide to form toxic hypohalous acids. Its biological functions are mainly attributed to its peroxidase activity, though other non-peroxidase activities have been suggested, including damage to nasal sinus mucosa. Mast cell release of histamine and platelet aggregation has been attributed to EPO.[25] It has potent cytotoxic effects capable of killing parasites[26] and bacteria.[27,28] Eosinophil peroxidase can also cause neutrophil accumulation in the airway, by autocrine stimulation of IL-8 secretion by eosinophils.[3] Interluken-8 has also been shown to behave as a chemo-attractant for eosinophils in atopic patients.[3,29]

*3.4. Eosinophil derived neurotoxin.* Eosinophil derived neurotoxin is a member of the RNase, a multigene family localized to the matrix of the specific granules, sharing a large amino acid homology with ECP, 70% for the 'pre' form of both.[30,31] Eosinophil derived neurotoxin, however, is a more potent RNase than ECP.[32] It is an 18.5 kDa, single chain polypeptide,[30,35] and because of a relatively lower number of arginine residues in the protein, is up to 100 times more acidic than either human MBP or ECP. This fundamental difference may also cause its reduced cytotoxicity.[31] The protein can be found in basophils, mononuclear cells and possibly neutrophils, and may be secreted by the liver.[33-35] Eosinophil derived neurotoxin, despite its name, is a much less potent neurotoxin than ECP, requiring concentrations of 100 times greater to induce the 'Gordon pheomenon' in experimental animals.[3] An important function of EDN is the paralysis of Schistosoma Mansoni larvae, thus preventing further infiltration of the parasite.[3]

*3.5. Charcot-leyden crystals.* These highly characteristic needle-shaped crystal structures were first described in tissue from a patient with leukemia.[36] They can also be

observed in the sputum of asthmatic patients.[37] This protein is localized from the primary granules of mature eosinophils[38] and also present in basophils in a roughly equal amount.[39,40] The crystals are colorless, hexagonal, and bi-pyramidal, 20-40 µm in length and 2-4 mm across, and are routinely found in the feces and sputum of animals with severe gastrointestinal and respiratory eosinophilia.[41] The protein is 17.4 kDa, hydrophobic and possesses lysophospholipase activity.[42-44]

*3.6. Cysteinyl leukotrienes.* The cysteinyl leukotrienes (LTs) are produced by lipoxygenation of arachadonic acid.[45] There are 4 types of leukotriene, LTB4, LTC4, LTD4, and LTE4. Eosinophils and mast cells are the main infiltrating cell types capable of producing and liberating leukotrienes in the asthmatic airway, although resident cells, such as bronchial vascular endothelium may also be induced to produce leukotrienes, by transcellular metabolism.[45] The biological effects of the LTs are diverse and profound, and include stimulation of airway smooth muscle contraction, edema of the airway wall, and stimulation of mucous secretion.[46] In bronchial challenge testing, inhaled LTs have a more prolonged bronchoconstrictor effect than metacholine or histamine[47] and occur with much smaller concentrations of agonist. Of the different types of leukotrienes, LTE4 appears to be more provocative in inducing a drop in forced expiratory volume (FEV1) compared to LTD4 and LTC4.[47] Interestingly, there is also evidence that aspirin sensitive asthmatics are even more responsive to LTE4 than other asthmatics, indicating a role for leukotrienes in the pathogenesis of aspirin-sensitive asthma.[45] Leukotrienes are detectable in the serum, bronchoalveolar lavage (BAL) fluid, and urine of asthmatic subjects in higher concentrations than normal subjects.[45] Studies of urinary leukotriene concentrations during exacerbation of asthma found no correlation with asthma severity, yet over 60% of asthmatics presenting with emergency acute asthma exacerbation had elevated urinary LTE4 compared to normal matched subjects. As a therapeutic intervention, both leukotriene receptor antagonists and leukotriene synthesis inhibitors have been used in clinically. Improvements in the lung function of chronic stable asthmatics have been demonstrated in several randomized, placebo-controlled clinical trials of LTs receptor agonist, and leukotriene synthesis inhibitors over 4-weeks and 6 months treatment periods.[45] These trials demonstrated improvements in the lung function of approximately 15% reduced bronchodilator use, fewer courses of oral corticosteroid therapy, and in one study, a higher asthma-specific quality of life score when compared with placebo. Indeed, a recent trial of Montelukast (a leukotriene receptor agonist) was found to reduce eosinophil numbers in sputum, reduce symptoms, and increase the peak expiratory flow (PEF) in asthmatic patients.[48] It is clear that the LTs play a significant role in the pathogenesis of asthmatic inflammation.

*3.7. Reactive oxygen species (ROS).* Reactive oxygen species are capable of damage to proteins, lipids and nucleic acids, and are highly reactive components of the normal inflammatory response to pathogens.[49] They are produced by activated macrophages, eosinophils, neutrophils and mast cells. Reactive oxygen species include the superoxide anion ($O2^-$), the hydroxide radical (OH), and hydrogen peroxide ($H_2O_2$). They can initiate the oxidation of arachadonic acid, creating lipid mediators such as the LTs. Oxidative stress has been shown to induce expression of the transcription factor nuclear factor kappa ß (NFκb), which leads to the increased transcription of several inducible genes such as nitric oxide synthase, $COX_2$ and several cytokines, including IL-8 and regulated on activation, normal T-cell expressed and secreted (RANTES).[49] Thus, ROS can be considered important inflammatory mediators in allergic inflammation. The physiological effects of ROS are diverse, and include bronchoconstriction, airway hyper-responsiveness (which is inhibited by antioxidants and superoxide dismutase), and epithelial cell damage indirectly by stimulating eosinophil release of EPO.[49] Evidence for the potential role of ROS in asthma pathogenesis comes from experiments showing eosinophil release of superoxide anion which is greater in asthmatics than controls.[49] Studies have associated reduced dietary intake of antioxidant vitamins with an increased risk of developing AHR and 'wheezy illness'.[50-52]

*3.8. Platelet activating factor.* Platelet-activating factor (PAF) is produced by several cells, including platelets, basophils, eosinophils and neutrophils. Although PAF was first described as a powerful stimulator of platelet aggregation, it has several effects. One of the most important is its role in the allergic and inflammatory responses. This lipid mediator is derived from hydrolysis of membrane phospholipids by phospholipase A2 to produce lyso-PAF and a subsequent acetylation by an acetyltransferase to produce active PAF. The active form can induce bronchoconstriction, airway microvascular leakage and eosinophil chemotaxis.[53] It has also been demonstrated to promote adhesion of neutrophils and eosinophils to vascular endothelial cells[54,55] and inducing the release of eosinophil peroxidase from eosinophils.[56] Alveolar macrophages and monocytes have been shown to produce TNF and IL-1, respectively, when exposed to PAF.[57,58]

*3.9. Cytokines and chemokines.* Eosinophils can produce 18 growth factors and regulatory or proinflammatory cytokines[59] including IL-2,[60] IL-4,[61]

IL-5,[62] and granulocyte macrophage colony stimulating factor (GM-CSF).[63] They also generate the chemokines eotaxin, macrophage inflammatory protein (MIP)-1α, monocyte chemotactic protein (MCP)-1 and -3 RANTES. These molecules are stored by the eosinophil in their granules for subsequent release. These eosinophil derived factors can act both in a paracrine and autocrine manner to influence on the allergic and asthmatic inflammatory response.

***4. Eosinophil cell surface receptors.*** Eosinophils express a variety of receptors on their plasma membrane, including receptors involved in adhesion. They also express receptors for cytokines, complement and immunoglobulins[64] (**Table 1**).

*4.1. Adhesion molecules.* Receptors involved in eosinophil adhesion can be divided into selectins (L-, P-, and E- selectin), Integrins (α4β1, very late antigen (VLA)-4, and α4β7, CD11a/CD18, CD11b/CD18, and CD11c/CD18) and immunoglobulin-like structures (ICAM-1/-3, PECAM).

*4.2. Cytokine receptors.* Most cytokine receptors consist of 2 or more subunits, one of which is ligand-specific. The other sub-units are associated with signal generation and transduction. Several cytokine receptors use a common signal transducer, which might explain the redundancy of the cytokine functions. Interlukin-3, IL-5 and GM-CSF share a common β chain and have their own cytokine-specific α chains. The fact that it is only expressed on eosinophils and basophils makes the IL-5 receptor is fairly specific. In contrast, receptors for IL-3 and GM-CSF may be demonstrated on many types of hematopoietic cells.[65] According to which chemokines they bind, their receptors are named CCR or CXCR. Currently, 5 CXC receptors and 8 CC receptors have been described.[66] Eosinophils, basophils and activated lymphocytes express CCR-1 and CCR-3. Regulated on

**Table 1 -** Eosinophil adhesion molecules, cytokines and chemokine receptors and their ligands.

| Molecules expressed on eosinophil | Eosinophel receptor | Ligands |
|---|---|---|
| Adhesion molecules | L-selectin | MAdCAM-1, CD34 |
| | VLA-4 (CD49d/CD29) | VCAM-1, fibronectin |
| | VLA-6 (CD49f/CD29) | Laminin |
| | CD11a/CD18 | ICAM-1, -2, -3 |
| | CD11b/CD18 | ICAM-1, fibrinogen, C3bi |
| | CD11c/CD18 | Fibrinogen, C3bi |
| | □4ß7 | MAdCAM-1, VCAM-1, fibronectin |
| | ICAM-1/-3, PECAM | PECAM-1, Av□3 |
| | Sialyl Lewis X | E-selectin |
| Cytokines and chemokins | IL-2R (CD25) | IL_2 |
| | IL-3R□+ ß chain | IL-3 |
| | IL-5R□+ ß chain | IL-5 |
| | GM-CSFR□+ ß chain | GM-CSF |
| | CXCR-1 | IL-8, GCP2 |
| | CXCR-2 | IL-8, GRO□□□, ENA-78, NAP-2 |
| | CXCR-3 | IP-10, MIG |
| | CXCR-4 | SDF-1□ |
| | CCR-1 | MIP-1□, MIP-1□, MCP-1, MCP-3, RANTES |
| | CCR-2 | MCP-1, MCP-5 |
| | CCR-4 | SDF-1□ |
| | CCR-5 | MIP-1□, MIP-1□, MCP-1, MCP-3, RANTES |
| | CCR-6 | MIP-3□ |
| | CCR-7 | MIP-3□, SLC |
| | CCR-8 | I-309 |

IL - interlukin, IFN - interferon, TGF - tumor necrosis factor, MCP-1 - monocyte chemotactic protein 1, MIP-1□ - macrophage inflammatory protein 1□, PDGF - platelet derived growth factor, VEGR - vascular endothelial growth factor, RANTES - regulated upon activation, normal T-cell expressed and secreted, GM-CSF - granulocye-monocyte colony stimulating factor, VLA - very late antigen, ICAM - intracellular adhesion molecule, VCAM - vascular adhesion molecule, BCL - B cell lymphoma, CCR - cysteine-cysteine receptor, CXCR - cysteine-X-cysteine receptor

activation normal T cell expressed and secreted, MIP-1α, and MCP-3 are examples of chemokines binding to CCR-1. Cysteine-cysteine receptor-3 represents the main receptor for eotaxin and eotaxin-2, but it also binds RANTES, MCP-2, MCP-3, and MCP-4.[66-67]

*4.3. Other cell surface receptors.* Eosinophils express apoptosis-inducing structures such as CD45, CD69, and CD95 (Fas). CD45, which commonly referred to as the 'leukocyte-common antigen', is a transmembrane protein tyrosine phosphatase (PTPase) and appears to be the dominant plasma membrane-located phosphatase found on the nucleated cells of the hemopoietic lineage.[68] Although encoded by a single gene, alternative splicing of primary messenger RNA transcripts causes CD45 to be expressed in characteristic cell type-specific patterns as a protein with differently glycosylated multiple isoforms that include CD45RA, CD45RB, and CD45RO.[69] The protein tyrosine phosphatase activity of CD45 plays an essential role in intracellular signal transduction in the immune system.[70] A member of the C-type lectin family of surface receptors, CD69 is a type II integral membrane signalling receptor known to be involved with lymphocytes as an early activation antigen.[71] It is found on stimulated T cells, B cells, and natural killer (NK) cells. CD69 gene has been mapped to the chromosome 12 p13-p12 humans, and following sequencing has been identified as a member of the Ca2+ dependent lectin superfamily.[72] Fas (APO-1/CD95) is a membrane glycoprotein belonging to the tumor necrosis factor receptor family.[73] Fas antigen is expressed on the surface of a variety of cell types, including thymocytes, T and B cells, monocyte, macrophages, keratinocytes, eosinophils, and epithelial and endothelial cells.[74-76]

***5. Eosinophil recruitment.*** Seven hours after allergen challenge during the late phase response, eosinophils increase in sputum samples of asthmatics, and this is associated with the appearance of eosinophil- basophil progenitors, and eosinophilia in peripheral blood.[77] Progenitor CD34+ cells bear the IL-5 receptor (IL-5R) with increased responsiveness to IL-5 suggesting they are primed toward the development of eosinophils.[78] It is known that IL5 is produced during the inflammatory response within lung tissue in asthma and that it acts to increase the production of eosinophils by a direct hormonal action on bone marrow.[79] That eosinophil progenitors and eosinophil growth factors IL-3, IL-5 and GM- CSF are observed within the asthmatic lung suggests the presence of some in-situ eosinophil differentiation.[80] The tissue migration of eosinophils is a one-way process, as there is no mechanism to export eosinophils once they have entered the tissues;[81,82] therefore the regulation of eosinophil migration into the lung is of crucial importance in the pathogenesis of asthma.[79,81] Local chemo-attractant factors cause the migration of eosinophils into the airways.[83] Chemotactic substances acting on eosinophils include various chemokines (MIP-1α, RANTES, eotaxin, eotaxin2, macrophage derived chemokine (MDC), MCP-2, MCP-3, MCP-4, IL-8 and IL-16), lipid mediators (LTB4 and PAF), and anaphylatoxins (C3a and c5a).[84-86] That patients with asthma have an increased eosinophil count due to increased eosinophilopoiesis and also an increased rate of release from bone marrow. Eosinophil egress from the blood vessels involves complex interactions between adhesion molecules on the eosinophils with counter ligands on endothelial cells.[87] Subsequent adhesive events include tethering and rolling on the endothelial surface, firm adhesion and transendothelial migration. Weak bonds between P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) assist the initial reversible tethering and rolling of eosinophils on the endothelium. They also assist with very late activation antigen-4 (VLA-4) through the involvement of vascular cell adhesion molecule-1 (VCAM-1). Weibel-palade bodies store preformed P selectin intracellularly until histamine and PAF cause them to relocate to the surface of the endothelium.[88] After tethering and rolling of eosinophils, activation is mediated by chemoattractants.[89] Migration of the tethered cells along the endothelium is fostered by chemoattractants. In support of this process, chemokines are deposited in a solid phase, with their concentration gradient generating activation, diapedesis, and migration into the tissue.[90] This activation causes up-regulation of β2-integrins (Mac-1 and leukocyte function-associated antigen-1 LFA-1) and β1-integrin (VLA-4). Whilst β2-integrins bind to ICAM-1 on the endothelium, β1-integrin binds to VCAM-1, and this causes cessation of movement, that is critical for transmigration.[91] The accumulation of eosinophils is not solely dependent on adhesion molecules. A number of cytokines and chemokines are implicated in the selective accumulation of eosinophils in the asthmatic airway.[92] Interlukin-4 and IL-13 induce expression of VCAM-1, whereas TNF-α and IL-1 induce expression of ICAM-1 on the surface of endothelial cells.[93-95] Interluken-13 has been found in BAL following allergen provocation of asthmatic subjects, which strongly correlated with the increase in eosinophil numbers,[96] and mRNA expression for IL-13 was detected in bronchial biopsies from both allergic and non-allergic asthmatic subjects.[97] Transient activation of VLA-4 by RANTES increasing the adhesiveness of the eosinophils to VCAM-1. However, MCP-3 stimulation results in a conformational change of Mac-1, which causes an increase in ICAM-1 adhesion.[98] Binding of the CC chemokines (eotaxin, eotaxin2, RANTES and MCP-3) to their G-protein-coupled receptors generates polymerization, a process

controlled by calcium influx. Breakdown of actin leads to the formation and retraction of lamellipodia, which assist movement of the migrating cells.[99] Migration across the endothelium further requires the presence of matrix metalloprotease-9 (MMP-9), which degrades type IV collagen, entactin, proteoglycans, and elastin, and thus allowing eosinophil penetration through the basement membrane.[100] The precursor form of MMP-9 is highly expressed in eosinophils, and enzyme activation is triggered whenever eosinophils adhere to endothelial or epithelial cells.[101] The high availbility of this enzyme combined with its ability to degrade epithelial adhesion molecules, epithelial basement membrane collagen and proteoglycans gives it a central responsibility in the remodeling of airways.[102] Once they have migrated through the endothelium, eosinophils encounter extracellular matrix (ECM) proteins, which seem likely in playing an important part in the regulation of eosinophil activation.[103]

***6. Role of IL-5 in allergic eosinophilic disease.*** Interlukin-5 is produced by a number of cell types, and is essential for the production, maturation, accumulation, activation and persistence of eosinophils.[104] As a result of its efficacy and selectivity, IL-5 appeared to be an ideal drug development target for inhibition to blunt or prevent the eosinophil toxicity in the asthmatic lung. Several animal models of asthma provided good evidence that inhibiting the actions of IL-5 using specific monoclonal antibodies can reduce blood and bronchoalveolar eosinophilia caused by allergic challenge or chronic diseases. Thus, by unilaterally inhibiting IL-5 it could reasonably be presumed and we could suppress at least one of putative causes of asthma, namely tissue damage secondary to eosinophil accumulation during airways inflammation.[105] However, several clinical trials with humanized antibodies against IL-5 have reported negative clinical outcomes following treatment of asthmatic patients. The first study was randomized, double-blinded, placebo-controlled, parallel-group designed to validate the safety and activity of the humanized anti-IL-5 mAb mepolizumab (SB240563).[106] The authors concluded that treatment with anti-IL-5 mAb significantly reduced blood eosinophils and sputum eosinophils for at least 4 weeks but did not have any significant effect on allergen challenge and questioned the role of eosinophils in this process.[107] The validity of patient selection in this study has also been questioned as the subjects had mild asthma that, if treated with GCs rather than mepolizumab, would likely have resulted in the same negative clinical outcome.[108] Recently, a clinical trial of a humanized anti-IL-5 mAb mepolizumab in mild asthma was carried out.[109] The antibody is shown to have a potential to inhibit the increases in the numbers of airway and bone marrow eosinophils, but asthmatic symptoms in patients was not affected. Kips et al[110] have examined the lung biopsy samples from the treatment group contained intact tissue eosinophils with large quantities of eosinophil granule proteins and found that there is no clinical benefit following mepolizumab treatment. Similar results were reported with the humanized anti-IL-5 mAb reslizumab (SCH55700) in patients with severe asthma who had not been controlled by inhaled corticosteroid use. These authors reported profound reduction in circulating eosinophils, but no significant improvement in clinical measures of asthma. An alternative to humanized anti-IL-5 mAb is the use of molecular modelling of the IL-5 receptor $\alpha$-chain to develop specific receptor antagonists. Recently such a compound (YM-90709) has been shown to be a relatively selective inhibitor of the IL-5R.[111] However, it has been shown that targeting IL-5 does not improve established asthma; a result observed in a number of animal models of asthma. It has been suggested that the disappointing clinical outcomes with humanized anti-IL-5 mAb casts doubt on the important of the eosinophil as a key effector cell in asthma. However, these opinions can neither be used to support nor refute the role of eosinophils in the pathogenesis of allergic asthma. Furthermore, asthma is a complex disorder and eosinophils are likely to be more important in some forms of asthma than others. There is an increasing evidence that the eosinophil may be important in the pathophysiology of airway remodelling. For example, thickening of sub-epithelial basement membrane was associated with increase in bronchial mucosal eosinophils in severe asthmatics through the release of substances, particularly TGF$\beta$, involved in ECM protein deposition.[112] Flood-Page et al[109] demonstrated that treatment of asthmatics with mepolizumab, which specifically reduced the airway eosinophil numbers, significantly reduced the expression of tenascin, lumican, and procollagen III in the bronchial mucosal reticular basement membrane when compared with placebo. Furthermore, anti-IL-5 treatment causes a substantial reduction in the number of airway eosinophils expressing mRNA for TGF-ß1 as well as lowers the concentration of TGF-ß1 in BAL fluid.[109] The authors concluded that eosinophils might contribute to tissue remodelling processes in asthma by regulating the ECM protein deposition and anti-IL-5 mAb may prove useful in preventing this. Interestingly, a more recent study demonstrated that treatment of wild type mice with neutralizing anti-IL-5 mAb almost prevented sub-epithelial and peribronchial fibrosis suggesting that eosinophils are involved in allergen-induced sub-epithelial and peribronchial fibrosis probably by producing TGF-ß1.[113] Although anti-IL-5 mAbs are extremely effective in the long-term

reduction of eosinophils, it has not effect on airway hyper-responsivenss. This approach nonetheless merits further study in patients with symptomatic asthma. It also highlights the importance of developing more effective anti-eosinophil strategies for asthma therapy.

***7. Activation of the eosinophil.*** Eosinophils become activated with hypodense phenotype after arrival at inflammatory sites in the airways.[114] When activated, eosinophils express a variety of inflammatory-related receptors for cytokines, chemokines, immunoglobulins and complement. During this process, complement-derived anaphylatoxins C3a and C5a bind to specific cellular receptors, which activates the respiratory burst in eosinophils. C5a represents a chemo-attractant for neutrophils and eosinophils. By inducing the release of granule proteins in eosinophils, and by generating free oxygen radicals that cause damage to the airways, it represents an important metabolic activator process.[67] Secretory IgA-IL-8 complex represents a potent eosinophil chemoattractant. It stimulates degranulation. The secretory component controls its potency.[115] Interlukin-3, IL-5, GM-CSF are the cytokines that simulate eosinophilopoiesis and can activate their effector functions. They bind to a receptor with common β-chain and different α chains.[116] Interlukin-3, IL-5, GM-CSF prime the eosinophil response to chemo-attractants. They also increase degranulation, promote longer survival (by inhibition of apoptosis), and cause a greater production free oxygen radicals. Furthermore, eosinophils express the EG2 epitope (activated EG2 + eosinophils)[117] when activated by IL -5.

***8. Apoptosis.*** Apoptosis and necrosis are discrete methods of cell death. Necrosis is a chaotic, unregulated loss of membrane integrity, which results in the spillage of intracellular contents.[118] Necrosis of an eosinophil, which possesses such a potent array of cytotoxic mediators, is very harmful to the surrounding airway tissue.[118] In contrast, apoptosis is a regulated process of self-destruction program that maintains the integrity of the outer membrane, thus preventing spillage of the cellular contents.[119] Apoptosis is a complex, tightly regulated and active cellular process. The phases of apoptosis are accompanied by well-recognized morphological changes including: shrinkage of the nucleus and the collapses of cytoplasm into crescents along the nuclear envelope and blebbing of the plasma membrane. The main pathways of apoptosis are commonly referred as the intrinsic and extrinsic pathways. The intrinsic pathway of cell death centers on mitochondria. Multiple signals converge on mitochondria, including DNA damage, hypoxia and oxidative stress, causing the release of cytochrome c (cyt c) from mitochondrial membrane and activation of other apoptogenic proteins in the cytosol. Upon release into the cytosol, cyt c binds to apoptosis activating factor (Apaf-1) triggering its oligomerization into a heptameric complex that binds pro-caspase-9, forming a multi-protein structure known as the "apoptosome". Caspase recruitment domains (CARDs) control the binding of Apaf-1 to pro-caspase-9 via homotypic CARD-CARD binding. Thereafter, the activation of apoptosome-associated cell death protease caspase-9 generates a proteolytic cascade involving the cleaving of downstream effector proteases such as pro-caspase-3.[120-122] The extrinsic apoptotic pathway differs in that as it requires TNF family death receptors in order to trigger apoptosis. One class of TNF family receptors contains a cytosolic death domain, which allows them to interact with downstream adaptor proteins, and which links these receptors to intracellular specific caspases. After ligand binding, the TNF family receptors (such as Fas, TNF), which contain the cytosolic death domains cluster in membranes, and recruiting both a death domain (DD) and a death effector domain (DED) on FADD. The death effector domain of FADD binds to DED-containing procaspse-9 and -10, forming a death inducing signalling complex called 'DISC', and this results in caspase activation[123-125] (**Figure 1**).

***9. Eosinophil and apoptosis.*** Our knowledge of the complex mechanisms responsible for the accumulation of eosinophils is now considerable. Critical stages include enhanced differentiation and release of eosinophils from bone marrow into the circulation, and their selective accumulation in the conducting airways.[126,127] In asthma, eosinophils that accumulate in the airways have no way of returning to the circulation, therefore their death and subsequent removal by apoptosis or PCD followed by their recognition and phagocytosis by macrophages or resident cells.[127-129] In addition, the phagocytosis of necrotic eosinophils is known to induce a pro-inflammatory pattern of cytokine and mediator secretion by the macrophages, whereas phagocytosis of apoptotic eosinophils induces an anti-inflammatory profile of cytokine and mediator secretion.[130]

Eosinophils have been convincingly shown to undergo apoptosis when cultured in vitro. The classic morphological changes synonymous with apoptosis controlled by the cells program apoptotic pathways include: cytoplasmic condensation, internucleosomal cleavage of DNA by endogenous endonucleases yielding a typical 'DNA ladder', together with cell volume shrinkage-hence the term 'shrinkage necrosis' formerly used to describe apoptosis. The decrease in size of apoptotic eosinophils by as much as 60% accommodates greater uptake by phagocytes.[131]

*9.1 Induction of apoptosis via eosinophil-receptors.* Many studies have investigated the effects of membrane receptor ligation on the induction of apoptosis in

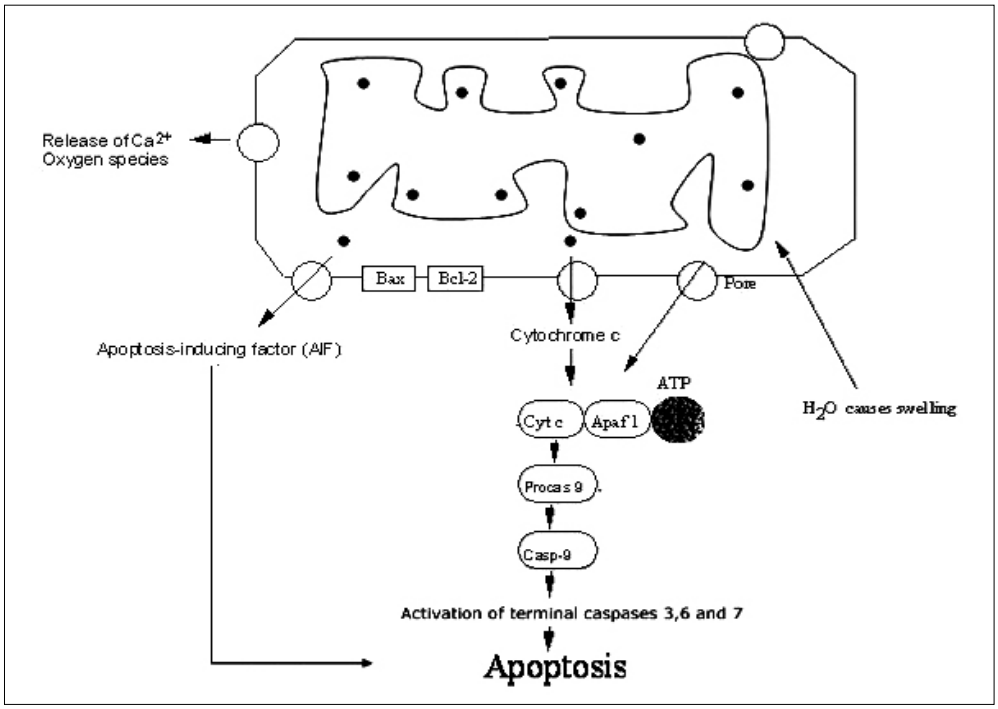


**Figure 1 -** A model showing the role of caspases in apoptosis. This figure was originally published in Saudi Medical Journal 2005; 26: 1679-1690.[173] Cyt c - cytochrome c, Apaf 1 - apoptosis activating factor, ATP - adenosine, triphosphatase.

eosinophils. Monoclonal antibody-dependent ligation of the membrane receptors CD69,[71] CD45,[132] and siglec-8[133] increased the rate of constitutive apoptosis in isolated peripheral blood eosinophils (PBE). Other membrane receptors seem to be able to differentially regulate eosinophil survival and/or apoptosis depending on the manner in which the signal is delivered to the receptor. For example, ligation of FcγRII (CD32) low-affinity IgG receptor by anti-CD32 mAb or aggregated IgG inhibited eosinophil apoptosis and prolonged survival as a result of autocrine production of GM-CSF. In contrast, if either anti-CD32 mAb or human IgG were immobilized on tissue culture plates, ligation of the FcγRII induced eosinophil apoptosis, an effect which was blocked by anti-β2 integrin (CD18) monoclonal antibodies.[134] A number of studies have demonstrated that ligation of the cell-death receptor Fas (APO-1/CD95), with either monoclonal antibody or with Fas ligand also induced eosinophil apoptosis.[127] but Fas expression is differentially regulated in human eosinophils depending on the pattern of cytokine expression in their surroundings.[135] The Th1 cytokines IFN-γ and TNF-α increase functional expression of the Fas receptor, although this effect is reversed by addition of IL-3, IL-5 or GM-CSF,[135] and increased Fas ligand-mediated apoptosis was observed in the eosinophils stimulated with IFN-γ or TNF-α.[136] These findings suggest that Fas-dependent apoptosis may be reduced in the pervading Th2 pattern of cytokine expression in the asthmatic airway.

*9.2 Induction of eosinophil apoptosis. 9.2.i Drugs.* Glucocorticoids are the foundation of anti-inflammatory therapy of asthma.[137] They cause a dramatic reduction of eosinophil numbers in vivo[138] and exert their actions either by acceleration of eosinophil apoptosis and clearance by lung macrophages,[139] or by reducing expression of the pro-inflammatory cytokines, or both. One study demonstrated that in vitro corticosteroid treatment of eosinophils in nasal polyp tissue sections increased apoptosis induction.[140] Several other commonly used asthma therapies also have effects on eosinophil apoptosis, including bronchodilator theophylline, which was shown to increase apoptosis in eosinophils cultured with IL-5,[141] and β2-agonists agonists such as salbutamol, which may block the anti-apoptotic effects of corticosteroids on eosinophils.[142] Moreover, the sulphonylureas,[143] cyclosporins A and H[144] and the local anaesthetic lidocaine[145] have also been shown to inhibit eosinophil survival by inducing apoptosis. Finally, the rationale for pursuing eosinophil apoptosis as a valid option for targeting asthmatic inflammation is strengthened by the observation that a GM-CSF receptor analogue E21R induced apoptosis in human eosinophils.[146] These findings suggest that the GM-CSF receptor may differentially regulate apoptosis and survival of eosinophils in the asthmatic airway.

**Table 2 -** Eosinophil survival and apoptosis signals.

| Survival | Apoptosis |
|---|---|
| Interlukin-3 | Glucocorticoids |
| Interlukin-5 | Bax |
| Interlukin-9 | Bcl-Xs |
| GM-CSF | Fas (CD95) |
| Bcl-XL | CD45 |
| Bcl-2 | Genistein |
| Mcl-1 | Staurosporin |
| Nitric oxide | Sulphonylureas |
| Interferon-□ | Cyclosporins A and H |
| Prostaglandin E2 | Theophylline |
| Interlukin-13 | Lidocaine |
| Interlukin-15 | TGF-ß |

TGF - tumor necrosis factor, GM-CSF - granulocye-monocyte colony stimulating factor, Bcl - B cell lymphoma, Mcl - myeloid cell leukemia-1

*9.2.ii Other factors.* The cytokine IL-4 was shown to enhance the constitutive rate of apoptosis in eosinophils cultured with IL-3, IL-5 and GM-CSF.[147] Interlukin-4 is a key Th2 cytokine, which stimulates the class switch in B cells from IgG to IgE to promote allergic inflammation. It is thought to be involved in a negative feedback mechanism to reduce allergic inflammation, and evidence for this comes from a study in which allergen challenge of mildly asthmatic subjects resulted in a reduction in IL-4 positive cells in inflamed airway tissue.[148,149] Transforming growth factor (TGF)-β, a pleiotropic cytokine that has a number of inhibitory effects on pro-inflammatory cells, induced apoptosis and loss of viability in eosinophils cultured with IL-3, IL-5, or GM-CSF. Higher concentrations of IL-5 overcame the effect of TGF-β suggesting that in vivo, a balance of the effect of cytokines might exist with TGF-ß acting by opposing the viability-enhancing effect of cytokines IL-5.[150] Moreover, nitric oxide (NO) was found to disrupt Fas receptor-mediated eosinophil apoptosis,[151] and asthmatics have higher levels of NO in their exhaled air than non-asthmatics.[152] Nitric oxide production by eosinophils may also increase expression of the Th2 cytokines, thus inhibiting apoptosis.[148]

***10. Eosinophil persistence.*** It has been appreciated for many years that culture of eosinophils in vitro with IL-3, IL-5, and GM-CSF enhances their survival for up to 2 weeks by inhibition of apoptosis. In addition, IL-13 has been shown to enhance eosinophil survival[153,154] especially when in combination with TNF-ß.[155] Recently, eosinophil viability enhancing effects have been reported for IL-9[156] and IL-15.[157] These cytokines are present in higher concentrations in the asthmatic airway tissue.[158] The significance of eosinophil persistence, or delayed apoptosis, in asthmatic inflammation is demonstrated by the finding that PBE from patients with atopic dermatitis survived for longer than eosinophils from normal controls.[159] Vignola et al[160] have found that tissue production of GM-CSF was greater in patients with asthma as compared to controls and those with chronic bronchitis. This production correlated with both the frequency of non-apoptotic eosinophils and macrophages and the severity of asthma. Eosinophils also exhibit autocrine production of viability-enhancing cytokines GM-CSF, IL-3 and IL-5 through interactions with the proteins of the extracellular matrix.[81] More recently, platelets have been shown to delay apoptosis in eosinophils through the release of GM-CSF.[161] Moreover, other resident lung cells including mast cells,[162] or IL-1β–stimulated airway smooth muscle cells[163] elaborate GM-CSF, which in turn enhances eosinophil survival, while upper airway tissue eosinophils isolated from nasal polyps show enhanced survival when cultured in vitro compared with peripheral blood eosinophils.[164] Furthermore, ligation of membrane receptor CD40, which is expressed by eosinophils, has been shown to enhance eosinophil survival as a consequence of autocrine GM-CSF release.[165] This study also established that tissue eosinophils resident in nasal polyp tissue had high expression of CD40.[166] The ligand for CD40, CD40L, is expressed by CD4+ T cells, which are also present in nasal polyp tissue, which suggests an intriguing potential for a further relationship between eosinophils and T cells (**Table 2**).

*10.1 The regulation of eosinophil apoptosis by Bcl-2 family proteins.* The Bcl-2 family is a large family of proteins that regulates apoptosis and caspase activation in many cellular systems. The family consists of both pro- and anti-apoptotic proteins. Bcl-2 and Bcl-xL inhibit cell death, whereas other members, such as Bax, Bad and Bcl-xs, promote apoptosis. There are several studies that utilised reverse transcription polymerase chain reaction (RT-PCR), immunoblotting, immunochemistry and flow cytometry demonstrating constitutive expression of Bcl-2[167,168] or Bax and Bcl-x[169] whereas a decrease in Bcl-xL messenger RNA and protein levels was found to be associated with eosinophil apoptosis. As mentioned above, ligation of CD69 mAb induces apoptosis in human eosinophils and this appears to be dependent on a Bcl-2-dependent death signal.[170] These studies are all increasing our knowledge of the intracellular mechanisms that regulate eosinophil survival or apoptosis-induction in the asthmatic lung **Table 2**.

*10.2 Eosinophil apoptosis in vivo.* Despite the mass of in vitro evidence regarding the potential role of eosinophil apoptosis, there are surprisingly few in vivo studies available of eosinophil apoptosis in asthma. Corticosteroid treatment of asthmatic patients not only

generates clinical improvement but apoptotic eosinophils can also be detected in their (induced) sputum, as well as alveolar macrophages which exhibit evidence of eosinophil engulfment.[137] Duncan et al[171] demonstrated that reduced apoptosis in eosinophils present in induced sputum significantly correlates with increased asthma severity as defined by airflow obstruction and symptom scores. Jang et al[172] demonstrated that apoptotic eosinophils were inversely correlated with severity of FEV1/FVC ratio, but estimated apoptosis by bcl-2 expression, the role of which is controversial in eosinophil apoptosis. In a study involving bronchial biopsies, Druilhe et al[168] showed that steroid-treated asthmatics had lower eosinophil counts than untreated asthmatics, and augmented apoptotic eosinophils with greater expression of Bcl-2, Fas and epithelial cell Fas-ligand. Thus, steroid treatment of asthmatic patients induces eosinophil apoptosis not only by a direct effect but also via enhancement of the expression of Fas ligand by epithelial cells, while enhanced expression of Bcl-2 might contribute to survival of the epithelium. In another study involving bronchial biopsy specimens, Vignola et al[160] demonstrated that the numbers of apoptotic eosinophils in bronchial biopsies inversely correlated with asthma severity as measured by the Aas chronic asthma grading score. They also found that GM-CSF levels correlated with asthma, which correlated with lower levels of apoptotic eosinophils. Taken together, these observations provide evidence that apoptosis induction in eosinophils and their subsequent phagocytic removal is a rational avenue for development of novel therapies for asthma.

In conclusion, selective eosinophil accumulation in the tissues of the lung, with activation and release of their potent pro-inflammatory arsenal of granule-derived basic proteins, mediators, cytokines and chemokines, is characteristic of asthmatic inflammation. A greater understanding of the intracellular events controlling mAb-dependent apoptosis induction in CE or differentiated EoL-1 may yield potential avenues of research for the development of more targeted and effective therapy for asthma and allergy.

## References


1. Simon HU. Molecules involved in the regulation of eosinophil apoptosis. ***Chem Immunol Allergy*** 2006; 91: 49-58.
2. Walsh GM. Human eosinophils: their accumulation, activation and fate. ***Br J Haematol*** 1997; 97: 701-709.
3. Venge P. Eosinophils. Asthma: Basic Mechanisms and Clinical Management. In: Barnes PJ, Rodger IW, Thomson NC, editors. San Diego (CA): Academic Press; 1998. p. 1-33.
4. Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. ***Annu Rev Med*** 1993; 44: 85-101.
5. Spry CJF. Eosinophils: A comprehensive review and guide to the scientific and medical literature. Oxford: Oxford University Press; 1998.
6. Denburg JA. The origins of basophils and eosinophils in allergic inflammation. ***J Allergy Clin Immunol*** 1998; 102: S74-S76.
7. Weller PF. Eosinophils: structure and functions. ***Curr Opin Immunol*** 1994: 6: 85-90.
8. Walsh GM. Eosinophil granule proteins and their role in disease. ***Curr Opinion Hematol*** 2001: 8: 28-33.
9. Barker RL, Gleich GJ, Pease LR. Acidic precursor revealed in human eosinophil granule major basic protein cDNA. ***J Exp Med*** 1988: 168: 1493-1498.
10. Wasmoen TL, Bell MP, Loegering DA, Gleich GJ, Prendergast FG, McKean DJ. Biochemical and amino acid sequence of human eosinophil granule protein MBP. ***J Biol Chem*** 1988: 263: 12559-12563.
11. Gleich GJ, Loegering DA, Maldonado JE. Identification of a major basic protein in guinea pig eosinophil granules. ***J Exp Med*** 1973: 137: 1459-1471.
12. Gleich GJ, Loegering DA, Mann KG, Maldonado JE. Comparative properties of the Charcot-Leyden crystal protein and the major basic protein from human eosinophils. ***J Clin Invest*** 1976: 57: 633-640.
13. Plager DA, Stuart S, Gleich GJ. Human eosinophil granule major basic protein and its novel homolog. ***Allergy*** 1998; 53 (45 Suppl): 33-40.
14. Fryer AD, Adamko DJ. Effects of inflammatory cells on neuronal M2 muscarinic receptor function in the lung. ***Life Sciences*** 1999: 64: 449-455.
15. Olsson I, Venge P. Cationic proteins of human granulocytes. I. Isolation of the cationic proteins of the granules of the leukemic myeloid cells. ***Scand J Heamatol*** 1972: 9: 204-214.
16. Rosenberg HF, Dyer KD, Tiffany HL, Gonzalez M. Rapid evolution of a unique family of primate ribonuclease genes. ***Nature Genetics*** 1995: 10: 219-223.
17. Venge P, Bystrom J. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. ***Clin Exp Allergy*** 1999: 29: 1172-1186.
18. In't Veen JC, Smits HH. Lung function and sputum characteristics of patients with severe asthma during an induced exacerbation by double-blind steroid withdrawal. ***Am J Respir Crit Care Med*** 1999: 160: 93-99.
19. Björk A, Venge P, Peterson CG. Measurements of ECP in serum and the impact of plasma coagulation. ***Allergy*** 2000; 55: 442-448.
20. Niimi A, Matsumoto H. Serum measurement of eosinophil cationic protein in the management of asthma. ***Curr Opin Pul Med*** 1999: 5: 111-117.
21. Carlson MG, Peterson CG, Venge P. Human eosinophil peroxidase: purification and characterization. ***J Immunol*** 1985; 134: 1875-1879.
22. Ten RM, Pease LR, McKean DJ, Bell MP, Gleich GJ. Molecular cloning of the human eosinophil peroxidase. Evidence for the existence of a peroxidase multigene family. ***J Exp Med*** 1989; 169: 1757-1769.
23. Egesten A, Alumets J, von Mecklenburg C, Palmegren M, Olsson I. Localization of eosinophil cationic protein, major basic protein, and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. ***J Histochem Cytochem*** 1986; 34: 1399-1403.
24. Olsson I, Egesten A, Gullberg U, Lantz M, Strömberg K, Winqvist I. The biosynthesis of neutrophil and eosinophil granule proteins. ***Folia Histochem Cytobiol*** 1986; 24: 89-97.
25. Yoshikawa S, Kayes SG, Parker JC. Eosinophils increase lung microvascular permeability via the peroxidase-hydrogen peroxide-halide system. Bronchoconstriction and vasoconstriction unaffected by eosinophil peroxidase inhibition. ***Am Rev Respir Dis*** 1993; 147: 914-920.

26. Slungaard A, Mahoney JR. Thiocyanate is the major substrate for eosinophil peroxidase in physiologic fluids. Implications for cytotoxicity. Thiocyanate is the major substrate for eosinophil peroxidase in physiologic fluids. Implications for cytotoxicity. ***J Biol Chem*** 1991; 266: 4903-4910
27. Bujak JS, Root RK. The role of peroxidase in the bactericidal activity of human blood eosinophils. ***Blood*** 1974: 43: 727-736.
28. Jong EC, Henderson WR, Klebanoff SJ. Bactericidal activity of eosinophil peroxidase. ***J Immunol*** 1980: 124: 1378-1382.
29. Wu W, Chen Y, d'Avignon A, Hazen SL. 3-Bromotyrosine and 3,5-dibromotyrosine are major products of protein oxidation by eosinophil peroxidase: potential markers for eosinophil-dependent tissue injury in vivo. ***Biochemistry*** 1999; 38: 3538-3548.
30. Gleich GJ, Loegering DA, Bell MP, Checkel JL, Ackerman SJ, McKean DJ. Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein: Homology with ribonuclease. ***Proc Natl Acad Sci USA*** 1986: 83: 3146-3150.
31. Barker RL, Loegering DA, Ten RM, Hamann KJ, Pease LR, Gleich GJ. Eosinophil cationic protein cDNA. Comparison with other toxic cationic proteins and ribonucleases. ***J Immunol*** 1989: 143: 952-955.
32. Gullberg U, Widegren B, Arnason U, Egesten A, Olsson I. The cytotoxic eosinophil cationic protein (ECP) has ribonuclease activity. ***Biochem Biophys Res Commun*** 1986: 139: 1239-1242.
33. Rosenberg HF, Tenen DG, Ackerman SJ. Molecular cloning of the human eosinophil-derived neurotoxin: a member of the ribonuclease gene family. ***Proc Natl Acad Sci USA*** 1989: 86: 4460-4464.
34. Ten RM, Butterfield JH, Kita H, Weiler DA, Fischkoff S, Ishizaka T, et al. Eosinophil differentiation of human umbilical cord mononuclear cells and prolonged survival of mature eosinophils by murine EL-4 thymoma cell conditioned medium. ***Cytokine*** 1991: 3: 350-359.
35. Wilde CG, Snable JL, Scott RW, Loegering DA, Gleich GJ. Identification of eosinophil-derived neurotoxin as the major protein constituent of human basophil granules (Abstract). ***FASEB J*** 1992: 6: A1722.
36. Charcot JM, Robin C. Observation de leucocythemie. ***CR Mem Soc Biol*** 1953: 5: 454-450.
37. Carson HJ, Buschmann RJ, Weisz-Carrington P, Choi YS.Identification of Charcot-Leyden crystals by electron microscopy. ***Ultrastruct Pathol*** 1992: 16: 403-411.
38. Dvorak AM, Letourneau L, Login GR, Weller PF, Ackerman SJ. Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to a distinct crystalloid-free granule population in mature human eosinophils. ***Blood*** 1988: 72: 150-158.
39. Ackerman SJ, Weil GJ, Gleich GJ. Formation of charcot-leyden crystals by human basophils. ***J Exp Med*** 1982: 155: 1597-1609.
40. Tanabe K, Takahashi K, Maeda M, Kimura I. Formation of Charcot-Leyden crystals by human basophils in sputum and peripheral blood. ***Acta Med Okayama*** 1993: 47: 85-90.
41. Zucker F. Eosinophil structure and maturation. In: Mahmoud AAF, Austen KF, editors. The Eosinophil in Health and Disease. New York, (NY): Grune and Stratton; 1980. p. 43-60.
42. Weller PF, Goetzl EJ, Austen KF. Identification of human eosinophil lysophospholipase as the constituent of Charcot-Leyden crystals. ***Proc Natl Acad Sci USA*** 1980: 77: 7440-7443.
43. Weller PF, Bach DS, Austen KF. Biochemical characterization of human eosinophil Charcot-Leyden crystal protein (lysophospholipase). ***J Biol Chem*** 1984: 259: 15100-15105.
44. Weller PF, Bach D, Austen KF. Human eosinophil lysophospholipase: The sole protein component of Charcot-Leyden crystals. ***J Immunol*** 1982: 128:1346-1349.
45. Drazen JM. Cysteinyl Leukotrienes. In: Barnes PJ , Rodger IW, Thomson NC, editors. Asthma: Basic Mechanisms and Clinical Management. New York (NY): San Diego, Academic Press: 1998. p. 281-290.
46. O'Byrne PM. Why does airway inflammation persist? Is it leukotrienes? ***Am J Respir Crit Care Med*** 2000: 161: S186-187.
47. Schmidt D, Rabe KF. The role of leukotrienes in the regulation of tone and responsiveness in isolated human airways. ***Am J Respir Crit Care Med*** 2000: 161: 62-67.
48. Pizzichini E, Leff JA, Reiss TF, Hendeles L, Boulet LP, Wei LX, et al. Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. ***Eur Respir J*** 1999: 14: 12-18.
49. Barnes PJ, Chung KF. Other mediators of asthma. In: Barnes PJ, Rodger IW, Thomson NC, editors. Asthma: Basic Mechanisms and Clinical Management. New York (NY): San Diego, Academic Press: 1998: p. 343-357.
50. Greene LS. Asthma, oxidant stress, and diet. ***Nutrition*** 1999: 15: 899-907.
51. Fogarty A, Britton J. The role of diet in the aetiology of asthma. ***Clin Exp Allergy*** 2000; 30: 615-627. Erratum in: Clin Exp Allergy 2000; 30: 1334.
52. Hijazi N, Abalkhail B, Seaton A. Diet and childhood asthma in a society in transition: a study in urban and rural Saudi Arabia. ***Thorax*** 2000; 55: 775-779.
53. Chung KF. Platelet-activating factor in inflammation and pulmonary disorders. ***Clin Sci (Lond)*** 1992; 83: 127-138.
54. Garcia JG, Azghani A, Callahan KS, Johnson AR. Effect of platelet activating factor on leukocyte-endothelial cell interactions. ***Thromb Res*** 1988; 51: 83-96.
55. Kimani G, Tonnesen MG, Henson PM. Stimulation of eosinophil adherence to human vascular endothelial cells in vitro by platelet-activating factor. ***J Immunol*** 1988; 140: 3161-3166.
56. Kroegel C, Yukawa T, Dent G, Chanez P, Chung KF, Barnes PJ. Platelet-activating factor induces eosinophil peroxidase release from purified human eosinophils. ***Immunology*** 1988; 64: 559-561.
57. Dubois C, Bissonnette E, Rola-Pleszczynski M. Platelet-activating factor (PAF) enhances tumor necrosis factor production by alveolar macrophages. Prevention by PAF receptor antagonists and lipoxygenase inhibitors. ***J Immunol*** 1989; 143: 964-970
58. Bonavida B, Mencia-Huerta JM, Braquet P. Effect of platelet-activating factor on monocyte activation and production of tumor necrosis factor. ***Int Arch Allergy Appl Immunol*** 1989; 88: 157-160.
59. Lacy P, Moqbel R. Immune effector functions of eosinophils in allergic airway inflammation. ***Curr Opin Allergy Clin Immunol*** 2001; 1: 79-84.
60. Levi-Schaffer F, Barkans J, Newman TM, Ying S, Wakelin M, Hohenstein R, et al. Identification of interleukin-2 in human peripheral blood eosinophils. ***Immunology*** 1996; 87: 155-161.
61. Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Wakelin M, et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. ***J Immunol*** 1995; 155: 4939-4947.

62. Desreumaux P, Janin A, Colombel JF, Prin L, Plumas J, Emilie D, et al. Interleukin 5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. ***J Exp Med*** 1992; 175: 293-296.
63. Moqbel R, Hamid Q, Ying S, Barkans J, Hartnell A, Tsicopoulos A, et al. Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. ***J Exp Med*** 1991; 174: 749-752.
64. Tachimoto H, Bochner BS. The surface phenotype of human eosinophils. ***Chem Immunol*** 2000; 76: 45-62.
65. Miyajima A, Mui AL, Ogorochi T, Sakamaki K. Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. ***Blood*** 1993; 82: 1960-1974.
66. Murphy PM. Chemokine receptor cloning. ***Methods Mol Biol*** 2000; 138: 89-98.
67. Elsner J, Kapp A. Regulation and modulation of eosinophil effector functions. ***Allergy*** 1999; 54: 15-26.
68. Thomas ML. The leukocyte common antigen family. ***Annu Rev Immunol*** 1989; 7: 339-369.
69. Pulido R, Sánchez-Madrid F. Glycosylation of CD45: carbohydrate composition and its role in acquisition of CD45R0 and CD45RB T cell maturation-related antigen specificities during biosynthesis. ***Eur J Immunol*** 1990; 20: 2667-2671.
70. Clark EA, Ledbetter JA. Leukocyte cell surface enzymology: CD45 (LCA, T200) is a protein tyrosine phosphatase. ***Immunol Today*** 1989; 10: 225-228.
71. Walsh GM, Williamson ML, Symon FA, Willars GB, Wardlaw AJ. Ligation of CD69 induces apoptosis and cell death in human eosinophils cultured with granulocyte-macrophage colony-stimulating factor. ***Blood*** 1996; 87: 2815-2821.
72. Schnittger S, Hamann J, Dannenberg C, Fiebig H, Strauss M, Fonatsch C. Regional sublocalization of the human CD69 gene to chromosome bands 12p12.3-p13.2, the predicted region of the human natural killer cell gene complex. ***Eur J Immunol*** 1993; 23: 2711-2713.
73. Cosman D. A family of ligands for the TNF receptor superfamily. ***Stem Cells*** 1994; 12: 440-455.
74. Krammer PH, Dhein J, Walczak H, Behrmann I, Mariani S, Matiba B, et al. The role of APO-1-mediated apoptosis in the immune system. ***Immunol Rev*** 1994; 142: 175-191.
75. Matsue H, Kobayashi H, Hosokawa T, Akitaya T, Ohkawara A. Keratinocytes constitutively express the Fas antigen that mediates apoptosis in IFN gamma-treated cultured keratinocytes. ***Arch Dermatol Res*** 1995; 287: 315-320.
76. Druilhe A, Cai Z, Hailé S, Chouaib S, Pretolani M. Fas-mediated apoptosis in cultured human eosinophils. ***Blood*** 1996; 87: 2822-2830.
77. O'Byrne P. Asthma pathogenesis and allergen-induced late responses. ***J Allergy Clin Immunol*** 1998; 102: S85-S89.
78. Hamid QA, Minshall EM. Molecular pathology of allergic disease: I: lower airway disease. ***J Allergy Clin Immunol*** 2000; 105 (1 Pt 1): 20-36.
79. Wardlaw AJ. Molecular basis for selective eosinophil trafficking in asthma: A multistep paradigm. ***J Allergy Clin Immunol*** 1999; 104: 917-926.
80. Christodoulopoulos P, Cameron L, Durham S, Hamid Q. Molecular pathology of allergic disease. II: Upper airway disease. ***J Allergy Clin Immunol*** 2000; 105 (2 Pt 1): 211-223.
81. Walsh GM, Wardlaw AJ. Eosinophil interactions with extracellular matrix proteins: effects on eosinophil function and cytokine production. In: Bochner BS, editor. Adhesion Molecules in Allergic Disease. New York: Marcel Dekker, Inc; 1997. p. 187-200.
82. Walsh GM, Sexton DW, Blaylock MG, Convery CM. Resting and cytokine-stimulated human small airway epithelial cells recognize and engulf apoptotic eosinophils. ***Blood*** 1999; 94: 2827-2835.
83. Venge P. The eosinophil granulocyte in allergic inflammation. ***Pediatr Allergy Immunol*** 1993; 4 (4 Suppl): 19-24.
84. Seminario MC, Gleich GJ. The role of eosinophils in the pathogenesis of asthma. ***Curr Opin Immunol*** 1994; 6: 860-864.
85. Gutierrez-Ramos JC, Lloyd C, Gonzalo JA. Eotaxin: from an eosinophilic chemokine to a major regulator of allergic reactions. ***Immunol Today*** 1999; 20: 500-504.
86. Teran LM. CCL chemokines and asthma. ***Immunol Today*** 2000; 21: 235-242.
87. Bochner BS. Cellular adhesion and its antagonism. ***J Allergy Clin Immunol*** 1997; 100: 581-585.
88. Howarth PH. What is the nature of asthma and where are the therapeutic targets? ***Respir Med*** 1997; 91 Suppl A: 2-8.
89. O'byrne PM, Postma DS. The many faces of airway inflammation. Asthma and chronic obstructive pulmonary disease. Asthma Research Group. ***Am J Respir Crit Care Med*** 1999; 159 (5 Pt 2): S41-S63.
90. Brandtzaeg P, Farstad IN, Haraldsen G. Regional specialization in the mucosal immune system: primed cells do not always home along the same track. ***Immunol Today*** 1999; 20: 267-77.
91. Rothenberg ME. Eosinophilia. ***N Engl J Med*** 1998; 338: 1592-1600.
92. Riffo-Vasquez Y, Pitchford S, Spina D. Cytokines in airway inflammation. ***Int J Biochem Cell Biol*** 2000; 32: 833-853.
93. Stanciu LA, Djukanovic R. The role of ICAM-1 on T-cells in the pathogenesis of asthma. ***Eur Respir J*** 1998; 11: 949-957.
94. Holgate ST. The cellular and mediator basis of asthma in relation to natural history. ***Lancet*** 1997; 350 Suppl 2: SII5-SII9.
95. de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. ***J Allergy Clin Immunol*** 1998; 102: 165-169.
96. Kroegel C, Julius P, Matthys H, Virchow JC Jr, Luttmann W. Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. ***Eur Respir J*** 1996; 9: 899-904.
97. Humbert M, Durham SR, Kimmitt P, Powell N, Assoufi B, Pfister R, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. ***J Allergy Clin Immunol*** 1997; 99: 657-665.
98. Nickel R, Beck LA, Stellato C, Schleimer RP. Chemokines and allergic disease. ***J Allergy Clin Immunol*** 1999; 104 (4 Pt 1): 723-742.
99. Alam R. Chemokines in allergic inflammation. ***J Allergy Clin Immunol*** 1997; 99: 273-277.
100. Mautino G, Capony F, Bousquet J. Balance in asthma between matrix metalloproteinases and their inhibitors. ***J Allergy Clin Immunol*** 1999: 104: 530-533. Review.
101. Ohno J, Ohtani H, Nitta Y, Suzuki J, Hoshi H, Honma M, et al. Eosinophils as a source of matrix metalloproteinase 9 in asthmatic airway inflammation. ***Am J Respir Cell Mol Biol*** 1997: 16: 212-219.
102. Holgate ST. The inflammation-repair cycle in asthma: the pivotal role of the airway epithelium. ***Clin Exp Allergy*** 1998: 28 Suppl 5: 97-103.
103. Bjornsdottir US, Cypcar DM. Asthma: an inflammatory mediator soup. ***Allergy*** 1999: 54 Suppl 49: 55-61.
104. Sanderson CJ, Urwin D. Interleukin-5: a drug target for allergic diseases. ***Curr Opin Investig Drugs*** 2000: 1: 435-441.

105. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. ***Respir Res*** 2001: 2: 71-79.
106. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effect of interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness and the late asthmatic response. ***Lancet*** 2000: 356: 2144-2148.
107. O'Byrne PM, Inman MD, Parameswaran K. The trials and tribulations of IL-5, eosinophils, and allergic asthma. ***J Allergy Clin Immunol*** 2001: 108: 503-508.
108. Lipworth BJ. Eosinophils and airway hyper-responsiveness. ***Lancet*** 2001: 357: 1446.
109. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. ***Am J Respir Crit Care Med*** 2003: 167: 199-204.
110. Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HA, Postma DS, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. ***Am J Respir Crit Care Med*** 2003: 167: 1655-1659.
111. Morokata T, Ida K, Yamada T. Characterization of YM-90709 as a novel antagonist which inhibits the binding of interleukin-5 to interleukin-5 receptor. ***Int Immunopharmacol*** 2002: 2: 1693-1702.
112. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, 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: 1001-1008.
113. Tanaka H, Komai M, Nagao K, Ishizaki M, Kajiwara D, Takatsu K, et al. Role of interleukin-5 and eosinophils in allergen-induced airway remodeling in mice. ***Am J Respir Cell Mol Biol*** 2004: 31: 62-68.
114. Weller PF. The immunobiology of eosinophils. ***N Engl J Med*** 1991: 324: 1110-1118.
115. Djukanovic R. Asthma: a disease of inflammation and repair. ***J Allergy Clin Immunol*** 2000: 105: S522-S526.
116. Borish L, Rossenwasser LJ. Update on cytokines. ***J Allergy Clin Immunol*** 1996: 97: 719-733.
117. Bagley CJ, Lopez AF, Vadas MA. New frontiers for IL-5. ***J Allergy Clin Immunol*** 1997: 99: 725-728.
118. Persson CG, Erjefalt JS. "Ultimate activation" of eosinophils in vivo: lysis and release of clusters of free eosinophil granules (Cfegs). ***Thorax*** 1997: 52: 569-574.
119. Haslett C. Granulocyte apoptosis and its role in the resolution and control of lung inflammation. ***Am J Respir Crit Care Med*** 1999: 160: S5-S11.
120. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. ***Cell*** 1997: 91: 479-489.
121. Vieira HL, Haouzi D, El-Hamel C, Jacotot E, Belzacq AS, Brenner C, et al. Permeabilization of the mitochondrial inner membrane during apoptosis: impact of the adenine nucleotide translocator. ***Cell Death Differ*** 2000: 7: 1146-1154. Review.
122. Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, et al. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. ***J Exp Med*** 1995: 182: 367-377.
123. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, et al. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. ***Cell*** 1996: 85: 817-827.
124. Boldin MP, Goncharov TM, Goltsev YV, Wallach D. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1 and TNF receptor induced cell death. ***Cell*** 1996: 85: 803-815.
125. Nagata S, Golstein P. The Fas death factor. ***Science*** 1995: 267: 1449-1456.
126. Leung DYM. Molecular basis of allergic disease. ***Mol Genetics Metab*** 1998: 63: 157-167. Review.
127. Walsh GM. Advances in the immunobiology of eosinophils and their role in disease. ***Crit Rev Clin Lab Sci*** 1999: 36: 453-496.
128. Cohen JJ. Apoptosis: mechanisms of life and death in the immune system. ***J Allergy Clin Immunol*** 1999: 103: 548-554.
129. Giles KM, Hart SP, Haslett C, Rossi AG, Dransfield I. An appetite for apoptotic cells? Controversies and challenges. ***Br J Haematol*** 2000: 109: 1-12.
130. Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. ***Lancet*** 1999: 354: 541-545.
131. Beauvais F, Michel L, Dubertret L. Human eosinophils in culture undergo a striking and rapid shrinkage during apoptosis. ***J Immunol*** 1992: 148: 2207-2216.
132. Blaylock MG, Sexton DW, Walsh GM. Ligation of CD45 and the isoforms CD45RA and CD45RB accelerates the rate of constitutive apoptosis in human eosinophils. ***J Allergy Clin Immunol*** 1999: 104: 1244-1250.
133. Nutku E, Aizawa H, Hudson SA, Bochner BS. Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. ***Blood*** 2003: 101: 5014-5020.
134. Kim JT, Schimming AW, Kita H. Ligation of Fc□RII (CD32) pivotally regulates survival of human eosinophils. ***J Immunol*** 1999: 162: 4253-4259.
135. Luttmann W, Opfer A, Dauer E, Foerster M, Matthys H, Eibel H, et al. Differential regulation of CD95 (Fas/APO-1) expression in human blood eosinophils. ***Eur J Immunol*** 1998: 28: 2057-2065.
136. Luttmann W, Dauer E, Schmidt S, Marx O, Hossfeld M, Matthys H, et al. Effects of interferon-gamma and tumour necrosis factor-alpha on CD95/Fas ligand-mediated apoptosis in human blood eosinophils. ***Scand J Immunol*** 2000: 51: 54-59.
137. 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: 237-243.
138. Schleimer RP. Effects of glucocorticoids on inflammatory cells relevant to their therapeutic applications in asthma. ***Am Rev Resp Dis*** 1990: 141: S59-S69. Review
139. 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: 4422-4428.
140. Saunders MW, Wheatley AH, George SJ, Lai T, Birchall MA. Do corticosteroids induce apoptosis in nasal polyp inflammatory cells? In vivo and in vitro studies. ***Laryngoscope*** 1999: 109: 785-790.
141. Rabe KF, Dent G. Theophylline and airway inflammation. ***Clin Exp Allergy*** 1998: 28 Suppl 3: 35-41. Review.
142. Nielson CP, Hadjokas NE. Beta-adrenoceptor agonists block corticosteroid inhibition in eosinophils. ***Am J Respir Crit Care Med*** 1998: 1998: 157: 184-191.
143. Bankers-Fulbright JL, Kephart GM, Loegering DA, Bradford AL, Okada S, Kita H, et al. Sulfonylureas inhibit cytokine-induced eosinophil survival and activation. ***J Immunol*** 1998: 160: 5546-5553.

144. Kitagaki K, Nagai H, Hayashi S, Totsuka T. Facilitation of apoptosis by cyclosporins A and H, but not FK506 in mouse bronchial eosinophils. ***Eur J Pharmacol*** 1997: 337: 283-289.
145. Okada S, Hagan JB, Kato M, Bankers-Fulbright JL, Hunt LW, Gleich GJ, et al. Lidocaine and its analogues inhibit IL-5-mediated survival and activation of human eosinophils. ***J Immunol*** 1998: 160: 4010-4017.
146. Iversen PO, Robinson D, Ying S, Meng Q, Kay AB, Clarke-Lewis I, et al. The GM-CSF analogue E21R induces apoptosis of normal and activated eosinophils. ***Am J Resp Crit Care Med*** 1997: 156: 1628-1632.
147. Wedi B, Raap U, Lewrick H, Kapp A. IL-4-induced apoptosis in peripheral blood eosinophils. ***J Allergy Clin Immuno***l 1998: 102: 1013-1020.
148. Walsh GM. Eosinophil apoptosis: mechanisms and clinical relevance in asthmatic and allergic inflammation. ***Br J Haematol*** 2000: 111: 61-67.
149. Nonaka M, Nonaka R, Woolley K, Adelroth E, Miura K, Okhawara Y, et al. Distinct immunohistochemical localization of IL-4 in human inflamed airway tissues. IL-4 is localised to eosinophils in vivo and is released by peripheral blood eosinophils. ***J Immunol*** 1995; 155: 3234-3244.
150. Alam R, Forsythe P, Stafford S, Fukuda Y. Transforming growth factor □ abrogates the effects of hematopoietins on eosinophils and induces their apoptosis. ***J Exp Med*** 1994: 179: 1041-1045.
151. Hebestreit H, Dibbert B, Balatti I, Braun D, Schapowal A, Blaser K, et al. Disruption of fas receptor signaling by nitric oxide in eosinophils. ***J Exp Med*** 1998: 187: 415-425.
152. Alving KE, Weitzberg E, Lundberg JM. Increased amounts of nitric oxide in exhaled air of asthmatics. ***Eur Respir J*** 1993: 6: 1268-1370.
153. Luttmann W, Knoechel B, Foerster M, Matthys H, Virchow JC Jr, Kroegel C. Activation of human eosinophils by IL-13. Induction of CD69 surface antigen, its relationship to messenger RNA expression, and promotion of cellular viability. ***J Immunol*** 1996: 157: 1678-1683.
154. Horie S, Okubo Y, Hossain M, Sato E, Nomura H, Koyama S, et al. Interleukin-13 but not interleukin-4 prolongs eosinophil survival and induces eosinophil chemotaxis. ***Internal Med*** 1997: 36: 179-185.
155. Luttmann W, Matthiesen T, Matthys H, Virchow JC Jr. Synergistic effects of interleukin-4 or interleukin-13 and tumor necrosis factor-□ on eosinophil activation in vitro. ***Am J Resp Cell Mol Biol*** 1999: 20: 474-480.
156. Gounni AS, Gregory B, Nutku E, Aris F, Latifa K, Minshall E, et al. Interleukin-9 enhances interleukin-5 receptor expression, differentiation, and survival of human eosinophils. ***Blood*** 2000: 96: 2163-2171.
157. Hoontrakoon R, Chu HW, Gardai SJ, Wenzel SE, McDonald P, Fadok VA, et al. Interleukin-15 inhibits spontaneous apoptosis in human eosinophils via autocrine production of granulocyte macrophage-colony stimulating factor and nuclear factor-kappaB activation. ***Am J Respir Cell Mol Biol*** 2002: 26: 404-412.
158. Sun XM, MacFarlane M, Zhuang J, Wolf BB, Green DR, Cohen GM. Distinct caspase cascades are initiated in receptor-mediated and chemical-induced apoptosis. ***J Biol Chem*** 1999: 274: 5053-5060.
159. Wedi B, Raap U, Lewrick H, Kapp A. Delayed eosinophil programmed cell death in vitro: A common feature of inhalant allergy and extrinsic and intrinsic atopic dermatitis. ***J Allergy Clin Immunol*** 1997: 100: 536-543.
160. Vignola AM, Chanez P, Chiappara G, Siena L, Merendino A, Reina C, et al. Evaluation of apoptosis of eosinophils, macrophages and T-lymphocytes in mucosal biopsy specimens of patients with asthma and chronic bronchitis. ***J Allergy Clin Immunol*** 1999: 103: 563-573.
161. Raiden S, Schettini J, Salamone G, Trevani A, Vermeulen M, Gamberale R, et al. Human platelets produce granulocyte-macrophage colony-stimulating factor and delay eosinophil apoptosis. ***Lab Invest*** 2003: 83: 589-598.
162. Levi-Schaffer F, Temkin V, Malamud V, Feld S, Zilberman Y. Mast cells enhance eosinophil survival in vitro: role of TNF-ß and granulocyte-macrophage colony stimulating factor. ***J Immunol*** 1998: 160: 5554-5562.
163. Hallsworth MP, Soh CPC, Twort CH, Lee TH, Hirst SJ. Cultured human airway smooth muscle cells stimulated by interleukin-1□ enhance eosinophil survival. ***Am J Resp Cell Mol Biol*** 1998: 19: 910-919.
164. Ramis I, Finotto S, Dolovich J, Marshall J, Jordana M. Increased survival of nasal polyp eosinophils. ***Immunol Lett*** 1995: 45: 219-221.
165. Yssel H, Abbal C, Pene J, Bousquet J. The role of IgE in asthma. ***Clin Exp Allergy*** 1998: Suppl 5: 104-118.
166. Ohkawara Y, Lim KG, Xing Z, Glibetic M, Nakano K, Dolovich J, et al. CD40 expression by human peripheral blood eosinophils. ***J Clin Invest*** 1996: 97: 1761-1766.
167. Ochiai K, Kagami M, Matsumura R, Tomioka H. IL-5 but not interferon-gamma inhibits eosinophil apoptosis by upregulation of Bcl-2 expression. ***Clin Exp Immunol*** 1997: 107: 198-204.
168. Druilhe A, Arock M, Le Goff L, Pretolani M. Human eosinophils express bcl-2 family proteins: modulation of Mcl-1 expression by IFN-gamma. ***Am J Respir Cell Mol Biol*** 1998: 18: 315-322.
169. Dewson G, Walsh GM, Wardlaw AJ. Expression of Bcl-2 and its homologues in human eosinophils: Modulation by interleukin-5. ***Am J Resp Cell Mol Biol*** 1999: 20: 720-728.
170. Foerster M, Haefner D, Kroegel C. Bcl-2-mediated regulation of CD69-induced apoptosis of human eosinophils: identification and characterization of a novel receptor-induced mechanism and relationship to CD95-transduced signalling. ***Scand J Immunol*** 2002: 56: 417-428.
171. Duncan CJA, Lawrie A, Blaylock MG, Douglas JG, Walsh GM. Reduced eosinophil apoptosis in induced sputum correlates with asthma severity. ***Eur Resp*** 2003: 22: 484-490.
172. Jang AS, Choi IS, Parks CS. Immunohistochemically stained activated eosinophils in sputum in patients with asthma. ***Respiration*** 2000: 67: 183-188.
173. Alenzi FQ. Apoptosis and diseases: regulation and clinical relevance. ***Saudi Med J*** 2005; 26: 1679-1690.