REVIEW

Human eosinophils as potent inflammatory cells and their apoptosis

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Abstract

A significant association was established between the eosinophil and a number of disease conditions, including helminthiasis, allergy, asthma, drug hypersensitivity, certain neoplasias, and graft rejection. Activation of eosinophils and release of proinflammatory lipid mediators, cytokines, free oxygen radicals, highly-charged cationic proteins contribute to the onset and maintenance of tissue inflammation. Eosinophil accumulation in blood and tissues has been related to a defect in their apoptotic death. Decisive events during the apoptotic process involve mitochondrial permeabilization and caspase activation. Clearance of apoptotic cells depends on the ability of phagocytes to recognize their cell targets and, subsequently, to engulf them. ***(Tab. 2, Ref. 32.)***

Key words: eosinophil activation, eosinophil-derived mediators, eosinophilia, apoptosis, inflammation.

Eosinophils were originally described in the blood as "coarse granule cells" by Wharton Jones in 1846 and the term "eosinophil" was first used by Paul Ehrlich (1879) on the basis of their strong avidity for the acid-aniline dye eosin. They are non-dividing, fully differentiated and granulated leukocytes with a diameter of approximately 8 μm and bi-lobed nucleus (Weller, 1994). Eosinophils are considered important effector cells of infections with helminthic parasites, atopic diseases such as asthma, allergic rhinitis and atopic dermatitis, drug hypersensitivity, certain neoplasias, and graft rejection. They may have a wide spectrum of biological activities, especially in inflammation and there is evidence for enhanced eosinophil differentiation and production from bone marrow, selective adherence and chemotaxis, accumulation in blood and tissue, local activation and prolonged survival (Druilhe et al, 1998; Moqbel and Lacy, 1998; Hamid and Minshall, 2000; Hamid et al, 2003).

Activation of eosinophils and their mediators

After migration through the endothelium, eosinophils come into contact with extracellular matrix proteins that are likely to play important roles in the regulation of eosinophil activation. Activated eosinophils express a number of receptors for cytokines, chemokines, immunoglobulins and complement, mediated intracellular signaling pathways (RAS-RAF-mitogen-activated protein kinases, Janus kinases-signal transducers and activators of transcription, phosphatidylinositol 3-kinase and nuclear factor-Kappa B pathways), and their clinical significance in eosinophil-related diseases (Filipovi et al, 2001; Wong et al, 2002). Cytokines, particularly interleukin (IL)-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF), are thought to regulate eosinophil priming, activation, and survival. IL-9 may potentiate in vivo eosinophil function by increasing their survival and IL-5-mediated differentiation and maturation (Wills-Karp, 2000; Gounni et al, 2000; Esnault and Malter, 2002). Woerly et al (2002) demonstrated that IL-13 is synthesized and released from CD28-stimulated eosinophils and that eosinophil-derived IL-13 is bioactive. These findings suggest that through the release of IL-13 eosinophils could induce the influx of inflammatory cells, thereby sustaining their own recruitment.

The morphology of activated eosinophils varies from that of primed cells (stimulated without undergoing active secretion with only subtle differences in organelle structures) to that of fully activated, degranulating cells. Degranulation is a crucial event in the activation of the eosinophil. Eosinophils can release their secretory granule contents by three possible mechanisms:

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Tab. 1. Eosinophil-derived mediators.

Granule cationic proteins	MBP, ECP, EDN, EPO
Eicosanoids	PGE_2, PGD_2, PGF_2, LTA_4, LTB_4, LTC_4, LTD_4, LTE_4, HPETE, HETE, TXB_2
Platelet-activating factor	PAF
Cytokines	IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-11, IL-12, IL-16, TGF-α, TGF-β , GM-CSF, TNF-α, RANTES, MIP-1α, PDGF-B
Respiratory burst product	O_2-, H_2O_2
Others	CLC protein, collagenase, arylsulfatase B, histaminase, phospholipase D, catalase

MBP — major basic protein, ECP — eosinophil cationic protein, EDN — eosinophil-derived neurotoxin, EPO — eosinophil peroxidase, PG — prostaglandin, LT — leukotriene, HPETE - hydroperoxyeicosatetraenoic acid, HETE — hydroxyeicosatetraenoic acid, TX — thromboxane, PAF — platelet-activating factor, IL — interleukin, TGF — transforming growth factor, GM-CSF - granulocyte macrophage-colony stimulating factor, TNF — tumor necrosis factor, MIP — macrophage inflammatory protein, PDGF — platelet-derived growth factor, CLC — Charcot-Leyden Crystal

1) necrosis, 2) piecemeal degranulation, and 3) compound exocytosis. *In necrosis*, many eosinophils at sites of inflammation appear disorganised, with cells displaying nuclear lysis, centralization of granules, and loss of integrity of granules and plasma membrane. Release of intact or disrupted granules into the interstitium is likely to have toxic effects on the surrounding cells. *In piecemeal degranulation*, numerous small vesicles bud off from the larger secondary granules and move to the plasma membrane for fusion, thereby causing gradual emptying of the secondary granules and move to the outside of the cell. *In regulated exocytosis*, the crystalloid granules fuse directly with the plasma membrane prior to releasing their contents to the outside of the cell. This phenomenon is regarded as the classic form of regulated secretion, which is well characterised in anaphylactic degranulation of mast cells and basophils (Moqbel and Lacy, 1999; Erjefält et al, 1999).

The specific granules of eosinophils contain potent cytotoxic proteins. Release of these proteins into the extracellular space is believed to cause a variety of tissue disturbances such as epithelial damage, extravasation of plasma, airway hyperresponsiveness and contribute to the onset and maintenance of tissue inflammation. The cytotoxic and proinflammatory mediators, such as reactive oxygen species, major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin, as well as eicosanoids, cytokines and chemokines, are released from activated eosinophils (Tab. 1) (Moqbel and Lacy, 1998; James and Nijkamp, 1999; Gounni et al, 2000; Hamid et al, 2003; Lacy et al, 2003).

Eosinophil apoptosis

Apoptosis, the programmed cell death, is an active and energy dependent physiological process that can be triggered in every normal cell, the counterpart to cell proliferation and a part of tissue and organ development. The maintenance of haematopoietic cell homeostasis is closely related to the ability of the cell and the environment to maintain an appropriate balance between proapoptotic and anti-apoptotic stimuli. Dysregulation of apoptosis can lead to pathophysiologic changes that result in either loss of cells (acquired immunodeficiency syndrome, degenerative diseases) or accumulation of cells (cancer, autoimmune and inflammatory diseases). Delayed eosinophil apoptosis has been considered an important mechanism contributing to eosinophilia, major feature of inflammatory airway diseases (asthma, rhinitis) (Druilhe et al, 1998; Haslett, 1999; Simon, 2000; Kankaanranta et al, 2000; Druilhe et al, 2003). Contrasting the absence of significant eosinophil apoptosis in the lung tissue, eosinophils are clearly eliminated by migration into the airway lumen where they do undergo apoptosis. Once in the lumen, cells will be mixed with other components of airway discharges and be finally removed by normal physiologic clearance mechanisms such as mucociliary transport or coughing. Furthermore, a substantial portion of the luminal eosinophils may die through apoptosis, that may be a major mode of removal of these cells from lung tissues (Uller et al, 2001).

Tab. 2. Characteristic of apoptosis.

Changes in cell size and granularity
Chromosome condensation
DNA fragmentation
Changes in plasma membrane permeability
Cell surface modification (externalization of phosphatidylserine)
Formation of apoptotic bodies

Several studies have identified a number of triggers of apoptosis in eosinophils – transforming growth factor (TGF)-ß, Fas, CD69 and CD30 ligation. Inhibition of eosinophil apoptosis can be achieved by at least two mechanism – increased expression of eosinophil survival factors and disruption of death signals (Walsh, 1997; Fahy et al, 1998; Luttmann et al, 2000; Matsumoto et al, 2004). Apoptosis in eosinophils is envisioned as a three-step process: 1) *premitochondrial phase* involving oxidant-mediated effects, 2) *mitochondrial phase* during which mitochondrial membrane function is lost and cytochrome c and other proapoptotic proteins are released, and 3) *postmitochondrial phase* during which these released mitochondrial proteins activate caspases, other proteases, and nucleases. The death of the cell is thought to result from the proteolytic degradation of important cellular proteins that in turn leads to the characteristic morphologic and biochemical changes of apoptotic cells, including reduction of cellular volume, chromosome condensation, and DNA fragmentation (Tab. 2) (Simon and Alam, 1999; Walsh and Isner, 2000; Ravagnan et al, 2002; Létuvé et al, 2002; Gardai et al, 2003).

Apoptosis is associated with the swift recognition of intact cells by macrophages or resident cells followed by their engulfment and degradation. The rapid phagocytosis of apoptotic eosinophils prevents local tissue injury or inflammation. Unlike necrosis which is characterized by loss of cell membrane integrity and uncontrolled release of harmful cellular contents, apoptotic cells are phagocytosed intact. The engulfment of apoptotic eosinophils exerts profound effects on the functional ability of the ingesting macrophage, inducing an anti-inflammatory cytokine and

mediator profile, i.e. TGF-ß and prostaglandin E_2. In contrast, ingestion of necrotic eosinophils induces a proinflammatory cytokine and mediator profile, i.e. release of thromboxane B_2 and GM-CSF. The apoptosis and phagocytosis can be specifically regulated pharmacologically and could be exploited to develop new therapeutic intervention for a variety of immune and inflammatory diseases. The induction of eosinophil apoptosis and subsequent clearance by phagocytic cells are regarded as new approaches for accelerating the resolution of tissue inflammation in clinical situations related to allergy (Walsh et al, 1999; Zhang et al, 2002; Druilhe et al, 2003; Erjefalt et al, 2004).

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