Review Article

APOPTOSIS: A THERAPEUTIC STRATEGY IN EOSINOPHILIA

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Abstract

Eosinophils have crucial role in allergic inflammation and asthma. Prolong survival and pathological accumulation at the site of inflammation exclusively related to destructive activity of these pathogenic granulocytes by releasing tissue toxic and inflammatory granule proteins. Any deficiency in eosinophil apoptosis may put in to condition of eosinophilia. Thus, removal of eosinophils by inducing apoptosis has been proposed as potential anti inflammatory strategy in eosinophilia. Apoptosis proves to be beneficial for development of future airway drugs. For therapeutic instances, many reviews illustrate the importance of glucocorticoids as potent anti-inflammatory agent and known to induce apoptosis in eosinophils. Apart from glucocorticoids, theophylline and histamine are well studied apoptosis inducing agents in eosinophil related inflammation. This review elaborates the anti-inflammatory characters of apoptosis in asthma and allergic inflammation.

Key words. Apoptosis, Eosinophilia, inflammation

Introduction:

Eosinophilia is a provision in which peculiarly elevated amounts of eosinophils are found in either blood or in tissues and their number exceed from 600 cells per micro liter (μ L) of blood. Generally blood does not have a large number of eosinophils. Body may produce additional amount in reaction to any allergic disorder, skin inflammation, and parasitic infections. They can also increase in response to bone marrow disorders. Condition of Eosinophilia or hyper-eosinophilia due to molecular defect or any imperfection of immunomodulators may lead to organ damage [1]. Apoptosis is an automatic cell death and indispensable procedure to sustain cell turnover and helps to uphold the dynamic condition of cellular homeostasis. Throughout the progression of apoptosis, all the active cellular contents are remained enclosed by cell

membrane since it is a typical pathway through which malfunctioning and dangerous materials are eliminated without causing impairment to the body like necrosis [2]. It has been reported that dysregulated apoptosis can escort to pathological accumulation of eosinophils in bronchial tissue grounding allergic diseases such as asthma. Blemish in apoptosis throw in to chronic tissue eosinophilia. Elimination of activated eosinophils, lymphocytes by inducing apoptosis and enhancing phagocytic removal is one of the potential remedial approaches for eosinophilia [3,4]. Apoptosis proves to be valuable for the advancement of novel therapeutic marks [3,4]. It is highly desirable to develop such drugs that specifically induce eosinophil apoptosis triggering the resolution of unwanted eosinophilic inflammatory responses.

Programmed cell death

Apoptosis is an important development to keep correct cell numbers. Kerr was the scientist who used the phrase "apoptosis" in 1972 [5]. He defined it as internal program of events and sequence of morphological changes by which cell commits suicide, cells shrink and condense ultimately split up. Prior to an absolute crumple of nucleus in eosinophils, chromatin turns into enormously condensed and the cell disintegrates into apoptotic bodies [6]. As a result small membrane bounded apoptotic fragments are commonly phagocytised without losing the cell membrane, otherwise contents of these apoptotic fragments may have injurious possessions to the adjoining tissues like necrosis. In mammals, apoptosis helps in scheming special steps of the growth which may escort to the deletion of entire structure (e.g., the tail of the developing human embryos).

Allergic inflammation due to delayed eosinophils apoptosis

Mainly accrual of eosinophils is associated with pathological stipulation in the lungs due to tardy apoptosis. Assorted transcription factors and the eosinophilopoietins like Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interleukin-3 (IL-3), and IL-5 permit the manufacturing and endurance of eosinophils [7]. Under the allergy reactions distinctive activated eosinophils roam to the affected tissues and hoard at the place of irritation and also legalize Th2 cytokines manufacturing and participate in the airway altering. These specific eosinophilopoietins up regulate segregation of eosinophils within bone marrow and prolong survival in blood and lungs for enagement [3]. Increased survival and decreased apoptosis grounds for augmentation and determination of eosinophils in

asthma [8,9,10,11]. As eosinophils enclose cytotoxic secretory products therefore have imperative ability of inducing inflammation by releasing tissue toxic and inflammatory mediators [12]. Cytotoxic agents are eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN), eosinophil peroxidase (EPO), Major basic protein (MBP), eosinophil cationic protein (ECP) and oxygen radicals [13,14].

There is an augmented proof that T cells play very crucial task in eosinophilia [15] and subgroup of idiopathic eosinophilia [16,17]. The occurrence of Th2 cells is elevated in allergic persons [18]. Triggered Th2 cells manufacture eosinophil survival Cytokines for instance IL-3, IL-5 and GM-CSF. These survival cytokines are most responsible factors for tissue eosinophilia [19,20]. Certain other factors such as tumor necrosis factor alpha (TNF- α), Interferon gamma (IFN- γ), IL-13, and complement factor C5a also regulate eosinophils apoptosis and protract continual existence in vitro [21,22].

Fig.1. Schematic diagram of apoptotic morphology This figure illustrates sequences of

apoptotic morphology during which (a) cell loses its tonicity (b) chromatin turns into very condensed appearance (c) cell shrinks and condenses (d) cell disintegrates into apoptotic bodies and finally apoptotic fragments are phagocytised.

Expression of IL- 5 in eosinophils endurance and conscription

In order to promote eosinophil mediated responses, including attraction, activation, survival at the site of inflammation and release into peripheral blood circulation IL-5 is strong stimulus [23]. IL-5 provides selective increase eosinophil accumulation [16]. Under homeostatic conditions, IL-5 is not important for eosinophils increase along with demarcation. The importance of IL-5 is obvious from allergen sensitized, IL-5 lacking mice. These mice were not capable of raising eosinophilia [24]. However, additionally IL-5 is over expressed in transgenic mice that developed eosinophilia[4].

Increased IL-5 expressions are also associated with hyper eosinophilic syndrome. In allergic diseases, IL-5 up regulates B-cell lymphoma-extra large (Bcl-xL) as well as Mcl-1, however does not amend Bcl-2, Bcl-2-associated X protein (Bax) or Bcl-xS levels in eosinophils [25]. Over expression of Bax induces eosinophil apoptosis in contrast to Bcl-2 [25]. IL-5 mediate important anti-apoptotic signaling events including, activation of tyrosine kinases and transcription comprising JAK2, Lyn, phosphatase SHP2 and Raf-1 [26,27,28,29] where JAK2 and Lyn are central for eosinophil propagation and continued existence. The IL-5 receptor includes exclusive ligand obligatory alpha-subunit which is primarily articulated by eosinophils along with b-chain which is communal with GM-CSF and IL-3 receptors and the signaling is mediated by way of JAK/STAT pathway and by activation of Phosphatidylinositol 3-kinases (PI-3K) [30]. Activation of STAT proteins and tyrosine kinases are receptive cytokine mediated signals for the activation of Bcl-2 members as well as inhibitor of apoptotic proteins (IAP) families [31,32]. Recombinant sIL-5Ra (soluble form of IL-5Ra) tightly binds with IL-5 and discourages the binding of IL-5, except IL-3

or GM-CSF to surface IL-5R so helps in survival of eosinophils. Augmented levels of sIL-5Ra in serum are also studied in eosinophilia while IL-5Ra expression is decreased [33]. Other than JAK/ STAT pathway, IL-5 activates (Ras-ERK) and other cytokines [34,35].

Fig.2. IL-5 signaling mediated by way of JAK/STAT pathway. (A) The alpha-subunit of IL-5 receptor is articulated by eosinophils along with b-chain which is communal with GM-CSF and IL-3 receptors and the signaling is mediated by way of JAK/STAT pathway and by activation of PI-3K. (B) IL-5 mediate signaling events that include, activation of tyrosine kinases and transcription comprising JAK2, Lyn, phosphatase SHP2 and Raf-1. (C) Activation of STAT proteins and tyrosine kinases are receptive cytokine mediated signals for the activation of Bcl-2 members as well as inhibitor of apoptotic proteins (IAP) families. (D) Other than JAK/ STAT pathway, IL-5 activates (Ras-ERK) and other cytokines.

Anti IL-5 therapy illustrates advantage in obvious diminution in peripheral blood eosinophilia [36,37]. Anti-IL-5 therapy of asthma patients escorts to an inhibition of eosinophil maturation within bone marrow with decline of eosinophil progenitors in bronchial mucous membrane [38]. IL-5 also increases eotaxin effect by working in synergistic manner. Eotaxin along with IL-5 contribute to the pathogenesis of asthma followed by C-C chemokine receptor type 3 (CCR3) receptor mediated response [39]. In view of the fact that both IL-5 and eotaxin rule eosinophilia so

use of virus like particles (VLP) based vaccines against IL-5 and eotaxin abrogate eosinophilia [40]. Remedial mediators targeting IL-5/IL-5R comprise humanized mAbs toward IL-5 itself are verified to be efficient in the treatment of eosinophilia [41,42].

CCR3, interleukin receptor alpha, or the transcription factor GATA-1 can be used as markers to identify eosinophils as these receptors are expressed by eosinophils. CCR3 receptor involves Eotaxin, RANTES which are important chemokines and some other chemokines and certain complement factors such as C3, C5a and Paf [43,44]. Allergic encouragement consequences in eminent ranks of serum IL-5 that increases release of eosinophils into peripheral blood circulation [45] which directs to the eosinophil progenitor's increase plus their demarcation into adult cells. Eosinophils liberated into the peripheral blood circulation and get attached to fibronectin. Fibronectin is an extracellular matrix protein. This binding is mediated by integrins and selectins on the eosinophils. Finally eosinophils are recruited to the bronchial wall and prolong survival cause inflammation to the lung.

The published data clearly demonstrate that IL-4 also provoke eosinophils apoptosis apart from IL-5, IL-3, GM-CSF.

Cytokines and chemokines such as Eotaxin and RANTES control eosinophil progenitors to differentiate and mature in bone marrow [46]. Surface inhibitory receptors like Siglec-8 also organize eosinophil growth and survival in coordination with apoptosis [1]. Introduction of eosinophils to IL-33, similar to IL-5, can result in a parallel extent of augmentation of Siglec-8-induced apoptosis. In addition to IL-5, IL-33 is recently recognized cytokine that promotes eosinophilia. They have synergistic outcomes on apoptosis. IL-33 possesses many similar characteristics to that of IL-5. IL-33 triggers airway inflammation through its receptor ST2 [47].

The eosinophils move into the peripheral blood where they act in response to chemotactic signals turned out from allergen-specific cells. Eosinophils interact with endothelium and this interaction is settled by means of Integrin VLA-4. Integrin VLA-4 promotes eosinophil activation, adhesion and transition and binding of PSGL-1 to P-selectin, where selectins and their ligands promote binding and rolling of eosinophils. IL-13 in turn increases the appearance of integrin VLA-4 along with P-selectin on eosinophils. The eosinophils tightly stick on to the endothelium and flattened on the surface. The eosinophils endure transmigration from endothelial cells to the connective tissues and interact with matrix proteins. Finally eosinophils migrate toward their eventual destination and on degranulation, release toxic mediators that disrupt cells in bronchial epithelium.

Fig.3. Recruitment and activation of eosinophils (A) Eosinophils travel into the peripheral blood and interact with endothelium by means of Integrin VLA-4 which promotes eosinophil activation, adhesion and transition and binding of PSGL-1 to P-selectin, (B) where selectins and their ligands promote binding and rolling of eosinophils. (C) IL-13 in turn increases the appearance of integrin VLA-4 along with P-selectin on eosinophils. (D) The eosinophils tightly stick on to the endothelium and flattened on the surface then migrate from endothelial cells to the connective tissues and interact with matrix proteins. (E) Finally eosinophils migrate toward their eventual destination and on degranulation, release toxic mediators that disrupt cells in bronchial epithelium.

TNF α survival factor.

TNF- α is involved in eosinophil endurance activity followed by eosinophil respiratory burst, [22] degranulation, [48] and IL8 production [49]. During inflammatory reactions TNF α is produced from monocytes, natural killer cells, lymphocytes which inhibits eosinophils apoptosis [50]. TNF R-1 (p60) and TNF-II (p80) are the receptors articulated by eosinophils and liable to mediate the effects of TNF α [51]. TNF α is capable of triggering the activation and translocation of nuclear factor kappa B (NF- κ B) as well as activator protein 1 (AP-1). AP-1 regulates gene expression of GM-CSF and enhances its production as well as liberation [52,53]. In the presence of TNF α eosinophils apoptosis can be enhanced by inhibition of NF- κ B as NF- κ B arbitrate enormous collection of proteins which are concerned in inflammatory reactions, continual existence and propagation of cells. The action of TNF α is mediated through G proteins coupled receptors, in turn commencement of ACP, kinases proteins family and phospholipases, by way of cAMP production [50].

Role of leptin in eosinophilia

Structurally Leptin resembles by GM-CSF, IL-11, IL-6, IL-12, plus IL-15 [54]. Leptin mediates proliferation by the inhibition of apoptosis in various types of cells comprising T cells [55]. Leptin synthesis is amplified in asthma and additional inflammatory ailment [56,57]. Leptin mediates its anti-apoptotic role by the activation of P13 kinase along with Mitogen activated protein kinases (MAPK) pathways. Leptin over dues Bax and Bid cleavage thus inhibits cytochrome C pathway of mitochondria by slowing down the Caspases proteins activity [58].

Apoptosis inducing factors

The trail of apoptosis is controlled by a variety of factors. Specific types of death receptors, for instance Fas/AIP-1, TNF R1, along with ICE are responsible to mediate apoptosis in broader array of cells. Bcl-2 and other inhibitor proteins transduce anti apoptotic signals in eosinophils [59]. Bcl-2 apoptotic protein regulates the intrinsic pathway and inhibits the action of caspase 3. Eosinophils articulate considerable levels of Bcl-xL as well as Bax however hinder Bcl-xS and Bcl-2 [60]. Bcl-2 down regulates apoptosis imposed via p53 along with c-myc [61]. Conversely Bax; an activator protein binds with Bcl-2 and restrains the effects of Bcl-2 and up regulates apoptosis. Eosinophils mostly express Bax at elevated range [62]. Bcl-xl and Bax are involved in the commencement of proteolytic caspase cascades. Caspase 3, caspase 8, along with 9 cascade mediate apoptosis in eosinophils [63]

Eosinophils apoptosis endorsed by Fas receptor

Fas commencement endorses eosinophils apoptosis as Fas dependant apoptosis is susceptible for eosinophils. The appointment of Fas is concerned during the amputation of infiltrating eosinophils from lungs [11,64,65]. Amongst the best characterized members of death receptors, CD95 (Fas) is exposed to encourage apoptosis in lymphocytes and mimicked by novel anti asthma drugs [11,66]. Apoptosis in eosinophils is followed by CD95 (Fas) and CD95L (FasL) ligand system that forms complex called DISC [67] and the activated T cells express these death receptors [68].

In the bronchial wall epithelium is chief site of Fas-ligand expression [8,69]. By inducing Fas stimulating monoclonal antibody, apoptotic morphology could be produced [70]. Lacking of endurance factor or inhibition of Fas trail cytokines slanted to Th-2 cells. Fas insufficiency holds up the ruling of eosinophilia in mouse model [64,71]. Fas mediated apoptosis can be

inhibited by the increase of PI3K which hampers caspase 8 cleavage by DISC [72].

Nitric oxide in inflamed lungs possesses both anti and pro eosinophilic properties. Compare to eosinophils haematopoietins, nitric oxide avoids apoptosis in eosinophils because of Fas receptor [60]. Upon Fas receptors mediated activation in chronic inflammatory response, nitric oxide as a releasing product can be significant in formative whether eosinophils stay alive or endure apoptosis. Asthma patients contain elevated level of NO in exhaled air [73]. Elevated level of NO has been recognized to be related with Fas receptor confrontation during nasal tissues cyst. Eosinophils cell death can be abolished with augmented appearance of enduring factors along with the interruption of DISC [59]. Fas ligation

stimulates two main types of intracellular apoptotic pathways[74]. In type I cells, activated CD95 (Fas) and CD95L (FasL) ligand system forms a complex called DISC [67]. DISC directs towards the enrollment of protease caspase 8 along with cleavage of caspase 3 through the activation of caspase 8. DISC evades mitochondria and grounds for apoptosis via cleavage of caspases. At the end of second kind of cells, caspase 8 is fewer triggered by DISC. DISC cleaves a pro-apoptotic Bid protein that actually depolarizes the mitochondrial membrane and allowing the release of cytochrome C in cytoplasm. Under the influence of cytochrome C apoptosome formation is facilitated by the sequential cleavage of caspase 9 and caspase 3 resulting in cell death.

Fig.4. Fas ligation motivated two main types of intracellular apoptotic pathways. (A) In type I cells, activated CD95 (Fas) and CD95L (FasL) ligand system forms a complex DISC. DISC evades mitochondria and grounds for apoptosis via cleavage of caspase 3 through the activation of caspase 8 and 10. (B) At the end of second kind of cells, DISC cleaves a pro-apoptotic Bid protein that actually depolarizes the mitochondrial membrane and allowing the release of cytochrome C in cytoplasm. (C) Under the influence of cytochrome C apoptosome formation is facilitated by the sequential cleavage of caspase 9 and caspase 3 resulting in cell death.

Glucocorticoids as anti-inflammatory agents

Generally receptors for glucocorticoid are present on eosinophils **possessing** anti inflammatory effects that reduce eosinophil numbers in lung and circulation by inducing apoptosis [1]. Glucocorticoids diminish the activation of cytokines that cause inflammation like TNF- α , IL5 and GMCSF which are strong stimulus of survival and prolonging of eosinophils [75]. Glucocorticoids play its primary function in asthma therapy. Novel antiasthma drugs might emerge the effect of eosinophil apoptosis [76,77]. Glucocorticoids in turn increase phagocytic knack of macrophages. Glucocorticoids restrain the discharge of chemo attractant cytokines and enduring factors [78]. How glucocorticoids encourage eosinophils apoptosis is feebly known but recent facts suggest that activation of MAPK by glucocorticoids along with NO may possibly induce apoptosis in eosinophils [79,80].

Histamine role in eosinophilia

Histamine is a critical mediator in response to allergic inflammation, released from mast cells and basophil cells [81,82]. Various functions of eosinophils are mediated by histamine as it potentially regulates anti-inflammatory action by suppressing eosinophil activation, migration, and degranulation. H4 receptor is accountable in support of conscription along with chemotaxis of eosinophils. Histamine at high concentration inhibits chemo-taxis via H2 receptor while at low concentration enhances chemo taxis via H1 receptor. Histamine regulates the immunity by reversing IL 5 induced eosinophil survival. Histamine

receptors show pro inflammatory as well as anti inflammatory responses based on the net effect of activated receptors subtypes. It is suggested that caspase 6, 1, 10 and 12 can endorse histamine effect on eosinophils apoptosis.

Conclusion

Eosinophils execute significant function in allergic inflammation and asthma. Pathological accumulation moreover prolongs survival at the site of inflammation exclusively related to destructive activity of these pathogenic granulocytes by releasing tissue toxic and inflammatory granule proteins. Novel therapeutic approaches can be acquired by the brief knowledge of eosinophil apoptosis. This will possibly grant us valuable means of treatments in allergic inflammation also eosinophilia. Corticosteroids are considered as strongest anti inflammatory agents for the treatment of asthma. Apart from glucocorticoids, theophylline and histamine are well studied apoptosis inducing agents in eosinophil related inflammation. It is expected that in the future, use of apoptosis inducing drugs will have striking approach for the treatment of allergic inflammation and asthma.

Competing interests

All authors have no institutional and financial competing interests

Author contributions

MQ design the study and RA wrote up the manuscript. MSM, MR and UAA critically reviewed the manuscript. All the authors have read and approved the final manuscript.

References

1. Valent P, Gleich GJ, Reiter A, Roufosse F, Weller PF, et al. (2012) Pathogenesis and classification of eosinophil disorders: a review of recent developments in the field. *Expert Rev Hematol* 5: 157-176.
2. Haslett C (1992) Resolution of acute inflammation and the role of apoptosis in the tissue fate of granulocytes. *Clin Sci (Lond)* 83: 639-648.
3. Kay AB, Barata L, Meng Q, Durham SR, Ying S (1997) Eosinophils and eosinophil-associated cytokines in allergic inflammation. *Int Arch Allergy Immunol* 113: 196-199.
4. Dent LA, Strath M, Mellor AL, Sanderson CJ (1990) Eosinophilia in transgenic mice expressing interleukin 5. *J Exp Med* 172: 1425-1431.
5. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26: 239-257.
6. Simon HU (1996) Novel therapeutic strategies via the apoptosis pathways to resolve chronic eosinophilic inflammation. *Cell Death Differ* 3: 349-356.
7. Rothenberg ME, Hogan SP (2006) Molecules involved in the regulation of eosinophil apoptosis. *Chem Immunol Allergy* 91: 49-58.
8. Druilhe A, Wallaert B, Tscopoulos A, Lapa e Silva JR, Tillie-Leblond I, et al. (1998) Apoptosis, proliferation, and expression of Bcl-2, Fas, and Fas ligand in bronchial biopsies from asthmatics. *Am J Respir Cell Mol Biol* 19: 747-757.
9. Vignola AM, Chanez P, Chiappara G, Siena L, Merendino A, et al. (1999) Evaluation of apoptosis of eosinophils, macrophages, and T lymphocytes in mucosal biopsy specimens of patients with asthma and chronic bronchitis. *J Allergy Clin Immunol* 103: 563-573.
10. Kodama T, Matsuyama T, Miyata S, Nishimura H, Nishioka Y, et al. (1998) Kinetics of apoptosis in the lung of mice with allergic airway inflammation. *Clin Exp Allergy* 28: 1435-1443.
11. Kankaanranta H, Moilanen E, Zhang X (2005) Pharmacological regulation of human eosinophil apoptosis. *Curr Drug Targets Inflamm Allergy* 4: 433-445.
12. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, et al. (1990) Eosinophilic inflammation in asthma. *N Engl J Med* 323: 1033-1039.
13. Djukanovic R, Wilson SJ, Howarth PH (1996) Pathology of rhinitis and bronchial asthma. *Clin Exp Allergy* 26 Suppl 3: 44-51.
14. Martin LB, Kita H, Leiferman KM, Gleich GJ (1996) Eosinophils in allergy: role in disease, degranulation, and cytokines. *Int Arch Allergy Immunol* 109: 207-215.
15. Simon HU, Grotzer M, Nikolaizik WH, Blaser K, Schoni MH (1994) High altitude climate therapy reduces peripheral blood T lymphocyte activation, eosinophilia, and bronchial obstruction in children with house-dust mite allergic asthma. *Pediatr Pulmonol* 17: 304-311.
16. Simon HU, Yousefi S, Dommann-Scherrer CC, Zimmermann DR, Bauer S, et al. (1996) Expansion of cytokine-producing CD4-CD8- T cells associated with abnormal Fas expression and hypereosinophilia. *J Exp Med* 183: 1071-1082.
17. Simon HU, Plotz SG, Dummer R, Blaser K (1999) Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. *N Engl J Med* 341: 1112-1120.
18. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, et al. (2004) Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 199: 1567-1575.
19. Owen WF, Jr., Rothenberg ME, Silberstein DS, Gasson JC, Stevens RL, et al. (1987) Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 166: 129-141.

20. Stern M, Meagher L, Savill J, Haslett C (1992) Apoptosis in human eosinophils. Programmed cell death in the eosinophil leads to phagocytosis by macrophages and is modulated by IL-5. *J Immunol* 148: 3543-3549.
21. Luttmann W, Knoechel B, Foerster M, Matthys H, Virchow JC, Jr., et al. (1996) 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* 157: 1678-1683.
22. Valerius T, Repp R, Kalden JR, Platzer E (1990) Effects of IFN on human eosinophils in comparison with other cytokines. A novel class of eosinophil activators with delayed onset of action. *J Immunol* 145: 2950-2958.
23. Hadjicharalambous C, Dent G, May RD, Handy RL, Anderson IK, et al. (2004) Measurement of eotaxin (CCL11) in induced sputum supernatants: validation and detection in asthma. *J Allergy Clin Immunol* 113: 657-662.
24. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG (1996) Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 183: 195-201.
25. Plotz SG, Dibbert B, Abeck D, Ring J, Simon HU (1998) Bcl-2 expression by eosinophils in a patient with hypereosinophilia. *J Allergy Clin Immunol* 102: 1037-1040.
26. Takatsu K, Takaki S, Hitoshi Y (1994) Interleukin-5 and its receptor system: implications in the immune system and inflammation. *Adv Immunol* 57: 145-190.
27. Sato S, Katagiri T, Takaki S, Kikuchi Y, Hitoshi Y, et al. (1994) IL-5 receptor-mediated tyrosine phosphorylation of SH2/SH3-containing proteins and activation of Bruton's tyrosine and Janus 2 kinases. *J Exp Med* 180: 2101-2111.
28. Ogata N, Kouro T, Yamada A, Koike M, Hanai N, et al. (1998) JAK2 and JAK1 constitutively associate with an interleukin-5 (IL-5) receptor alpha and beta subunit, respectively, and are activated upon IL-5 stimulation. *Blood* 91: 2264-2271.
29. Kagami S, Nakajima H, Kumano K, Suzuki K, Suto A, et al. (2000) Both stat5a and stat5b are required for antigen-induced eosinophil and T-cell recruitment into the tissue. *Blood* 95: 1370-1377.
30. Martinez-Moczygemba M, Huston DP (2003) Biology of common beta receptor-signaling cytokines: IL-3, IL-5, and GM-CSF. *J Allergy Clin Immunol* 112: 653-665; quiz 666.
31. Yousefi S, Hoessli DC, Blaser K, Mills GB, Simon HU (1996) Requirement of Lyn and Syk tyrosine kinases for the prevention of apoptosis by cytokines in human eosinophils. *J Exp Med* 183: 1407-1414.
32. Vassina EM, Yousefi S, Simon D, Zwicky C, Conus S, et al. (2006) cIAP-2 and survivin contribute to cytokine-mediated delayed eosinophil apoptosis. *Eur J Immunol* 36: 1975-1984.
33. Wilson TM, Maric I, Shukla J, Brown M, Santos C, et al. (2011) IL-5 receptor alpha levels in patients with marked eosinophilia or mastocytosis. *J Allergy Clin Immunol* 128: 1086-1092 e1081-1083.
34. Coffey PJ, Schweizer RC, Dubois GR, Maikoe T, Lammers JW, et al. (1998) Analysis of signal transduction pathways in human eosinophils activated by chemoattractants and the T-helper 2-derived cytokines interleukin-4 and interleukin-5. *Blood* 91: 2547-2557.
35. Hall DJ, Cui J, Bates ME, Stout BA, Koenderman L, et al. (2001) Transduction of a dominant-negative H-Ras into human eosinophils attenuates extracellular signal-regulated kinase activation and interleukin-5-mediated cell viability. *Blood* 98: 2014-2021.
36. Oldhoff JM, Darsow U, Werfel T, Bihari IC, Katzer K, et al. (2006) No effect of anti-interleukin-5 therapy (mepolizumab) on the atopy patch test in atopic dermatitis patients. *Int Arch Allergy Immunol* 141: 290-294.
37. Oldhoff JM, Darsow U, Werfel T, Katzer K, Wulf A, et al. (2005) Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. *Allergy* 60: 693-696.
38. Menzies-Gow A, Flood-Page P, Sehmi R, Burman J, Hamid Q, et al. (2003) Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational

arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol* 111: 714-719.

39. Shahabuddin S, Ponath P, Schleimer RP (2000) Migration of eosinophils across endothelial cell monolayers: interactions among IL-5, endothelial-activating cytokines, and C-C chemokines. *J Immunol* 164: 3847-3854.

40. Zou Y, Sonderegger I, Lipowsky G, Jennings GT, Schmitz N, et al. (2010) Combined vaccination against IL-5 and eotaxin blocks eosinophilia in mice. *Vaccine* 28: 3192-3200.

41. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, et al. (2009) Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 360: 973-984.

42. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, et al. (2009) Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 360: 985-993.

43. DiScipio RG, Daffern PJ, Jagels MA, Broide DH, Sriramarao P (1999) A comparison of C3a and C5a-mediated stable adhesion of rolling eosinophils in postcapillary venules and transendothelial migration in vitro and in vivo. *J Immunol* 162: 1127-1136.

44. Warringa RA, Mengelers HJ, Kuijper PH, Raaijmakers JA, Bruijnzeel PL, et al. (1992) In vivo priming of platelet-activating factor-induced eosinophil chemotaxis in allergic asthmatic individuals. *Blood* 79: 1836-1841.

45. Palframan RT, Collins PD, Severs NJ, Rothery S, Williams TJ, et al. (1998) Mechanisms of acute eosinophil mobilization from the bone marrow stimulated by interleukin 5: the role of specific adhesion molecules and phosphatidylinositol 3-kinase. *J Exp Med* 188: 1621-1632.

46. Klion AD, Nutman TB (2004) The role of eosinophils in host defense against helminth parasites. *J Allergy Clin Immunol* 113: 30-37.

47. Na HJ, Hudson SA, Bochner BS (2012) IL-33 enhances Siglec-8 mediated apoptosis of human eosinophils. *Cytokine* 57: 169-174.

48. Egesten A, Blom M, Calafat J, Janssen H, Knol EF (1998) Eosinophil granulocyte interaction with serum-opsonized particles: binding and degranulation are enhanced by tumor necrosis factor alpha. *Int Arch Allergy Immunol* 115: 121-128.

49. Yamashita N, Koizumi H, Murata M, Mano K, Ohta K (1999) Nuclear factor kappa B mediates interleukin-8 production in eosinophils. *Int Arch Allergy Immunol* 120: 230-236.

50. Camussi G, Albano E, Tetta C, Bussolino F (1991) The molecular action of tumor necrosis factor-alpha. *Eur J Biochem* 202: 3-14.

51. Vandenabeele P, Declercq W, Beyaert R, Fiers W (1995) Two tumour necrosis factor receptors: structure and function. *Trends Cell Biol* 5: 392-399.

52. Musso M, Ghiorzo P, Fiorentini P, Giuffrida R, Ciotti P, et al. (1996) An upstream positive regulatory element in human GM-CSF promoter is recognized by NF-kappa B/Rel family members. *Biochem Biophys Res Commun* 223: 64-72.

53. Gramigni C, Penco S, Bianchi-Scarra G, Ravazzolo R, Garre C (1998) An upstream negative regulatory element in human granulocyte-macrophage colony-stimulating factor promoter is recognised by API family members. *FEBS Lett* 440: 119-124.

54. Faggioni R, Feingold KR, Grunfeld C (2001) Leptin regulation of the immune response and the immunodeficiency of malnutrition. *FASEB J* 15: 2565-2571.

55. Fujita Y, Murakami M, Ogawa Y, Masuzaki H, Tanaka M, et al. (2002) Leptin inhibits stress-induced apoptosis of T lymphocytes. *Clin Exp Immunol* 128: 21-26.

56. Guler N, Kurerler E, Ones U, Tamay Z, Salmayenli N, et al. (2004) Leptin: does it have any role in childhood asthma? *J Allergy Clin Immunol* 114: 254-259.

57. Kimata H (2002) Elevated serum leptin in AIDS. *Allergy* 57: 179.

58. Najib S, Sanchez-Margalet V (2002) Human leptin promotes survival of human circulating blood

monocytes prone to apoptosis by activation of p42/44 MAPK pathway. *Cell Immunol* 220: 143-149.

59. Hashida R, Ohkura N, Saito H, Tsujimoto G (2007) The NR4A nuclear receptor family in eosinophils. *J Hum Genet* 52: 13-20.

60. Dibbert B, Daigle I, Braun D, Schranz C, Weber M, et al. (1998) Role for Bcl-xL in delayed eosinophil apoptosis mediated by granulocyte-macrophage colony-stimulating factor and interleukin-5. *Blood* 92: 778-783.

61. Ryan JJ, Prochownik E, Gottlieb CA, Apel IJ, Merino R, et al. (1994) c-myc and bcl-2 modulate p53 function by altering p53 subcellular trafficking during the cell cycle. *Proc Natl Acad Sci U S A* 91: 5878-5882.

62. Oltvai ZN, Millman CL, Korsmeyer SJ (1993) Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74: 609-619.

63. Nutku E, Hudson SA, Bochner BS (2005) Mechanism of Siglec-8-induced human eosinophil apoptosis: role of caspases and mitochondrial injury. *Biochem Biophys Res Commun* 336: 918-924.

64. Duez C, Tomkinson A, Shultz LD, Bratton DL, Gelfand EW (2001) Fas deficiency delays the resolution of airway hyperresponsiveness after allergen sensitization and challenge. *J Allergy Clin Immunol* 108: 547-556.

65. Druilhe A, Cai Z, Haile S, Chouaib S, Pretolani M (1996) Fas-mediated apoptosis in cultured human eosinophils. *Blood* 87: 2822-2830.

66. Walsh GM, Al-Rabia M, Blaylock MG, Sexton DW, Duncan CJ, et al. (2005) Control of eosinophil toxicity in the lung. *Curr Drug Targets Inflamm Allergy* 4: 481-486.

67. Peter ME, Krammer PH (2003) The CD95(APO-1/Fas) DISC and beyond. *Cell Death Differ* 10: 26-35.

68. Hebestreit H, Yousefi S, Balatti I, Weber M, Cramer R, et al. (1996) Expression and function of the Fas receptor on human blood and tissue eosinophils. *Eur J Immunol* 26: 1775-1780.

69. Gochuico BR, Miranda KM, Hessel EM, De Bie JJ, Van Oosterhout AJ, et al. (1998) Airway epithelial Fas ligand expression: potential role in modulating bronchial inflammation. *Am J Physiol* 274: L444-449.

70. Uller L, Rydell-Tormanen K, Persson CG, Erjefalt JS (2005) Anti-Fas mAb-induced apoptosis and cytolysis of airway tissue eosinophils aggravates rather than resolves established inflammation. *Respir Res* 6: 90.

71. Tong J, Bandulwala HS, Clay BS, Anders RA, Shilling RA, et al. (2006) Fas-positive T cells regulate the resolution of airway inflammation in a murine model of asthma. *J Exp Med* 203: 1173-1184.

72. Varadhachary AS, Peter ME, Perdow SN, Krammer PH, Salgame P (1999) Selective up-regulation of phosphatidylinositol 3'-kinase activity in Th2 cells inhibits caspase-8 cleavage at the death-inducing complex: a mechanism for Th2 resistance from Fas-mediated apoptosis. *J Immunol* 163: 4772-4779.

73. Ilmarinen-Salo P, Moilanen E, Kankaanranta H (2010) Nitric oxide induces apoptosis in GM-CSF-treated eosinophils via caspase-6-dependent lamin and DNA fragmentation. *Pulm Pharmacol Ther* 23: 365-371.

74. Barnhart BC, Alappat EC, Peter ME (2003) The CD95 type I/type II model. *Semin Immunol* 15: 185-193.

75. Barnes PJ (2001) Molecular mechanisms of corticosteroids in allergic diseases. *Allergy* 56: 928-936.

76. Ward C, Dransfield I, Chilvers ER, Haslett C, Rossi AG (1999) Pharmacological manipulation of granulocyte apoptosis: potential therapeutic targets. *Trends Pharmacol Sci* 20: 503-509.

77. Simon HU (2006) Molecules involved in the regulation of eosinophil apoptosis. *Chem Immunol Allergy* 91: 49-58.

78. Druilhe A, Letuve S, Pretolani M (2003) Glucocorticoid-induced apoptosis in human eosinophils: mechanisms of action. *Apoptosis* 8: 481-495.

79. Gardai SJ, Hoontrakoon R, Goddard CD, Day BJ, Chang LY, et al. (2003) Oxidant-mediated mitochondrial injury in eosinophil apoptosis: enhancement by glucocorticoids and inhibition by granulocyte-macrophage colony-stimulating factor. *J Immunol* 170: 556-566.

80. Zhang X, Msc, Moilanen E, Lahti A, Hamalainen M, et al. (2003) Regulation of eosinophil apoptosis by nitric oxide: Role of c-Jun-N-terminal kinase and

signal transducer and activator of transcription 5. *J Allergy Clin Immunol* 112: 93-101.

81. Akdis CA, Simons FE (2006) Histamine receptors are hot in immunopharmacology. *Eur J Pharmacol* 533: 69-76.

82. Jutel M, Blaser K, Akdis CA (2005) Histamine in allergic inflammation and immune modulation. *Int Arch Allergy Immunol* 137: 82-92.