Eosinophils in health and diseases
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Abstract
Eosinophils are multifunctional cells whose activities are associated with a single gene and have a diverse role in inflammation as well as in immunity (both innate and adaptive). Our understanding of this multifunctional leukocyte over the past two decades has enhanced from a definitely damaging cell to a cell actively participating in many physiological and pathological processes. Eosinophils are different from other granulocytes by their dense population of cytoplasmic crystalloid granules also known as secretory granules. These secretory granules contain full-bodied stores of various preformed cationic proteins. Numerous functions of eosinophils have been identified over the years, and there capabilities to synthesize, accumulate, and express a varied range of cytokines. The machineries leading to selective formation of preformed cytokines are especially beneficial targets when treatment is concerned. The present review discusses the recent and upcoming notion about the complexities in the structure of human eosinophils and also discusses its role in health and diseases, especially in precancer and cancer. Furthermore, an update on our knowledge of various stains and markers has been discussed in this review.

Introduction
Eosinophils were first defined by Paul Ehrlich in the year 1879 and he noticed their unusual nature to be stained by acidic dyes. Some basic characteristics of eosinophils are established and accepted. The pluripotent progenitor cells give rise to eosinophils after undergoing division and differentiation in various stages of development within the bone marrow. The phenotypically mature eosinophils are then released in the circulation were they are capable of being activated and recruited into tissues in response to various chemoattractants chiefly interleukin-5 (IL-5) along with eotaxin chemokines. Eosinophils have a half-life of about 18 h in the peripheral blood after which they migrate to the gastrointestinal tract or thymus and resides under homeostatic conditions. In response to stimuli like inflammation, progenitor cells in bone marrow differentiates to eosinophils after which they exit the bone marrow, wander into the blood, and subsequently, hoard in peripheral tissues, where their survival is prolonged. The association of eosinophils with asthma, allergic hypersensitivity, and certain neoplastic diseases has long being known but its exact role in health and diseases still needs to be properly understood. Thus, this review discusses the recent and upcoming notion about the complexities in the structure of human eosinophils and also discusses its role in health and diseases, especially in precancer and cancer. Furthermore, an update on our knowledge of various stains and markers has been discussed in this review.

Structure of Eosinophil
About 1-3% of total leukocyte count in blood is comprised of eosinophils. The diameter of an eosinophil is 10-12 μ and is characterized by its copious, coarse granular cytoplasm. These granules are reflective in nature and stain red with acid aniline dyes. The nucleus is bilobed connected by a bridge (spectacle shape). In transmission electron microscopy, eosinophil usually shows a segmented nucleus with heterochromatic regions and cytoplasm contains spherical, ovoid granules or secretory organelles that are just a small component housed in the cytoplasm. Four different types of granules can be appreciated which are secondary granules, primary granules, lipid bodies, and small granules, all of which contains a plethora of proteins, many with enzymatic activity. The secondary granules are composed majorly of eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), arylsulfatase, and hydrolytic enzymes. This unique granulocyte has various proinflammatory mediators with significant potential to commence and maintain
an inflammatory response. These include cytokines, cytotoxic granule proteins, chemokines, and lipid mediators. These cytokines discussed above are potent inducers of immune responses in various inflammatory diseases such as asthma and rhinitis.

**Eosinophil Trafficking**

The predominant population of eosinophils is of those present within the lamina propria of the gastrointestinal tract except the esophagus.\(^1\) The occurrence of eosinophils in the gastrointestinal tract is independent of lymphocytes and enteric flora indicating its unique regulation. The trafficking of eosinophils predominantly into the gastrointestinal tract along with its homing into the thymus, mammary gland and uterus are controlled by eotaxin-1. The trafficking of eosinophils into inflammatory sites has been shown to involve a number of cytokines (predominantly IL-4, IL-5 and IL-13), adhesion molecules (b1, b2 and b7 integrins), chemokines such as RANTES, eotaxins, and other recently identified molecules (e.g., acidic mammalian chitinase). Of the cytokines implicated in modulating leukocyte recruitment, mainly IL-5 and the eotaxins control eosinophil regulation. IL-5 controls growth, activation and survival of this granulocyte and has been shown to provide vital information for the proliferation and movement from the bone marrow into the lung following allergen exposure. Eotaxin-1 is vaguely related to eotaxin-2 and eotaxin-3 by having identical sequence of about 30% but is located in diverse chromosomal position. The explicit commotion of all eotaxins is regulated by the selective expression of chemokine receptor 3 (CCR3) receptor, principally expressed on eosinophils. Notably, the eotaxin chemokines along with IL-5 perform a significant function in the induction of tissue eosinophilia.\(^2\)

Experimental induction of skin and lung late phase cutaneous and pulmonary late-phase responses in humans has revealed that the eotaxin chemokines are produced by tissue-resident cells such as respiratory epithelial cells for lungs and fibroblasts for skin and allergen-induced infiltrative cells such as macrophages and eosinophils. First, eotaxin-1 is induced (6 h) following allergen challenge in the human lung thus correlating with early eosinophil recruitment; in contrast to eotaxin-2 which correlates with eosinophil build up in about 24 h. Both the above mentioned eotaxins induce an immediate wheal and flare linked with degranulation of mast cell and subsequent infiltrations by eosinophils, basophils, and neutrophils. The infiltration by neutrophils is likely to be mediated indirectly by degranulation of mast cell.\(^3\)

**Eosinophils in Health**

In recent years, it has been shown through various studies and researches that eosinophils performs various functions related to immunity including presentation of antigen and also has significant action in inflammatory responses by releasing series of cytokines and lipid mediators.

**Role of eosinophils in immune regulation**

Recent clinical and experimental investigations have shown that these multifunctional granulocytes can play a role as antigen-presenting cells which may be microbial, viral, parasitic antigen, and even superantigens. In response to staphylococcal superantigen (Staphylococcus enterotoxins A, B and E) stimulation, granulocyte-macrophage colony stimulating factor (GM-CSF)-treated eosinophils uphold T-cell proliferation. Eosinophils can also efficiently present soluble antigens to CD4+ T-cells, thereby enhancing T-cell proliferation and polarization. Adoptive transfer of antigen pulsed eosinophils results in eosinophil-dependent T-cell proliferation. It also regulates T-cell polarization by synthesizing an enzyme involved in oxidative metabolism of tryptophan, converting tryptophan to kynurenines. Kynurenines regulate Th1 and Th2 imbalance by promoting Th1 cell apoptosis.

**Association of eosinophils in healing of wounds**

Wound healing in simple terms is the effort of body to reinstate normal structure and function in response to an injury. Healing of skin wounds provides a classical example of regeneration and repair. The epidermis and dermis, which is the peripheral aspect of the skin, protects the internal structures from the external environment. If any injury takes place in this protective barrier, a series of biochemical events quickly sets into action to revamp the damage. Various growth factors - such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor α (TGF-α), fibroblast growth factor (FGF) 1, and 2 and EGF - are released which aid in wound healing.\(^3\) Eosinophils have been established to express and release both mediators of epithelial-mesenchymal transition such as TGF-α, FGF 1, FGF 2, PDGF, VEGF, matrix metalloproteinases (MMP) as well as other repair/remodeling factors, such as nerve growth factors, neuroepitides, and cytokines. TGF-α, PDGF, and VEGF have important functions in wound healing. TGF-α plays an important role in mitogenicity, angiogenicity, promotion of keratinocyte migration and autoinduction of TGF-α expression in keratinocytes, thus making it a significant component to be present in healing of injuries of the skin.\(^4\) Although the exact means of action of eosinophils in healing of wounds is not known, various studies have shown that eosinophils are present in significant number at wound sites and it releases important growth factors which promote wound healing.

**Eosinophils in Diseases**

**Role of eosinophils in precancer and cancer**

The relations between the tumor tissue and adjacent stroma have been major area of concern in cancer research with numerous
features such as varying degree of infiltration of inflammatory cells, increased angiogenesis, microinvasion, and desmoplastic stromal reaction documented as significant features of the general biology and behavior of the tumor cell population. In underlying stroma, the most invasive area has gained a lot of attention as it acts as a prognostic indicator in human malignancies. The invasive tumor front (ITF) offers a fruitful environment to assess the interactions between tumor cells and the adjacent stroma. The ITF has the cells with maximum destructive ability of the tumor population. The ITF houses the most aggressive cells of the tumor population, moreover these cells show lack of cell cohesion and lesser degree of differentiation in contrast with additional areas of the tumor. Studies have shown consistent infiltration of eosinophils in the peritumoral and intratumoral sites, especially at the invasive front area. Inflammatory cell infiltration of mononuclear cells, neutrophils has been seen in oral cancers, nevertheless, when eosinophils exist, it forms the principal inflammatory cell population. Eosinophils have been found to be located in intratumoral and peritumoral site in various cancers apart from oral cancers such as of larynx, pharynx, lung, intestine, and genitourinary tract. Kargahi et al. in their study compared the quantity of tumor-associated tissue eosinophils (TATE) in normal tissue and dysplastic tissue and observed rise in the quantity of eosinophils in dysplasia compared to normal tissue. It was also seen that number of TATE increased as the severity of dysplasia increased. Similarly, Jain et al. in their study noted a substantial rise in the quantity of tissue eosinophils in different degrees of dysplasia. This may suggest that eosinophil has role in tumor promotion but it may also be thought that the quantitative increase in eosinophils is to provide protection to the host against the dysplastic condition.

Various studies were done to assess the role of eosinophils in cancer which showed a definite quantitative rise in eosinophils compared to normal tissue and dysplastic tissue. Jain et al. did a study in which they analyzed the number of eosinophils in oral cancer with metastasis, without metastasis and among different degrees of dysplasia. They found a noteworthy enhancement in the quantity of TATE in oral cancer without metastasis compared to oral cancer with metastasis. Similarly, Rahrobatan et al. in their study established that poorly differentiated oral squamous cell carcinoma (OSCC) had less number of eosinophilic infiltration compared to well and moderately differentiated OSCC. Various studies have shown a better prognosis with increase in the number of TATE. In contrast to above findings, few studies have also shown poor prognosis with increasing number of TATE. Falconieri et al. and Tostes Oliveira et al. have found that severe eosinophilia was largely related to connective tissue invasion. Similarly, Alrawi et al. established amplified eosinophil number in aggressive tumor compared to non-invasive tumors of head and neck region. Hence, TATE shows dual nature of tumor promoting and tumor inhibiting activity.

Th2 response is chiefly related to the primary recruitment and stimulation of eosinophils toward the tumor microenvironment. ILs, such as IL-4 and IL-13, are secreted by the Th2 cells on stimulation, which in turn are strong inducers of eosinophil chemoattractant eotaxin. Eotaxin chemokines bind with the CCR3, which is a major eosinophil chemokine receptor and attracts the eosinophils to the tumor microenvironment. Once eosinophils are recruited to the tumor microenvironment, these recruited eosinophils also secrete certain chemokines which attracts further eosinophils from the blood stream and hence this cycle continues.

Other chemotactic factors are also involved for eosinophil activation like neutrophil peptides, histamine and eosinophilic chemotactic factor A in mast cells, eosinophil stimulator and promoter substances in lymphocytes and C5a complement which additionally draw eosinophils to the tumor microenvironment. Eosinophils show role in tumor promotion through IL-4, IL-10 and pro-angiogenic factors like VEGF, FGF, TNF-α, GM-CSF, TGF-β, and IL-8, 92-kd gelatinase (of the MMPs family) which destroy the basement membrane and the extracellular component, thus promoting stromal invasion, prostaglandin E2 which is produced and related to aggressive behavior shares its forerunner with prostaglandin D2, and additionally, in vitro experiments reported that ECP degrades the myofibrillar proteins as well as membrane-associated cytoskeletal proteins; reinforcing the hypothesis that eosinophil can contribute to muscle fiber degradation in tissue damage, particularly in invasion of malignant tumor.

Various hypotheses on the action of eosinophils show their tumoricidal activities like EPO, a cytotoxic protein released by the above-mentioned granulocytes causes lysis of the tumor cells directly. Eosinophils also enhance the permeability of tumor cells thus helping in infiltration of tumoricidal cytokines into the tumor cells and ultimately causing lysis. This is done by ECP which create pores in cell membranes thus allowing the passage of tumoricidal cytokines into tumor cells. IL like IL-4 inhibits tumor growth by its antiangiogenic property; IL-13 prevents tumor growth by inhibiting interferon γ secretion and CD8+ cytotoxic T lymphocyte activity whereas IL-10 causes stimulation of B-cells. TGF-β inhibits the production of metalloproteinase (MMP) and consequently prevents the development of tumors. The anti-tumor effect of eosinophil is frequently linked to the expression of cytotoxic proteins including ECP, MBP, EPO and EDN, which has been linked to tumor cell apoptosis. Thus, eosinophils show dual characteristics of having both protumor and antitumor function.

Eosinophils are also associated with various diseases such as atopic and related diseases, drug associated eosinophilia, parasitic infections, mostly with helminthes, specific fungal infections, hypereosinophilic syndromes, eosinophilic ulcer of the oral mucosa, eosinophilic granuloma, lichenoid reaction, odontogenic cysts, leukemia, lymphomas, tumor associated mastocytosis, skin and subcutaneous diseases, Kimura’s disease, gastrointestinal diseases, neurologic diseases, pulmonary diseases, cardiac diseases, rheumatologic diseases, renal diseases, specific immune deficiency diseases, and transplant rejection.
Special Stains for Eosinophils

Special stains for staining of eosinophils are Giemsa stain, Wright’s stain, Carbol chromotrope, Astra blue-Vital new red, Sirius red, and Congo red. Meyerhols et al. in their study compared the efficacy of different stains for staining eosinophils in which they concluded that the best stain for staining eosinophils is Sirius red. Other stains following a descending order on its efficacy to stain eosinophils are Congo red, modified hematoxylin and eosin (H and E) and Astra blue/Vital new red stain. It was observed that the modified H and E, Congo red and Sirius red have advantage of less neutrophil and background staining thus enhancing identification of eosinophils even among dense inflammatory cells infiltrate. Although the cost of staining was comparable of all the above-mentioned stains, the time taken for staining was least for Sirius red and maximum for Astra blue/Vital new red stain.[16] Hence after considering all the above factors, it was concluded that the best stain for staining eosinophils is Sirius red. Debta et al. in their study also showed that Carbol chromotrope is a better stain compared to Congo red stain.[17]

Routine used H and E stained tissue sections can be used to identify intact eosinophils but problem arises when these granulocytes assumes an unusual morphology and that too if there is dense infiltration of other inflammatory cells. Even use of special stains are not so helpful in detection of such eosinophils, hence immunohistochemistry is the preferred approach in such cases. Hence, the useful immunohistochemical markers for eosinophils are (i) immunohistochemistry for EPO but it require the use of frozen tissue, (ii) eosinophil MBP antibody is very effective in labeling eosinophils in paraffinized tissue which is BMK-13. BMK-13 works by binding to MBP of both resting and activated eosinophils and thus can be used as an eosinophil marker. BMK-13 also reacts faintly with basophils as it contains small amount of this protein.[18]

Conclusion

The research on eosinophils and its functions is going to become one of the changing trends in the field of oncology in the near future. Recent studies have given new insights in the numerous roles played by eosinophils like its involvement as modulators of adaptive immunity by activating T-cells directly and its role in initiation and promulgation of varied inflammatory responses. Identification of eosinophil regulatory cytokines like IL-5 and eotaxin is a major breakthrough in the field of understanding the biology of eosinophils, its production and its localization under homeostatic condition and inflammation. In particular, an integrated mechanism involving Th2 cell-derived IL-5 regulating eosinophil expansion in the bone marrow and blood and Th2 cell-derived IL-13 regulating eotaxin production now explains the means by which T-cells regulate eosinophils. Based on these findings, it is predicted that targeted therapy against key eosinophil regulators (e.g., IL-5 antagonists and CCR3 antagonists) will likely transform medical management in eosinophilic patients.

References

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