

Review article

The rise of the phoenix: the expanding role of the eosinophil in health and disease

We have entered a new phase in the evolution of our understanding of the role of the eosinophil with a greater appreciation of novel potential functions that may be ascribed to this enigmatic cell type. This review not only provides an update to our current understanding of the various immunobiological roles for the eosinophil, but also attracts attention to some novel observations predicting functions beyond its putative effector role. These observations include the intriguing possibility that the eosinophil may possess the capacity to regulate the immune and inflammatory responses in diseases such as asthma.

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Like all facets of life, our understanding of the immunobiology of inflammation is undergoing progressive evolution. This evolution is relevant also to our ever-expanding appreciation of the biological relevance of various components of the immunological and inflammatory cascade in health and disease. The human eosinophil since its first description by Paul Ehrlich (1) experienced successive highs and lows in understanding its precise role and function in the complex milieu of allergic and asthmatic inflammation (2). As early as a century ago, the eosinophil was identified to be associated with various conditions, most of which we now recognize as Th2-related diseases. The 1970s and 1980s witnessed an upsurge in interest in the capacity of the eosinophil to adhere to and kill metazoan helminth parasites, *in vitro* (3, 4). Circa the same era, elegant clinical observations of Gleich and others established a close correlation between secreted eosinophil crystalloid granule-stored cationic proteins and tissue damage in asthma (5, 6). Subsequently, attention became focused on understanding eosinophil effector function. This was paralleled by extensive attempts to target the eosinophil as a major therapeutic strategy for asthma. Antieosinophil strategies were deemed particularly relevant as corticosteroids, choice therapies for asthma, down-regulate eosinophil counts in blood, sputum, bronchoalveolar lavage (BAL)

and airway tissue and in turn correlate with amelioration of disease severity and symptom improvement (7). Thus, began an era of great optimism around the potential for immunopharmacological interventions in the management of allergic airway diseases. The discovery of interleukin (IL)-5 in the 1980s as the most crucial cytokine in the regulation of growth and terminal differentiation of the eosinophil (8, 9) led to major pharmaceutical investments aimed at antagonizing IL-5 with a view to blocking the eosinophil influx into the tissue and presumably inhibiting its associated sequelae. Animal models, particularly simian, pointed optimistically to such a possibility (10). However, clinical trials with a humanized anti-IL-5 monoclonal antibody, Mepolizumab, concluded that targeting the eosinophil is far more complex than blocking its differentiation at the level of the bone marrow and blood (11). A safety trial of SCH55700, an alternate humanized anti-IL-5 monoclonal antibody further found limited clinical efficacy (12).

The resurrection of the eosinophil as an important effector cell in asthma

After the poor results of Mepolizumab, a number of reports have sought to explain the apparent failure of

such a potent antieosinophil treatment in the management of asthma. Beyond the fact that the study by Leckie et al. was well underpowered to appreciate statistical differences in the treatment group, a key factor was that the airways of the positive control group were not hyperreactive (13). Thus, the main outcome of airway hyperreactivity (AHR) could not be correctly analysed. A second study by Kips et al. was designed to establish safety of SCH55700. The authors emphasized that their study was not designed to discern differences in important clinical variables including forced expiratory volume in 1 s (FEV₁). Their protocol used a single dose of anti-IL-5 and studied clinical results a month after treatment. While blood eosinophils decreased after a single dose, sputum eosinophils did not. The authors noted that the study population was selected for its steroid-resistance rather than a more typical asthma phenotype. Whether these severely obstructed patients primarily had asthma or other secondary diseases may be questioned given the selection criteria. There is an increasing sense that the measurement of eosinophils in sputum or airway fluids, may not truly reflect the contributions of airway tissue eosinophils. Flood-Page et al. showed that Mepolizumab depleted <50% of bronchial tissue and bone marrow eosinophils in spite of its effect in reducing blood and BAL fluid eosinophils (14). It might, therefore, not be surprising to see a lack of effect of anti-IL-5 on airway hyperresponsiveness (AHR). While corticosteroids also do not fully deplete tissue eosinophilia, they do possess a wider range of inhibitory activity on eosinophils beyond IL-5 receptor inactivation (15, 16), which may be more relevant and will be discussed later. The Leckie paper also did not consider IL-5 receptor expression and function of the eosinophils obtained in airway fluid in comparison with blood or tissue eosinophils. Liu et al. demonstrated a marked reduction in the expression of messenger RNA of the surface IL-5 receptor (mIL-5R α) and its intracellular component (mIL-5R β) from BAL eosinophils compared with blood eosinophils (17). Further, in contrast to circulating eosinophils, BAL eosinophils do not release eosinophil-derived neurotoxin (EDN) when treated with IL-5 as do their blood counterparts, suggesting that the function of BAL eosinophils are IL-5-independent (18). Thus, airway tissue eosinophils may be less dependent on IL-5 for their survival and mediator release.

Eosinophil production and survival within tissues

From the human IL-5 data and in animal models, the ability of eosinophils to survive and function in the tissue is becoming an important focus. The development and maturation of eosinophils can occur *in situ* in peripheral sites of inflammation containing pre-existing increased tissue eosinophils. Eosinophil progenitors are released into the circulation to reach such tissue sites (19). Eosinophils can release granulocyte/macrophage-colony

stimulating factor (GM-CSF) in an autocrine fashion (20, 21), a cytokine which is stored in association with eosinophil granules (22). Other eosinophil-derived and stored cytokines [e.g. IL-4 (23), IL-13 (24, 25)] and chemokines [e.g. RANTES (26, 27)] may further amplify the inflammatory milieu. Thus, the local production of such eosinophil factors may be important in tissue eosinophil reactions beyond IL-5. The IL-5 and GM-CSF are produced also by local fibroblasts and epithelial cells. Eosinophils may enhance their own survival by directly stimulating CD4 T cells within tissue to produce IL-5. Nasal explants from atopic patients were shown to survive *ex vivo* using similar mechanisms to promote extramedullary eosinophil maturation and survival (28, 29). These, as well as lung explants of Brown-Norway rats, exhibited rapid (6 h) accumulation of major basic protein (MBP)-positive cells after allergen challenge of the explants *in vitro* (30). The major signalling pathway of these events is associated with IL-5 receptor ligation leading to phosphorylation of JAK-2 and Lyn kinases, decreased BAX translocation, and ultimately decreased apoptosis through activation of the caspase family of enzymes (31). Additionally, GM-CSF appears to have a strong role in inhibiting eosinophil apoptosis at the tissue level. Autocrine GM-CSF stimulation of eosinophils bound to fibronectin, via α 4 integrin, promoted eosinophil survival for 2 weeks (21). Recently, Lee et al. demonstrated that eosinophils, when instilled into the trachea of IL-5 knockout mice, not only survive in the absence of IL-5, but in concert with CD4 (+) T cells, migrate back into lung, and reconstitute the asthma phenotype of wild-type antigen challenged animals (32). Overall, while IL-5 is essential in the maturation and differentiation of eosinophils in the bone marrow (33), the recruitment to tissues and function within tissues may be IL-5-independent.

Tissue localization and activation status of the eosinophil in the lung

Eosinophils have a variety of toxic proteins and pro-inflammatory cytokines, which are released under highly regulated conditions at the appropriate target site. When considering the role of the eosinophil in AHR, we must understand the mechanisms of AHR and then consider where any single cell type would be positioned to cause such effect. While the smooth muscle layers of asthmatic airways can become hypertrophied and more fibrous with time, whether the muscle is intrinsically more hyperresponsive to stimulation remains unclear (34).

Smooth muscle contraction is controlled via M3 muscarinic receptor stimulation with acetylcholine released from parasympathetic nerves (35). The AHR measured by inhalation of methacholine or histamine (PC20) involves sensory nerve stimulation of the smooth muscle via the vagus efferent nerves (36, 37). In humans

with AHR, these neural pathways are abnormally sensitive (38). One reason for the abnormality of the neural control of the airway smooth muscle is the loss of M2 receptor function (M2R). The M2Rs act by limiting the release of acetylcholine (ACh) onto the smooth muscle M3R (39). Thus, loss of M2R leads to uncontrolled smooth muscle contraction and subsequent bronchoconstriction as seen in asthma (40).

In histopathological samples of patients with asthma, eosinophils can be found clustered around the vagal nerve ganglia in the lung (41). As well, positive staining for extracellular eosinophil MBP has been detected in the vicinity of these nerves (41). *In vitro*, eosinophils bind to parasympathetic nerve endings via the intracellular adhesion molecule (ICAM)-1 receptor, the expression of which is increased in sensitized nerve cultures (42). Further, eosinophil binding to nerves in culture increases the release of acetylcholine via an M2R mechanism (42). In guinea-pig models of antigen sensitization followed by challenge (43) and antigen sensitization followed by virus infection (44), the release of MBP from eosinophils has been shown to cause M2R dysfunction and hyperreactivity. The development of eosinophil-mediated M2R dysfunction after virus infection occurs only in sensitized animals. The reason may be related to the increased number of eosinophils found in closer proximity to the parasympathetic nerves of sensitized guinea-pigs compared with nonsensitized animals (45). In both sensitized and nonsensitized animals, virus infection causes a dramatic decrease in the number of eosinophils stained in the airway (45). Thus, in addition to the MBP data, airway eosinophils appear to respond to virus infection. When CD8⁺ T cells are depleted in both sensitized and nonsensitized animals prior to viral infection, the disappearance of eosinophils in the airway histology is prevented (45). CD8⁺ T-cell depletion also prevented the development of virus-induced M2R dysfunction but only in the group that received prior sensitization. The failure to stain eosinophils in the airway of these virus-infected animals could be related to eosinophil degranulation by cytolysis (46, 47). Virus-induced eosinophil cytolysis would disperse the granules and decrease the number of whole cells, making the staining of eosinophils more difficult by conventional means. Thus, if eosinophils in both sensitized and nonsensitized animals respond to the viral infection with mediator release, the damaging effect on the airway nerves would be likely in sensitized animals where eosinophils are in closest proximity. Overall, studying the airway pathology with more focused attention on the position of eosinophils and their activation status demonstrates subtle but important differences in the possible mechanism of AHR.

In normal, nonallergen-sensitized guinea-pigs, M2R dysfunction also develops after virus infection, but in contrast, virus-induced loss of M2R function in nonallergen-sensitized animals is *not* mediated by eosinophils (44, 48). Such virus-induced M2R dysfunction is seen in

nonatopic humans (38), which may be secondary to increased interferon (IFN)- γ . The IFN- γ is higher in nonallergen virus-infected animals (49) and humans (50), it positively correlates with AHR in humans (51), and it decreases M2R expression in nerve cell culture (52).

Developing better animal models

It is generally agreed that there are different subtypes of asthmatic patients with apparently different degrees of eosinophil activity. Thus, a key goal in asthma research must be the development of more realistic animal models to reflect the subtypes of disease. The mouse model of eosinophil-related asthma may be questioned because murine eosinophils do not appear to degranulate as similar to human eosinophils *in vivo* (53). Further, the ability to generate the 'asthma phenotype' is quite dependent on the genetic background of the mouse used (54). While constitutive overexpression of recombinant IL-5 (55) or IL-5-deficient mice (56) have demonstrated a strong association of eosinophilia with many of the features compatible with human asthma, alternate mouse models can generate contradictory results (57, 58).

Part of the claim against a role for eosinophil in AHR comes from work in mice where, IL-13 appears to induce AHR independent of eosinophilia (57, 59). Many of the studies make their measurements of AHR through the use of enhanced pause (Penh) rather than true changes in airway resistance. This mechanism of selective IL-13-induced AHR is not understood. IL-13 receptors may exist on noninflammatory cell types including smooth muscle cells (60). IL-13 increases smooth muscle contraction *in vitro* (61). Venkayya et al. demonstrated that AHR could be generated in a mouse by injecting the airways with recombinant IL-13 (62). The AHR occurred within 6 h and was not associated with the development of any inflammation including eosinophilia. The effect was also seen in both mast cell-deficient mice and mice-deficient in T and B cells. Thus, assumptions of a lack of a role of eosinophils or apparently any other inflammatory cell type in AHR must be carefully examined before applying data from IL-13-related mouse models to humans.

The time course of the model is also important. Mathur et al. showed that while anti-IL-5 could abolish established airway eosinophil infiltration, it could not abolish established AHR, a result compatible with human data (63). While this was a negative report on the importance of eosinophils, it could also suggest that if active eosinophilic inflammation has occurred in the airway, a period of healing or repair may be necessary before resolution of established AHR. Even the role of IL-13 in AHR was questioned in a chronic asthma model (64), which again suggests the time course of a model can be crucial to understanding mechanisms.

Eosinophil modulation of tissue inflammation

Beyond direct tissue damage, the contributions of eosinophil-derived cytokines and other secretory products are becoming evident. The secretion of IL-4 and IL-13 by infiltrating Th2 lymphocytes has been strongly associated with the development of the asthmatic phenotype of inflammation (65). Although many of these cytokines are produced by lymphocytes, reports have demonstrated the ability of eosinophils to synthesize, store and secrete an array of cytokines [e.g. IL-4 (66, 67) and IL-13 (24, 25)] and chemokines [e.g. RANTES (68)]. Eosinophils may also influence airway function by producing effects on airway remodelling through tenascin production and the release of transforming growth factor (TGF)- β . Phipps et al., using an allergen-induced cutaneous model of asthmatic inflammation, showed that the release of TGF- β and IL-13 by eosinophils contribute to airway remodelling (69). Thus, the release of mediators from eosinophils could affect the general Th2 phenotype of inflammation.

In addition, eosinophils may directly influence the function of lymphocytes. Eosinophils express co-stimulatory molecules essential for interaction with lymphocytes (70). Studies in mice have shown that eosinophils are capable of transmigrating to lymphoid tissues and presenting antigens to lymphocytes (71, 72). Although antigen presentation to naïve T cells by eosinophils is still controversial, there is a consensus that eosinophils can present antigen to *previously* activated T cells, supporting the notion that eosinophils can further influence established immune responses (73).

Eosinophils may also be involved earlier in the ontogeny of the immune response. For example, studies by Throsby et al. showed that thymus-homing eosinophils actively participate in major histocompatibility complex (MHC) class I-restricted deletion of autoreactive T cells during early neonatal period (74). The TGF- β , for which the eosinophil is a well-accepted source (75), is also related to T-lymphocyte subset development (76). Eosinophils synthesize and store a battery of chemokines, cytokines and growth factors that may influence T-cell selection in lymphoid tissue (77). Thus, cytokine production by lymphoid tissue-infiltrating eosinophils may directly influence T-cell selection by dendritic cells as the cytokine microenvironment of dendritic cells may determine whether they induce T-cell tolerance or activation. Eotaxin, an eosinophil-specific CCR3 ligand, is constitutively expressed in the thymus and lymph nodes of mice (78). The specific recruitment of eosinophils into lymphoid tissues puts these cells in a vantage position to exert immunomodulatory effects on T cells in diseases associated with eosinophilia. Indeed, the induction by IFN γ of indoleamine 2,3-dioxygenase (IDO), the rate-limiting enzyme in the oxidative catabolism of tryptophan, has been shown to be a potent mechanism through which dendritic cells induce apoptosis and inhibit proliferation of T cells (79).

Lymphoid tissue-dwelling eosinophils may therefore indirectly influence T-cell apoptosis through synthesis and release of IFN γ , following the ligation of CD28 on eosinophils (80), with subsequent induction of IDO in dendritic cells. Interestingly, we have recently submitted work showing that eosinophils constitutively express IDO and induce T-cell apoptosis (S. O. Odemuyiwa, A. Ghahary, Y. Li, L. Puttagunta, S. Musat-Marcu and R. Moqbel, 2004, unpublished data). Thus, eosinophils may even directly influence T-cell function through the IDO mechanism (Fig. 1). Although the mechanism of eosinophil-induced T-cell modulation is currently poorly understood, the specific recruitment of cytokine-expressing eosinophils to lymphoid tissues may significantly influence the afferent limb of the immune response. This is a new and exciting area of research into the immunobiology of the eosinophil.

The eosinophil as a marker of allergic disease and asthma phenotyping

Atopic diseases such as asthma, dermatitis and rhinitis are classically associated with increased tissue eosinophils (81, 82). The presence of eosinophils has been correlated with disease severity and bronchial hyperresponsiveness (83). Despite this association there is significant heterogeneity amongst subgroups in asthma, and even within individual patients from season to season. Clearly different inflammatory phenotypes are present in asthmatics (84). For example, eosinophilic bronchitis (EB) is characterized by an increase in airway eosinophils, yet in contrast to asthma, AHR does not appear to be a feature. This raises the question: why is EB not associated with AHR if eosinophils contribute to AHR? In comparing mild asthma with EB, Brightling et al. found that while both groups had eosinophilia, the significant difference in airway pathology of the asthma patient was the presence of mast cells within the smooth muscle (85). This mast cell myositis was proposed as the cause of AHR in asthma, which suggested that AHR, a key feature of asthma, involves cells and mediators beyond the eosinophil.

Traditionally, mast cells are responsible for the acute phase of the asthmatic response via IgE-mediated histamine release and smooth muscle stimulation. Mild asthma by definition can have AHR and acute periods of bronchospasm, often allergy-related. In contrast, mild asthma should not have decreased lung function by spirometry or should it show exacerbations requiring hospitalization. Thus, it was important that in a subsequent paper by many of these same authors interested in mast cell myositis, that in moderate-to-severe asthmatics, management of eosinophils did make a difference in asthma symptoms and outcomes (86). After a run-in period where they attempted to gain a baseline measurement of control with systemic and

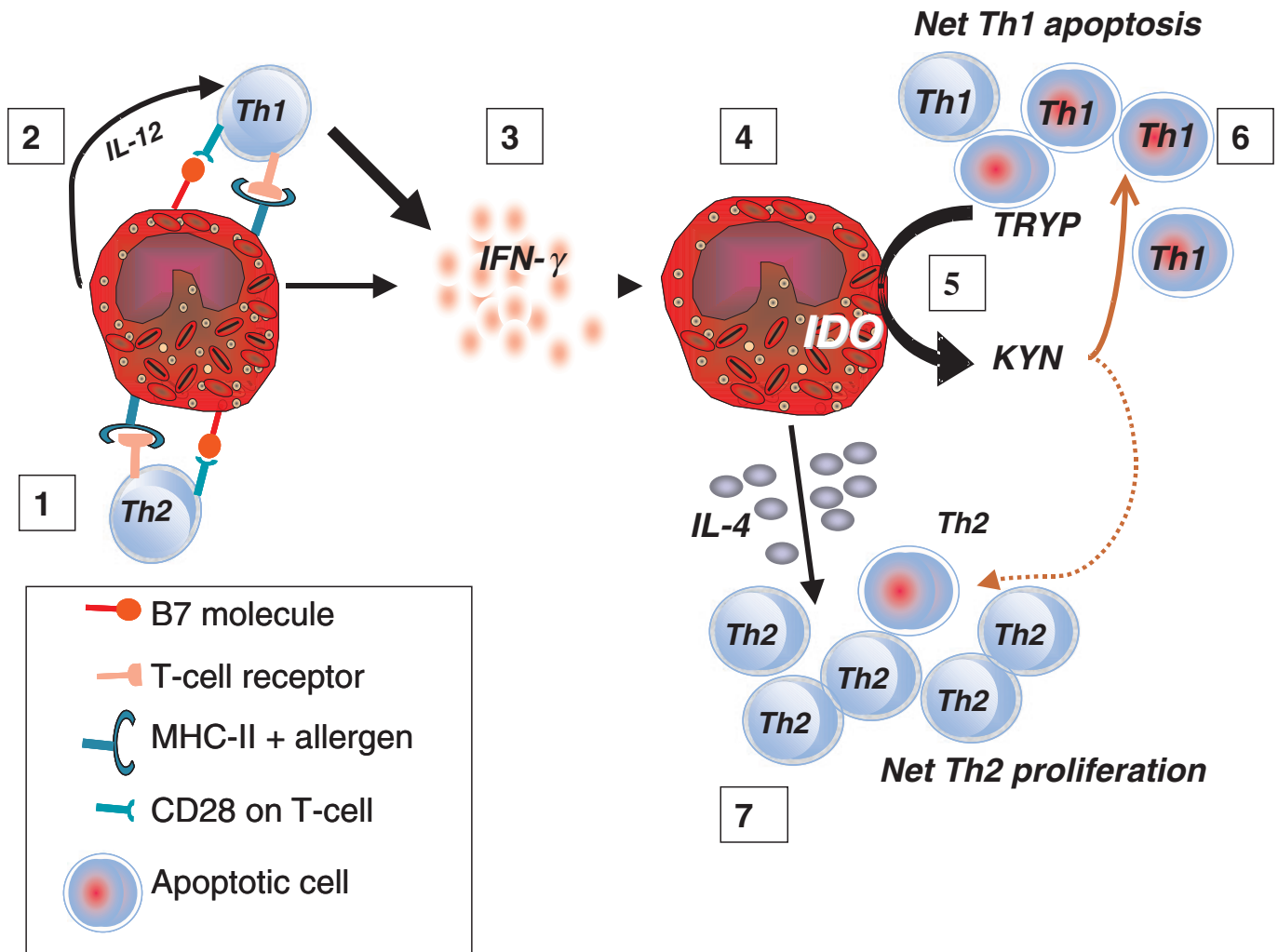


Figure 1. A proposed model of Th1-Th2 imbalance through IDO induction in lymph node-dwelling eosinophils. (1) Eosinophils present antigens to both Th1 and Th2 cells in the lymph node; (2) ligation of co-stimulatory molecules on eosinophils induces IL-12 release, which in turn induces Th1 cells to produce IFN- γ ; (3) ligation of co-stimulatory molecules on eosinophils activates them to release IFN- γ ; (4) IFN- γ induces IDO and IL-4 in eosinophils; (5) eosinophil-derived IDO converts tryptophan (TRYP) to kynurenine (KYN); (6) KYN induces apoptosis in a very high percentage of IFN- γ -producing Th1 cells which may contribute to enhanced eosinophil IDO production; (7) Th2 cells are spared from KYN-induced apoptosis by IL-4.

inhaled corticosteroids (ICS), patients with moderate-to-severe asthma were randomized to two groups. One group received standard but strict medical therapy based on guidelines of the British Thoracic Society (BTS). The other group was managed by the same guidelines but with the addition of regular sputum analyses of eosinophilia or nitric oxide (NO) production. The sputum group was closely monitored for direct evidence of eosinophil activity in the airway as a signal to adjust medication accordingly. Unlike the sputum group, the BTS group had only symptoms and lung function to guide therapy, which is the end result of inflammatory damage.

There was a very convincing improvement in the outcomes for the eosinophil controlled group. Sputum eosinophil number and NO production were decreased

by 63% and 48%, respectively, compared with the BTS group. The sputum group had lowered AHR, fewer exacerbations, less prednisone doses, fewer admissions to hospital, while receiving the same ICS dose. Further, in patients with low eosinophil numbers, the ICS dose was able to be lowered in the sputum group.

It appears that because the sputum group provided earlier information about the degree inflammation reflected by eosinophilia, their asthma could be controlled before eosinophil activity caused damage and subsequent clinical morbidity. Thus, just as the type of animal model is important in the study of eosinophils, depending on the clinical phenotype of the asthma patient, the role of the eosinophil may also vary. Knowing both the inflammatory profile and the clinical categorization of

the patient develops a clearer phenotype of the asthma patient and appears to contribute to improved asthma care.

The ultimate goal in asthma therapy will continue to be the development of the most effective anti-inflammatory strategy for individual patients. Thus, the focus may be to correlate the clinical picture of the asthmatic patient with the inflammation in the tissue; such correlations will provide distinct phenotypes of asthma. Eosinophils are extremely sensitive to the effects of glucocorticosteroids (GS). The subpopulation of asthmatics who have a primarily neutrophilic airway inflammation may be better served by an alternative agent to control inflammation than GS. Under-recognition of ongoing airway inflammation despite clinical remission is a problem for both patients and doctors (87). Lower airway inflammation can be evaluated safely and in a noninvasive fashion by measuring changes in induced sputum (88). The utilization of the latter in characterization also depends on the compartment analysed (sputum, blood, urine). Eosinophils from blood and BAL express different cell surface markers following allergen challenge. Correlating which compartment is the most relevant for clinical response to therapy will also be important. Evaluating asthmatics has now evolved from the research arena to clinical management (89, 90).

We are clearly approaching a period where the cellular profiling of individuals will be a commonplace practice in the clinical management of asthma. The role of phenotyping as part of routine clinical management is likely to expand shortly to include other airway diseases, including chronic obstructive pulmonary disease (COPD) and cystic fibrosis (91). While present national and international asthma guidelines do not advocate inflammatory cell characterization in choosing therapy, this likely will change as our understanding of the correlation of inflammatory and clinical asthma phenotypes continue to improve.

Beyond allergy and asthma

In addition to a potential role in immunomodulation, there is also emerging new information about the role of eosinophils in oncology. Some cross-sectional studies using morphometric immunohistochemical techniques to quantify eosinophils in tumours have concluded that tumour-associated tissue eosinophilia (TATE) has a positive prognostic influence on squamous cell carcinomas and pulmonary adenocarcinoma (92, 93). The mechanisms of eosinophil-induced tumour regression are poorly understood. However, expression of eotaxin by oral squamous cell carcinomas was found to specifically induce TATE in this tumour type (94). Whether this will reflect in a prognostic implication remains to be elucidated. Lung cancers show an eosinophil association in certain subtypes (95).

The likelihood of tissue invasion in some malignancies has been shown to be related to the presence of tissue eosinophilia (96). The role phenotyping will play in these populations requires further study.

Future direction in asthma therapy: the paradigm of convergence

Reversal of the chronic inflammatory response associated with asthma has been a traditional therapeutic goal in the management of this disease. Treatment with ICS interrupts ongoing local chronic inflammation in the respiratory airway, with significant remission of asthmatic symptoms following considerable improvement of measurable airway functions (97). However, a major disadvantage of corticosteroid therapy is the existence of a subset of asthmatics that are not responsive to corticosteroid therapy. Another issue with corticosteroids is the problem associated with preventing the systemic effects of locally administered corticosteroids. Recent case reports in hypereosinophilic syndrome patients resistant to corticosteroid therapy, demonstrate the role of targeting the eosinophil with anti-IL-5 therapy to gain clinical improvement (98). Further, adding anti-IL-5 therapy to these patients may have a corticosteroid sparing effect (99). Thus, alternative therapies targeting eosinophils and inflammatory mediators are being developed.

A major lesson learnt hitherto in the design of novel therapy against asthma is the realization that targeting a single inflammatory cell may not lead to a significant remission of asthma symptoms. Just as all patients do not uniformly present with all the same clinical features of asthma, the pathological inflammatory profile of specific cell types also appears to vary in the subjects studied. For example, some patients with asthma are not atopic and do not have a typical mast cell-related type-1 reaction to allergens, which may suggest that not all patients may respond to anti-IgE therapy. As well, some patients do not have prominent airway eosinophilia confirming the paradigm the asthma is a complex heterogeneous disease and part of the problem in treating the inflammation of asthma relates to the clinical phenotype of subjects selected for study. While conservative reductionist approaches have served us well in understanding the potential functions of various cells and molecules, they may not necessarily produce the best therapeutic options in a disease characterized by complex cellular and cytokine dyscrasia. It is very interesting that the most effective asthma drug to date, corticosteroids, targets multiple cell types involved in the chronic inflammation that characterizes asthma.

A strategy that targets a functional pathway common to all cellular infiltrates involved in asthma may finally produce the best drug to reduce considerably the burden of asthma in future. The development of such drugs will require further studies to understand the common func-

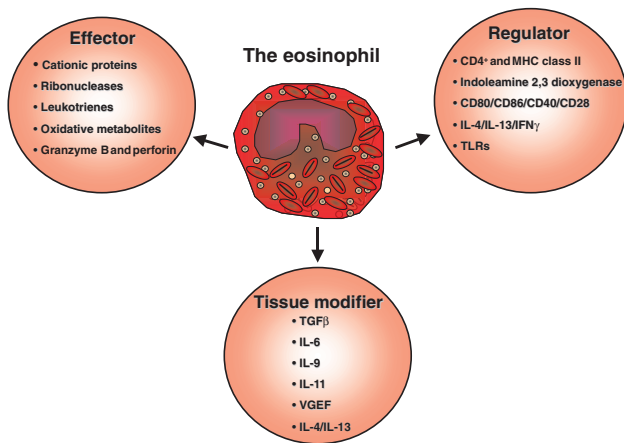


Figure 2. The ever-expanding role of the eosinophil in asthma and beyond.

tions of these cellular infiltrates. A painstaking dissection of the pathways involved in such functions should eventually identify the weakest link in the chain of events that can be targeted and exploited for drug development.

In conclusion, our ongoing attempt at identifying a precise role for the eosinophil in asthma is hampered by the realization that asthma (Fig. 2) is not a single clinical entity and should, therefore, not be expected to be associated with or dependent on a single responsible cell type. When better and more accurate phenotyping of asthma is achieved, a greater appreciation of patient-directed, rather than disease-directed therapies would likely emerge. It is our firm belief that as complex diseases will require complex therapeutic approaches, combination therapies may dominate future disease management to include antieosinophilic strategies.

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