

"Their's but to do and die":* Eosinophil longevity in asthma

With fascination, we have studied granulocyte recruitment in inflammation. Stimulated granulocytes migrate from the blood stream, roll, adhere to endothelium, and migrate into the tissues. Once there, they unleash an arsenal to do good (eg, kill bacteria), wreak havoc (eg, destroy host tissues), or both. Much of our focus has been on these initial events of recruitment and activation. Only in more recent years have we considered subsequent events (the "what next?") in inflammation. Once the effector leukocytes (neutrophils, eosinophils, basophils, or monocyte/macrophages) arrive in tissues, they do not emigrate. Importantly, restitution of tissue function requires not only interruption of the initiating signals but also removal of the inflammatory cells. So what is the ultimate fate of these recruited cells, and does that fate have bearing on the severity or chronicity of inflammation and disease?

A general hypothesis is that inhibition of apoptosis of inflammatory cells (see the review article by Cohen¹ in this issue of *The Journal*) contributes to "tissue load" of the cells (Fig 1). Tissue load of eosinophils, for example, is related to severity of asthma. Evolving data supports this hypothesis, including the article by Vignola et al² in this issue of *The Journal*.

Because granulocytes immigrate, but do not emigrate, tissue load or accumulation is a dynamic equilibrium of both cellular recruitment and longevity. An assumption of this model is that if infiltrating cells are long-lived, becoming (for awhile) resident cells, then they continue to churn out injurious products/cytokines that perpetuate inflammation. It is assumed that they are not benign old timers quietly living out their final days.

In the study by Vignola et al,² bronchial biopsy specimens from 30 patients with asthma were examined for the presence of apoptotic eosinophils, macrophages, and T lymphocytes. Data from these biopsy specimens were compared with those of 15 control subjects and 26 patients with chronic bronchitis. Unlike previous studies, in which subjects with relatively mild asthma underwent biopsy,³ in this study 6 subjects had moderate asthma (Aas score of 3) and 10 had severe asthma (Aas scores of

Abbreviation used

TGF: Transforming growth factor- β

4 and 5). All were without recent anti-inflammatory treatment. Similar to previous studies, relatively few apoptotic cells were found in biopsy specimens from asthmatic subjects, and the overall numbers were not different between asthmatic and control subjects (whose biopsy specimens contained fewer leukocytes). Importantly, however, Vignola et al² found that the absolute number of apoptotic cells was inversely correlated with clinical severity of asthma, as measured by Aas scores. This inverse relationship with clinical severity also held up for the ratio of apoptotic eosinophils to total eosinophils—the milder the asthma, the greater the percentage of eosinophils on biopsy specimens that were found to be apoptotic. Apoptotic macrophages were also demonstrated in the biopsy specimens of these patients, and again the ratio of apoptotic macrophages to total macrophages was inversely correlated to Aas scores. Additionally, these authors showed that tissue production of GM-CSF was greater in subjects with asthma than in control subjects or subjects with chronic bronchitis and, importantly, that GM-CSF production correlated with both the frequency of nonapoptotic eosinophils and macrophages and the severity of asthma. Thus Vignola et al² conclude that the accumulation of eosinophils and macrophages in asthma is likely due to not only increased recruitment of these cells into the airways but also to inhibition of their apoptosis by exposure to the prosurvival cytokine GM-CSF.

A critical look at the data raises interesting questions regarding cellular recruitment and apoptosis. In Fig 3 of the article by Vignola et al,² there appear 3 distinct subgroups in the eosinophil and macrophage data for asthmatic subjects. Examining the eosinophil data, half the group had no eosinophils per square millimeter at the time of biopsy and thus are represented in Fig 5 of that article by a ratio of 0 for apoptotic eosinophils to total eosinophils. Although the information is not shown, we can deduce by their sheer numbers that these subjects span the gamut of Aas scores. Putting sampling issues aside, this might suggest that for these patients, the fate of eosinophils is unimportant, raising the ever present question of heterogeneity in the pathology of asthma. A second subgroup is composed of those patients in Fig 3 with numerous eosinophils in biopsy specimens, a substantial number of which were apoptotic, giving ratios for Fig 5 that place them in the milder range of Aas

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**Charge of the Light Brigade*, Alfred Lord Tennyson.

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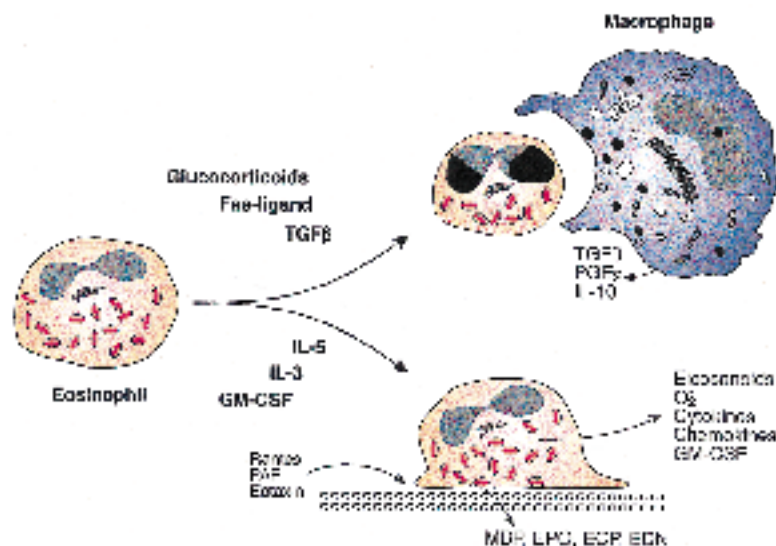


FIG 1. Pathways to eosinophil activation or apoptosis and clearance. *PAF*, Platelet-activating factor; *MBP*, major basic protein; *EPO*, eosinophil peroxidase; *ECP*, eosinophil cationic protein; *EDN*, eosinophil-derived neurotoxin.

scores. Finally, there are those patients with many eosinophils, none of which were apoptotic, represented by ratios of 0 for apoptotic to total eosinophils. We can make the likely inference that these are subjects with the most severe asthma (highest Aas scores) in Fig 5. Would other data, such as allergic status, chronicity of disease, or prior treatment, give insights and distinguish these groups clinically?

Interestingly, Vignola et al² found significantly more apoptotic cells in the biopsy specimens of patients with chronic bronchitis than in tissues from asthmatic patients, whereas the overall leukocytic cellularity (CD45-positive cells) was said to be similar. Many of the apoptotic cells in the biopsy specimens from patients with chronic bronchitis were not identified as either eosinophils or macrophages. However, of the identified eosinophils, although significantly fewer in number than those found in asthmatic patients (as would be expected), the percentage of eosinophils that were apoptotic was significantly greater in tissues from subjects with chronic bronchitis than in tissues from asthmatic subjects. This confirms what we would intuitively expect: a cell not particularly germane to the pathogenesis of chronic bronchitis would not receive the signals required for longevity. Macrophages, numerous in both disorders, were also found to be more often apoptotic in tissues from subjects with chronic bronchitis than in tissues from asthmatic subjects. Thus the authors hypothesize that given similar accumulation of leukocytes in asthma and chronic bronchitis, apoptosis of eosinophils and macrophages is delayed in asthma relative to chronic bronchitis. The data would again suggest that both recruitment and delayed apoptosis of eosinophils and macrophages are important in the accumulation of these cells and play a role in the resulting severity of asthma.

This conclusion is the simplest interpretation of the

data and may indeed be the correct interpretation. Unfortunately, biopsy specimens constitute a single snapshot in time and cannot assess cell recruitment and accumulation in a chronic process. One approach to better understand events of chronic inflammation is to study acute, self-limited inflammation and its resolution. To this end, Ying et al⁴ studied apoptosis of infiltrating cells after induction of the late-phase cutaneous response to allergen. This model achieves relatively synchronous leukocyte immigration in response to a time-limited stimulus. Ying et al found that resolution of the late-phase cutaneous response to allergen was associated with apoptosis of recruited neutrophils and eosinophils and the subsequent engulfment of these cells into tissue macrophages. By contrast, infiltrating T cells and macrophages did not undergo apoptosis and persisted in the skin long after resolution of the clinical response. Another limitation we face is a lack of markers for the age of recruited cells. For instance, given continuous cytokine production, we might expect infiltration by less mature cells elicited from the bone marrow. If eosinophils or macrophages have built in "biological clocks"¹ then the apoptosis of younger cells would appear to be delayed. The eosinophils of subjects with severe asthma may simply be spending their life span in the tissues rather than the bone marrow compared with the "middle aged" cells found in the tissues of subjects with mild asthma, control subjects, and subjects with chronic bronchitis. These hypotheses, delayed apoptosis and recruitment of less mature cells, are not mutually exclusive, and in fact are likely to be mutually operant in the perpetuation of an inflammatory process.

The outcome, either survival or apoptosis of a cell, is often seen as the balance between opposing survival and death signals.¹ Survival often requires the continuous presence of survival factors, which are frequently also

those that govern proliferation and differentiation of a given cell (eg, GM-CSF for eosinophils and macrophages as demonstrated by Vignola et al²). As in many examples, cells themselves may produce their own survival factor or factors when appropriately stimulated.⁵⁻⁷ Removal or inactivation of these survival factors allows the activation of the intrinsic death programs, with apoptosis the consequence. Studies by Simon et al⁸ have beautifully demonstrated eosinophil apoptosis in nasal polyp tissue explants on the exogenous addition of neutralizing antibody to IL-5. Similarly, we have demonstrated a pivotal role for GM-CSF expression in the enhanced monocyte survival in chronic atopic dermatitis.⁶ Treatment with glucocorticoids inhibits cytokine production from inflammatory and activated resident cells and results in inflammatory cell apoptosis. Recent studies have shown an increase in the proportion of apoptotic eosinophils in tissue biopsy specimens³ and sputum and the demonstration of macrophages containing eosinophilic material⁹ after corticosteroid administration in asthma. These data support the hypothesis that apoptosis induction is clinically relevant in the treatment of this disease.

What of other known prosurvival and proapoptotic signals? Vignola et al² looked for Bcl-2 expression, an oncogene product often associated with cell survival. They found that Bcl-2 was increased in the tissues from asthmatic subjects compared with those of control subjects and subjects with chronic bronchitis and that expression correlated with the severity of asthma. Although Bcl-2 can be associated with resident cells (mucous glands and smooth muscle), both protein and message were specifically found in T cells of the inflammatory infiltrate in subjects with asthma and chronic bronchitis. As in other studies,³ Bcl-2 serves as a marker of infiltrating T cells, which rarely appear to be apoptotic in asthma. Recent studies have shown that although Bcl-2 does not appear important in eosinophil survival, Bcl-x_L, a related family member, is upregulated by IL-5 and GM-CSF and is associated with enhanced survival of this cell type in vitro.¹⁰ Confirmation of its upregulation and role in eosinophil longevity in vivo is awaited.

Often it is not known whether a cell has a "biological clock" that is simply kept in check by prolife signals or whether a cell actively requires death signals coming either internally or from the surrounding tissues to become apoptotic. Along these lines, Vignola et al² looked for expression of P53, the product of the p53 gene, the "guardian of the genome" that is associated with induction of apoptosis. They found increased expression in biopsy specimens from patients with chronic bronchitis over that seen from control and asthmatic subjects and concluded that P53 contributes to the heightened apoptosis demonstrated in this disorder. However, without assigning the P53 immunoreactivity to a specific cell type and showing that it plays a role in the induction of apoptosis of that cell, this conclusion must remain conjectural. Similarly, the authors' discussion of the so-called "imbalance between the expression of Bcl-2 and P53" being important in delaying apoptosis in asthma

would appear at this point to be simplistic. Alternatively, specific delivery of death signals to inflammatory cells by glucocorticoid treatment (not just the downregulation of prosurvival cytokine production) is an intriguing and exploitable possibility in the reduction of airway inflammation.¹¹ Druilhe et al³ have recently shown that glucocorticoid treatment increased the expression of Fas ligand on bronchial epithelium while enhancing expression of Bcl-2 and possible survival of the epithelium. Upregulated Fas ligand may trigger the apoptotic death of Fas-bearing eosinophils. Functional Fas receptor expression on eosinophils varies among individuals with hypereosinophilia, and the survival cytokines (GM-CSF, IL-3, and IL-5) reportedly have no effect on Fas expression or Fas-induced apoptosis.^{12,13} Alternatively, nitric oxide confers resistance to Fas receptor-mediated eosinophil apoptosis.¹⁴ Transforming growth factor- β 1 (TGF β 1) production, augmented during glucocorticosteroid treatment, inhibits autocrine production of prosurvival cytokines by eosinophils in vitro, suggesting the possibility of a balance of these cytokines in vivo.¹⁵

The final event in the lives of most apoptotic cells, including those of inflammatory cell origin in the tissues, is recognition and removal by phagocytes.^{3,4,16,17} It is this clearance that is critical to the resolution or prevention of inflammation in vivo, not the apoptosis of the inflammatory cell itself. Two important mechanisms account for the anti-inflammatory effects of clearance. First, apoptotic cells are removed before lysis, which prevents the release of proinflammatory and immunogenic intracellular contents, as is seen in cellular necrosis or cytolysis¹⁸ in vivo and in apoptotic cells undergoing secondary necrosis in vitro. If phagocytosis is inhibited in vivo, it would not matter whether cells died by apoptosis or necrosis; the intracellular contents would be released, and inflammation would result. Vignola et al² attempted to assess clearance by examining expression of CD36; however, CD36 is only one of many receptors^{17,19} implicated in clearance by macrophages, and macrophages are not the only cells that can recognize and phagocytose apoptotic cells. Also, rates of phagocytic clearance cannot be assessed by looking at expression of surface markers alone. Secondly, as determined by in vitro studies, engulfment of apoptotic cells not only fails to induce proinflammatory mediators^{20,21} but stimulates the production of TGF β , and in some cases IL-10 and PGE₂, each of which inhibit production of proinflammatory cytokines, chemokines, and eicosanoids.²²⁻²⁴ At first glance, the production of TGF β might be considered counterproductive because it has been demonstrated to promote fibrosis in some models of chronic lung injury. However, TGF β is a veritable Janus of cytokines, and the balance of anti-inflammatory versus profibrotic activity may be related to timing and duration of release and the specific isoforms involved. We hypothesize that an early transient release of TGF β may be beneficial, whereas a delayed sustained release could result in airway fibrosis. Given these suppositions about clearance, one might reach different, but equally valid, conclusions about the

data of Vignola et al.² Chronic bronchitis results from impaired clearance of apoptotic cells. Apoptosis and clearance of inflammatory cells may be normal in asthma, but the disease results from the sheer numbers and/or activation state of the infiltrating leukocytes. It will be critical to determine mechanisms of clearance by macrophages and other cells in vivo. This will require well-defined models of inflammatory disease because it will be extremely difficult, if not impossible, to study in the clinical setting for both ethical and experimental reasons.

A variety of studies point to the intricacies of control of inflammatory cell longevity and the exquisite balance of prosurvival and proapoptotic signaling. The degree of complexity is not surprising given the need of tissues to both promote and curtail inflammation (ie, to contain perceived injury but also to limit tissue damage) and to allow healing and recovery of function. It is highly likely that dysregulation of these processes can lead to persistent inflammation and that targeted induction of cellular apoptosis could be a holy grail of anti-inflammatory therapy.²⁵

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