Thorax

Editorial

Regulation of granulocyte apoptosis and implications for anti-inflammatory therapy

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The elimination of unwanted cells is now regarded as an essential component of many normal biological programmes. These include embryonic remodelling, the removal of autoreactive thymic T cells, and the disposal of senescent enterocytes at the villus tip. Hence cell death, in particular apoptosis or "programmed cell death", appears to be a prerequisite for life.[1] In the particular example of inflammation many of the unique features of apoptosis, such as the retention of plasma membrane integrity, the loss of the cytotoxic and secretory capacity of the cell, and the ability of apoptotic cells to be recognised and phagocytically removed, suggest that this remarkable process may play a key role in limiting tissue injury and facilitating the successful resolution of inflammation.[2–5] Furthermore, the ingestion of apoptotic cells by macrophages, unlike the uptake of other biological and non-biological material, fails to induce a secretory or pro-inflammatory response.[6 7] Although a number of different cells within the lung have the capacity to undergo apoptosis (including the bronchial epithelial cell and type II pneumocyte[8 9]), the central role of the granulocyte as the primary effector cell in most forms of lung and airway wall inflammation, coupled with the huge potential for these cells to induce tissue injury if not cleared, marks the apoptotic capacity of these cells as being of particular importance in lung inflammation.

Despite the considerable interest generated by the discovery that neutrophils and eosinophils undergo constitutive apoptosis when aged in vitro,[2 10] a process that results in their ingestion by either professional[2 11] or semi-professional phagocytes (including fibroblasts[12]), only a relatively small number of studies have addressed the importance of this process in vivo. This in part reflects the observation that apoptotic cells appear to be cleared extremely rapidly in tissues and hence are not readily apparent at any one time, and secondly that genetic manipulation of this process has been hampered by the global importance of apoptosis, especially in fetal development. Despite this, apoptotic granulocytes are observed at sites of inflammation[4 5] and there is a substantial increase in the number of ingested and non-ingested apoptotic neutrophils in bronchoalveolar fluid in acute lung injury in neonates[13] and in the recovery phase of experimental lipopolysaccharide induced alveolitis.[14] More compelling evidence linking this event to the resolution of inflammation is the finding that the administration of corticosteroids in patients with acute severe asthma is associated with the appearance of apoptotic eosinophils in sputum, and this event parallels clinical improvement.[15]

An additional problem encountered in trying to track and quantify apoptosis in vivo is that many of the pro-inflammatory mediators and cytokines present at an inflamed site—for example, GM-CSF, C5a, IL-5—and the physicochemical characteristics of such an environment—for example, hypoxia—cause profound inhibition of granulocyte apoptosis.[10 16 17 18–21] Hence, eosinophil apoptosis is rarely encountered within nasal polyp tissue[22] and neutrophil apoptosis is not readily apparent in fluid recovered from an empyema cavity.[23] Moreover, it is now recognised that the inhibition of eosinophil apoptosis by agents such as IL-5, GM-CSF and other Th-2 derived cytokines represents a critical factor underlying the initial accumulation of these cells.[5 24 25] Such data also suggest that granulocyte induced tissue injury and chronic inflammation may reflect not only excessive granulocyte recruitment but also inhibition of normal apoptosis based clearance mechanisms. This concept is supported by data indicating that defective T cell killing, which involves autocrine or paracrine generation of Fas ligand (Fas-L) leading to activation of the death domain containing Fas receptor, may underlie chronic T cell activation and survival following antigen stimulation,[26] and that bronchoalveolar lavage fluid from patients with ARDS prolongs the survival of normal human neutrophils in vitro.[27]

Such a hypothesis suggests that a therapeutic strategy based on stimulating granulocyte apoptosis may offer a novel approach to promoting the resolution of inflammation. To date, however, it has been difficult to identify agents that stimulate granulocyte apoptosis since, despite the relatively rapid rate of constitutive apoptosis observed when these cells are cultured in vitro, the majority of priming and activating agonists inhibit rather than accelerate this process. This list includes lipopolysaccharide and the pro-inflammatory mediators IL-5, C5a, GM-CSF, IL-1β, IFNγ, and LTB_4.[10 16–20] Moreover, many of the well recognised routes to induce apoptosis in thymocytes and lymphocytes, such as the use of corticosteroids and the elevation of intracellular cAMP, have the opposite effect in neutrophils[28 29]—that is, they delay apoptosis— and it is possible that such observations explain in part the limited anti-inflammatory profile of β_2 adrenoceptor agonists and corticosteroids in certain "neutrophil dominant" forms of inflammation such as ARDS.[30] Recent data, however, have indicated that it is possible to drive apoptosis in these cells—for example, with TNFα, Fas-L, the ingestion of *E coli* or oil red particles[19 31–36]—and, most intriguingly, theophylline[37] which, unlike other agents that increase intracellular cAMP, causes a modest stimulation of neutrophil apoptosis. Hints that nature already utilises such a strategy to drive the removal of granulocytes from an inflamed site appear in studies showing that synovial fluid from patients with active rheumatoid arthritis, and bronchoalveolar fluid obtained from rabbits with experimental pneumococcal pneumonia, induce neutrophil apoptosis[38 39] (R Lawson and C Haslett, unpublished

observations). Moreover, the potential to manipulate this process therapeutically is supported by the ability of anti-Fas monoclonal antibodies delivered to the lungs of mice with allergen induced airways eosinophilia to cause an increase in the numbers of peroxidase positive macrophages in lavage fluid and a substantial reduction in the number of eosinophils in the airways.[33]

The study of granulocyte apoptosis in vitro has also revealed some very important differences in the regulation of apoptosis in neutrophils and eosinophils. Most notably, it is now clear that glucocorticoids accelerate eosinophil apoptosis yet delay this process in the neutrophil.[28] This observation may explain, at least in part, the ability of corticosteroids to resolve tissue eosinophilia in conditions such as asthma and pulmonary eosinophilia.[14] Other differences also exist in the regulation of apoptosis between these two cell types. For example, the anti-apoptotic factor Bcl_2 is readily detectable in eosinophils yet is absent from neutrophils,[21 36 40] and an increase in intracellular calcium enhances eosinophil apoptosis but delays neutrophil apoptosis.[41 42] Hence, despite the close proximity of these cells in phylogenetic terms, the prospect of being able to selectively target apoptosis in a particular inflammatory cell type appears to be a realistic goal.

Finally, while under in vitro conditions the uptake of apoptotic granulocytes by macrophages appears to be a rapid and highly efficient process, in certain pathological states this disposal system may become overwhelmed. Augmentation of the phagocytic capacity of inflammatory macrophages may therefore represent an additional option for intervention, and recent data demonstrating a major enhancement of macrophage ingestion of apoptotic neutrophils following CD44 ligation offers some support for this type of approach.[43]

Hence, the recognition that apoptotic cell death plays such a critical role in dictating the function and fate of inflammatory cells (and is amenable to exogenous regulation) has transformed our understanding of how "beneficial" and "deleterious" forms of inflammation may differ and offers a completely new avenue for therapeutic intervention in inflammation. Monitoring apoptosis in biological samples such as sputum should also provide a novel index of the success or otherwise of such strategies. The future goal of identifying the basis for the unique genetic control of granulocyte apoptosis may provide novel and selective therapeutic targets to drive this process and offer an explanation as to why the intensity, duration, and outcome of an inflammatory response differs so much between individuals.

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