Resolution of acute inflammation and the role of apoptosis
in the tissue fate of granulocytes

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INTRODUCTION

For centuries the inflammatory response was regarded as an entirely beneficial host response to injury or infection; for example, Elie Metchnikoff, the father of modern inflammatory cell biology, described inflammation as “a salutary response to some injurious influence”. In recent decades, however, it has become apparent that inflammation is also implicated in the pathogenesis of many diseases that have assumed prominence in developed societies. In the lung these include chronic bronchitis and emphysema, asthma, respiratory distress syndromes of the adult and neonate, the pneumoconioses and a variety of other fibrosing alveolitides. In most of these disorders, the persistent accumulation of inflammatory cells is associated with destruction of lung tissue or deposition of scar tissue in the delicate gas exchange tissues, either of which can lead to catastrophic loss of organ function. Analogous conditions in other organs include some forms of glomerulonephritis (which represent the bulk of kidney diseases causing renal failure requiring dialysis), rheumatoid arthritis, and myocardial infarction and reperfusion injury.

It is clear, however, that circumstances also exist whereby even massive inflammatory cellular reactions in tissues can resolve completely. Perhaps one of the best such examples is the inflammatory response in lobar streptococcal pneumonia. Historical studies [1] and more recent work using experimental models suggest that the enormous tissue load of inflammatory cells that accumulates in pneumococcal pneumonia is cleared without obvious residual tissue injury or the provocation of a significant scarring response. This is remarkable when it is considered that neutrophil granulocytes, monocytes and macrophages, which represent the bulk of extravasated cells, are now recognized to possess great potential for injuring tissues and promoting scar tissue formation. The definition of how inflammation normally resolves is likely to shed new light on the pathogenesis of persistent inflammation, which remains obscure despite much recent research into the initiation and amplification mechanisms. Moreover, the definition of resolution mechanisms may suggest novel therapeutic strategies which could be directed at the promotion of inflammatory resolution. By contrast with the mechanisms of initiation and amplification, however, comparatively scant attention has been paid to the process involved in the termination of inflammation. In his treatise Hurley [2] considered that acute inflammation may terminate by: development of chronic inflammation, suppuration, scarring or resolution. It is reasonable to suggest that all the alternatives to resolution are ‘non-ideal’ and could contribute to disease processes, particularly in organs whose function depends on the integrity of delicate exchange membranes.

The remainder of this article represents (of necessity) a largely theoretical consideration of some of the processes which are likely to occur in the resolution of inflammation, with particular attention being paid to recent work on the tissue fate of granulocytes.

For tissues to return to normal, all of the events involved in the evolution of inflammation must be reversed. In the very simplest example (Fig. 1) of an acute inflammatory reaction this must include: removal of the inciting stimulus, dissipation of the mediators generated by the inciting influence, cessation of granulocyte emigration from blood vessels, return of normal microvascular permeability, limitation of granulocyte secretion of potentially histotoxic and pro-inflammatory agents, cessation of the emigration of monocytes from blood vessels and
their mutation into inflammatory macrophages, and, finally, the removal of extravasated fluid, proteins, bacterial and cellular debris, neutrophils and macrophages. With the completion of these events, the stage should be set for the recovery of normal tissue architecture and function.

While each of these events will be considered briefly, attention is focused on the behaviour of the neutrophil. The neutrophil is the archetypal acute inflammatory cell, being essential for host defence but also implicated in the pathogenesis of many inflammatory diseases [3]. It is the first cell to arrive at the scene of tissue perturbation and some key early inflammatory events, including monocyte emigration [4] and the generation of inflammatory oedema [5], appear to depend upon the initial accumulation of neutrophils. Neutrophils contain a variety of agents with the capacity not only to injure tissues [6], but also to cleave matrix proteins into chemotactic fragments [7] with the potential to amplify inflammation by attracting more inflammatory cells. Thus, the neutrophil granulocyte, which has been the main focus of our research so far, appears to occupy a pivotal position in the initiation, amplification and resolution processes of inflammation. Termination of neutrophil emigration from blood vessels and the clearance of extravasated neutrophils and their products are prerequisites for resolution to occur, and are important events to consider in the control of inflammatory tissue injury generally.

MEDIATOR DISSIPATION

It is important to begin to understand how mediators are removed, inactivated or otherwise rendered impotent. This might occur by spontaneous decay, for example thromboxane A₂ and endothelial-derived relaxing factor (nitric oxide) are evanescent factors which are spontaneously unstable. Platelet-activating factor and C5a are inhibited in vitro by inactivating enzymes [8] and interleukin-8 (IL-8) is thought to become inactivated by binding to neutrophils. Reduction of mediator efficacy might also occur by reduction in concentration due to dilution in inflammatory oedema fluid or by reduction in target cell responsiveness, as exemplified by the down-regulation of receptors which occurs during desensitization of neutrophils to a variety of inflammatory mediators [9]. In vivo, it is also important to consider the local generation of factors exerting negative effects, e.g. neutrophil-immobilizing factor [10], which would tend to counteract the effects of chemotactic peptides at the site. A complex function such as neutrophil chemo taxis in response to a peptide such as C5a or IL-8 may thus be influenced by a number of factors, including the concentration of mediators and their inhibitors or inactivators, possible desensitizing mechanisms and the effects of negative agents. As with the blood coagulation cascade, there must exist natural constraints upon inflammatory cascades (such as cytokine generation), and the identification of natural agents, such as the interleukin-1 receptor antagonist, will not only help us to understand the control mechanisms but could also lead to novel approaches in anti-inflammatory therapy. A final requirement for the success of most of the above mechanisms is that the production of mediators must cease.

It must also be recognized that the inflammatory response has a plethora of redundant mechanisms.
For example, C5a, leukotriene B\(_4\) and IL-8 (and probably many more factors) are likely to be important neutrophil chemotaxins in vivo. Therefore, it will be necessary to consider how a variety of important mediators may act in concert at an inflamed site and to seek to appreciate the integrated impact of negative and positive stimuli on dynamic events in situ. The overall propensity for inflammation to persist would thus be expected to wane when the balance of mediator effects tips towards the inhibitory rather than the stimulatory, presumably as a result of the combination of some of the possible mechanisms mentioned above.

**CESSATION OF NEUTROPHIL AND MONOCYTE INFLUX**

Because other poorly understood factors, such as cell removal rates, may also exert major influences on the number of cells observed in 'static' histological sections, an accurate appraisal of neutrophil emigration kinetics requires the study of tagged populations of cells. When intravenous pulses of radiolabelled neutrophils were used to define the emigration profile of neutrophils from blood into acutely inflamed skin [11], joints [12] or lung [13], it was found that neutrophil influx ceased remarkably early (2–4 h), by contrast with the greatly prolonged influx that occurred in a model of inflammation which progressed to chronic tissue injury and scarring [14]. In an experimental model of lobar streptococcal pneumonia (R.J. Clark, H.A. Jones, R. Lawson & C. Haslett, unpublished work) in which a massive acute inflammatory pneumonia (Fig. 2a) develops over 48 h and resolves over the succeeding 5–7 days (Figs. 2b and 2c), we have found that intravenous pulses of \(^{111}\)In-labelled neutrophils fail to migrate by 24 h after the onset of pneumonia (Figs. 3a and 3b), which is remarkable considering the histological picture at 48 h (Fig. 2a). The cessation of neutrophil emigration occurring so early in the evolution of acute inflammation may therefore represent one of the earliest resolution events.

Several possible mechanisms could contribute to the normal cessation of neutrophil emigration in acute inflammation.

1. Chemotactic factor inhibitors could be generated locally and inactivate neutrophil chemotactic factors. Agents with the capacity to inactivate C5a activity in vitro have been isolated in plasma [8], but these factors have not been isolated, characterized or quantified at inflamed sites, and a plasma-derived inactivator is unlikely to account for cessation of neutrophil emigration in situations where extravascular protein leakage is minimal or absent.

2. Deactivation or desensitization may lead to extravasated neutrophils becoming unresponsive to further chemotactic factor stimulation [15]. This would be expected to occur at the centre of an inflamed site where the concentration of chemotaxins is highest, but it seems unlikely that this mechanism is involved in the cessation of neutrophils entering the site.

3. A negative feedback loop might operate, whereby neutrophils which have already accumulated exert an influence that prevents more neutrophils emigrating from the bloodstream.

4. Cessation of neutrophil emigration may simply occur after dissipation or removal of chemotactic factors from the inflamed site.

5. The cell layers (endothelial and epithelial) through which neutrophils emigrate could alter to form a ‘barrier’ to further neutrophil emigration. Neutrophils can migrate between endothelial cells [16] and epithelial cells [17] without causing obvious injury. This process, known as transmigration, involves different intercellular adhesive mechanisms than the initial neutrophil/endothelial adhesion step, together with the opening of intercellular endothelial or epithelial cell junctions by mechanisms which are as yet obscure.

The relative importance of these possible events in vivo is uncertain. In a skin model of inflammation it appeared that a desensitization mechanism was operating [11], and in some forms of human disease involving persistent inflammation there have been suggestions that chemotactic factor inhibitory agents may be important. However, in experimental arthritis [12] we found no evidence for a desensitization mechanism or for a chemotactic factor inhibitory mechanism. Cessation of neutrophil emigration to the joint coincided with the loss of chemotactants from the joint space, an event which was not dependent upon cellular accumulation at the site (evidence against a simple feedback mechanism).

Although the mechanism responsible for the loss of chemotactins was not identified, these observations suggest that the generation (and removal) of chemotactants are centrally important in the persistence and cessation of neutrophil emigration.

This is not to understate the probable role of ‘barrier cells’, such as microvascular endothelial cells, in the control of the initiation and termination of neutrophil emigration from the blood into tissues. It is clear that locally generated inflammatory mediators not only exert locomotor effects but may also act on inflammatory cells and endothelial cells to promote the expression and activation of surface molecules (adhesins) [18] which lead to the adhesive and capillary transmigration events characterizing the initiation of neutrophil emigration.

Neutrophil adhesins are upregulated very rapidly upon exposure to chemotaxins such as C5a and IL-8. It is now thought that L-selectin is important in the initial interaction with endothelial cells under conditions of shear stress which exist in vivo, whereas the neutrophil surface heterodimeric proteins (e.g. MAC-1, LFA-1 and P150.95) of the integrin family are especially important in the capillary transmigration process. Neutrophil adhesins must uncouple to permit the next stage of migration.
Fig. 2. Histological appearance of experimental pneumococcal pneumonia in the rabbit at different times after the instillation by fibreoptic bronchoscope of $10^8$ Streptococcus pneumoniae organisms into the apical segment of the right upper lobe. (a) Forty-eight hours: a profound acute inflammatory response with alveolar spaces packed with neutrophils and monocyte/macrophages (magnification $\times 400$). (b) Ninety-six hours: significant clearing (magnification $\times 1000$). (c) Seven days: the lung has returned to virtually normal appearance (magnification $\times 200$).

Fig. 3. Emigration of intravenously-delivered $^{111}$In-labelled neutrophils to experimental rabbit pneumococcal pneumonia. (a) When the pulse of labelled neutrophils was injected intravenously 6h after the instillation of bacteria into the right apical lung segment, the posterior view of the external y-camera scintigram taken 24h thereafter shows accumulation of labelled neutrophils in the upper lobe. (b) No detectable accumulation of labelled cells was seen when the intravenous pulse of labelled neutrophils was injected 24h after the instillation of bacteria. The liver and spleen, which are the main organs responsible for the 'physiological' removal of senescent neutrophils from the bloodstream, are also clearly seen in each case. Reproduced with permission from Holgate, S. T. & Church, M. K. Allergy. London: Gower Medical: 1993.

to proceed and must finally down-regulate in expression or function for emigration of the cell population to terminate. The molecular mechanism controlling the 'turn on' and 'turn off' signals of integrins are just beginning to be elucidated [19].

The endothelium plays much more than a passive role in these events; indeed, it has recently been suggested that endothelial cells may actively promote transmigration of the endothelium and broaching of their basement membrane by neutrophils [20]. Neutrophil adhesins interact with their counterparts on the endothelium [e.g. ELAM-1 (E-selectin), intercellular adhesion molecule-1, intercellular adhesion molecule-2], some of the pairings
having been identified, whereas others are uncertain. Endothelial adhesins are upregulated by locally generated mediators including interleukin-1 and tumour necrosis factor.

There has been no detailed research on adhesin expression in situ during the termination of neutrophil emigration, but in experimental arthritis it is clear that the inflamed site can respond to an inflammatory stimulus by permitting a further wave of neutrophil emigration [12]. Therefore any 'barrier' to cell adhesion or transmigration existing at the time of cessation of neutrophil emigration must be readily reversible, presumably by the further generation of inflammatory cytokines which would induce renewed expression/activation of endothelial adhesins at the same time as activating neutrophil locomotion. Thus, it is still reasonable to suggest that the identification of mechanisms controlling the local generation (and dissipation) of agents which promote chemotaxis and adhesion function is central to our understanding of the processes of termination and persistence of neutrophil emigration at inflamed sites.

Much less is understood of the control of monocyte emigration, although similar principles are likely to be applicable in defining the factors involved in the cessation of monocyte emigration.

RESTORATION OF NORMAL MICROVASCULAR PERMEABILITY

Classical ultrastructural studies demonstrated that neutrophil migration to inflamed sites is not necessarily associated with overt endothelial injury [1]. Nevertheless, in examples of acute inflammation including experimental pneumonia [22] there is morphological evidence of endothelial injury ranging from cytoplasmic vacuolation to areas of complete denudation and fluid leakage into alveolar spaces. However, the cell sheets retain the capacity for repair as the pneumonia resolves. Since there is evidence of at least some inevitable endothelial and epithelial injury even in 'beneficial inflammation', this is likely to be a pivotal point at which loss of the normal controls of tissue injury and repair could represent a major mechanism in the development of persistent inflammation and injury. Although the underlying processes are poorly understood, repair is likely to occur by a combination of local cell proliferation to bridge gaps, and the recovery of some cells from sublethal injury. Endothelial monolayers deliberately ‘wounded’ in vitro have a remarkable capacity to reform [23]. Little is known of how endothelial cells recover from sublethal injury, but epithelial cells in vitro appear to recover from hydrogen peroxide-induced injury by a mechanism which requires new protein synthesis [24]. These cytoprotective mechanisms have received little study, but their definition may provide ‘natural approaches’ to boosting local defences against excessive inflammatory injury.

CONTROL OF INFLAMMATORY CELL SECRETION

In order to clarify how tissue injury is limited at inflamed sites, we must also define mechanisms controlling the secretory behaviour of neutrophils, macrophages and other inflammatory cells. Although much is known of how phagocyte secretion is modulated in vitro [25], little is known of how their secretion is down-regulated or terminated in vivo. As is the case with chemotaxis, secretion in situ is likely to be modulated by the balance between stimulatory and inhibitory mediators. The simplest mechanism for termination, the cell exhausting its secretory potential, is unlikely since cells isolated from inflamed sites retain the capacity for further secretion upon stimulation [26]. Other factors which may contribute to down-regulation or termination of secretion are the exhaustion of internal energy supplies, receptor down-regulation, dissipation of stimuli, and, finally, death and removal of the cell itself. In a short-lived cell like the neutrophil granulocyte, which has a half-life in the blood of about 6 h, the death of the cell itself could represent an important mechanism in the final irreversible down-regulation of its secretory function. As is discussed in more detail below, we have discovered that the neutrophil has a mechanism available whereby it constitutively undergoes programmed cell death, or apoptosis (Figs. 4a and 4b). This process leads to removal of the intact senescent cell by local macrophages in a fashion which would tend to limit tissue injury and promote the resolution of inflammation [27]. During apoptosis the neutrophil retains its granule contents and membrane function but loses the ability to secrete granule contents in response to external inflammatory stimuli (M. K. B. Whyte, L. C. Meagher, J. MacDermot & C. Haslett, unpublished work). In culture, apoptotic neutrophils may retain their membrane integrity and contain their granule enzymes for many hours before final disintegration occurs [27]. Apoptosis therefore provides a mechanism which renders the neutrophil inert and functionally isolated from pro-inflammatory mediators present in its microenvironment, thus greatly limiting its destructive potential before it is removed by local phagocytes.

CLEARANCE PHASE OF INFLAMMATION

Once the inflammatory cells have completed their tasks in host defence, and the inciting influences, e.g. bacteria, have been effectively destroyed or rendered impotent, the site must be cleared of fluid, proteins, antibodies and bacterial or cellular debris, and finally, the key cellular players, neutrophils and inflammatory macrophages, must be removed before the tissues return to homoeostasis.
Fig. 4. Electron micrographs (magnification × 11 000) of (a) normal human neutrophil granulocytes and (b) an apoptotic human neutrophil. In (a) note the irregular outline of the cell, the granular appearance of the nuclear chromatin and the large numbers of cytoplasmic granules. In (b) note the characteristic change in the nuclear chromatin, the prominent nucleolus and the dilated endoplasmic reticulum. These are the classical ultrastructural features of apoptosis. But note also that large numbers of apparently intact granules remain in the cytoplasm.

Clearance of fluid, proteins and debris

Lymphatic drainage, and phagocytosis and pinocytosis by inflammatory macrophages, are key processes in this phase. Most fluid is probably removed via the lymph vessels, although reconstitution of normal haemodynamics may contribute by restoring the balance of hydrostatic and osmotic forces in capillaries in favour of net fluid absorption at the venous end of the capillary. Proteolytic enzymes from plasma exudate and inflammatory cell secretions are likely to break down any fibrin clot at the inflamed site, and products of this digestion are drained by the lymphatics which become widely distended as the removal of fluid and proteins increases.

The macrophage may also play an important role in this phase. It can remove fluids, which might contain a variety of proteins, by the remarkable process of pinocytosis, which in activated inflammatory macrophages can be amplified to a volume uptake of 25% of the cell surface per minute [28]. It has long been recognized that inflammatory macrophages also have a greatly increased phagocytic potential. They can recognize opsonized and non-opsonized particles and they express cell-surface receptors for a wide variety of altered and damaged proteins and cells [29]. The critical role of macrophages in the clearance phase of inflammation was first recognized by Metchnikoff more than a century ago, and we are now beginning to elucidate the molecular mechanisms of some of his seminal observations.

Clearance of neutrophils

Despite our current awareness of the immense histotoxic potential of the neutrophil and its contents, the fate of this cell in situ has received surprisingly little attention or formal study until recently. There is no evidence that extravasated neutrophils return to the bloodstream or that lymphatic drainage provides an important disposal route, and it is generally thought that they meet their fate at the inflamed site. It has been widely assumed that neutrophils inevitably disintegrate before their fragments are removed by local macrophages [2]. If this was the case, healthy tissues would as a rule be exposed to large quantities of disgorge neutrophil contents. However, since the classical work of Metchnikoff, who was the first to catalogue the cellular events of the evolution and resolution of acute inflammation in vital preparations, there has been clear evidence for an alternative, injury-limiting fate whereby intact senescent neutrophils are removed by macrophages [30]. Over the intervening decades there have been a number of sporadic reports which describe apparently intact neutrophils within macrophages in diseased tissues [31] and in bone marrow preparations [32]. In experimental peritonitis, the engulfment of neutrophils by macrophages [33] was observed and, more recently it has been suggested [34] that the bulk of extravasated neutrophils is removed by this process.

The cellular and molecular mechanisms controlling the removal of senescent granulocytes are now beginning to be defined. Experiments in vitro with human cells showed that monocyte-derived macrophages or macrophages isolated from an actively inflamed site (but not monocytes themselves or resident alveolar macrophages) were able to recognize and ingest neutrophils that had been cultured for 24h, but did not ingest freshly isolated neutro-
for harvesting and culturing human neutrophils with from aggregation in culture[36] allowed us to minimal cell activation and avoiding loss of cells. We have found that ageing granulocytes undergo constitutive fashion without the addition of external stimuli[37]. Apoptosis was first described in situations in which large numbers of unwanted cells are removed in a 'programmed' or physiological fashion. These include thymus involution, gut crypt cell turnover and embryonic tissue remodelling. By contrast with cell necrosis, large numbers of cells can be removed by apoptosis without causing local tissue injury or inciting an inflammatory response (which would clearly be an undesirable side effect in the developing embryo, for example). These analogies suggested that the availability of this mechanism in neutrophils might represent a tissue injury-limiting process which would be expected to be important in the control of inflammatory tissue injury and the normal resolution process of inflammation. It has now been shown that this process determines the recognition and phagocytosis of intact senescent neutrophils by macrophages[27], and we have clear evidence of this process occurring in acute arthritides[27], neonatal lung injury[38] and resolving experimental pneumonia (Fig. 5).

The rate of apoptosis in neutrophil populations in vitro can be markedly inhibited by inflammatory mediators such as C5a, formylmethionyl-leucyl-phenylalanine, lipopolysaccharide and granulocyte-macrophage colony-stimulating factor (GM-CSF) (A. Lee, M. K. B. Whyte & C. Haslett, unpublished work). This inhibition is not associated with cell necrosis; indeed, it confers extended functional longevity, suggesting that longevity is controlled through apoptosis and that the apoptotic process itself can be modulated by environmental influences which might be expected to prolong the functional life of the neutrophil at inflamed sites. Although the underlying mechanisms remain obscure there has been a recent resurgence of interest in the internal cell biology of apoptosis, in part stimulated by its likely importance in the clonal deletion of lymphocytes[39], tumour kinetics[40] and T cell cytotoxicity[41]. The cellular and molecular events are poorly understood, but classical ultrastructural changes are associated with DNA cleavage in a characteristic internucleosomal fragmentation pattern indicative of endogenous endonuclease activation and (as yet unidentified) changes in the cell surface which are responsible for recognition of the apoptotic cell by macrophages. The intracellular mechanisms linking these 'end-events' and the processes responsible for triggering apoptosis remain obscure. Recent work in lymphoid cells has suggested that elevation of cytosolic calcium may be involved in the early induction events[42], and in the Bcl-2 system part of a genetic control mechanism inhibiting apoptosis in B lymphocytes has been uncovered[43]. By contrast with lymphoid cells, however, elevation of cytosolic calcium concentration appears to inhibit rather than promote neutrophil apoptosis (M. K. B. Whyte, L. C. Meagher, S. J. Hardwick, J. S. Savill & C. Haslett, unpublished work). This might be predicted from the knowledge that most inflammatory mediators exert their effects on neutrophil function at least in part by triggering signal transduction processes which lead to a rise in cytosolic calcium concentration. We have now shown that elevating intracellular calcium concentration by calcium ionophores inhibits the rate of neutrophil apoptosis and that intracellular calcium chelators such as BAPTA and MAPTAM enhance the rate of neutrophil apoptosis (M. K. B. Whyte, L. C. Meagher, S. J. Hardwick, J. S. Savill & C. Haslett, unpublished work). Why the intracellular controls of apoptosis in neutrophils and lymphoid cells should be different is presently obscure, but is the subject of intense study. Clearly one of the major research goals in this field is the purification and characterization of the endonuclease(s) responsible for the DNA cleavage which characterizes apoptosis[44].

Studies in vitro demonstrate that macrophages ingest and degrade apoptotic neutrophils at a very rapid rate. In Fig. 6(a) a macrophage that has ingested four apoptotic neutrophils is shown. In order to obtain this electron micrograph, it was necessary to fix the preparation immediately after the aged neutrophils had settled on the macrophage monolayer, and even so one of the apoptotic neutrophils was in a state of early degradation. Within minutes (Fig. 6b) ingested neutrophils were markedly degraded to the point at which they were

Fig. 5. Electron micrograph (magnification ×8000) of experimental pneumococcal pneumonia at 72 h (in the early clearance phase) showing a macrophage that has engulfed an apoptotic neutrophil.

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Fig. 6. Macrophage ingestion of apoptotic neutrophils in vitro (A. Slater & C. Haslett, unpublished work). Magnification \( \times 11000 \).

(a) Electron micrograph of a macrophage shortly after the ingestion of four apoptotic neutrophils. One is already beginning to disintegrate. (b) In less than an hour, ingested apoptotic neutrophils are at an advanced stage of degeneration.

no longer recognizable as neutrophils. This rapidity of uptake and subsequent degradation is likely to explain why the magnitude of these processes in resolving inflammation or other examples of remodelling tissues [44] may not have been appreciated from observations based on microscopy of 'static' histological sections.

While the uptake of many other particles by macrophages leads to the release of pro-inflammatory arachidonic acid products and cytokines, even maximal uptake of apoptotic neutrophils does not cause macrophages to release thromboxane \( \text{B}_2 \) [45] or a variety of other pro-inflammatory mediators and cytokines (L. C. Meagher et al., unpublished work). This remarkable lack of macrophage responsiveness appears to be determined by the specific recognition mechanism employed, since the ingestion of opsonized apoptotic neutrophils causes release of thromboxane \( \text{B}_2 \) by macrophages of a magnitude comparable with the effect of opsonized zymosan [45]. These observations lend further credence to the hypothesis that neutrophil apoptosis is an important process in the resolution of inflammation, and add further significance to the elucidation of the mechanisms by which macrophages recognize apoptotic neutrophils. Macrophage engulfment of apoptotic neutrophils involves a novel phagocytic recognition mechanism, modulated by local charge and pH, which utilizes macrophage surface molecules not previously implicated in phagocytosis [29]. (The recognition mechanism is dealt with in detail by Dr Savill in the following article.) That recognition is inhibited by cationic particles and by conditions of acid pH may well be relevant to the control of inflammation since chronically inflamed sites have been shown to be acidic and if apoptotic neutrophils are not cleared effectively their further degeneration to necrotic granulocytes would be expected to promote the release of granule products, many of which are highly cationic, thus setting up a potential vicious cycle process tending to amplify inflammation.

This work has recently been extended to studies of the eosinophil granulocyte. This cell, which has been specifically implicated in the pathogenesis of allergic diseases such as asthma, has much of the histotoxic potential of the neutrophil but contains a number of additional agents, such as major basic protein and eosinophil cationic protein, which are also highly toxic to mammalian cells. It had been suspected for some time that eosinophils have a longer tissue life than neutrophils, a concept supported by the observation that eosinophils survive for several days in culture. Using necrosis (the inability to exclude vital dyes) as the marker of ultimate cell demise in culture, it was observed that eosinophil survival was prolonged by fibroblast-conditioned medium [46], GM-CSF [47] and interleukin-5 (IL-5) [48]. We have recently shown that eosinophils, ageing in culture, became apoptotic many hours before there was any evidence of necrosis [49]. Furthermore, the rate of eosinophil apoptosis was retarded by GM-CSF and IL-5, and apoptotic eosinophils were recognized specifically and phagocytosed by macrophages before they became necrotic [49]. These observations suggest that tissue eosinophil longevity is determined by apoptosis and that the rate of this process is modulated by inflammatory mediators such as IL-5, which may exert an apoptosis inhibitory effect on eosinophil, but not on neutrophil apoptosis.

**SUMMARY**

We have identified a pathway for granulocyte removal in tissues which is controlled by apoptosis. This process can be modulated by agents in the inflammatory microenvironment and leads to loss of neutrophil secretory function and its phagocytosis
by macrophages which utilize a novel recognition mechanism such that the macrophage does not release pro-inflammatory mediators. It is hypothesized that this represents an alternative fate to necrosis, and one which would tend to limit tissue injury and promote inflammatory resolution. This is not to suggest that granulocyte necrosis does not occur: even at sites of 'beneficial inflammation' some necrotic neutrophils may be seen. However, since the development of inflammatory disease is currently thought to result from a multifactorial, quantitative imbalance between potentially injurious inflammatory influences and tissue defences, it is reasonable to suggest that the balance between neutrophil apoptosis and necrosis could represent one of several possible pivotal points in the control of inflammation. Finally, as the specific trigger and induction processes which permit closely related cells to undergo apoptosis at markedly different rates are uncovered, it may be possible to design new anti-inflammatory therapeutic strategies directed towards causing specific cell types to 'commit suicide' and to be cleared by the mechanisms which 'nature intended'.

Thus we have begun to dissect the cellular events occurring in the resolution of acute inflammation. We believe that the rapid cessation of neutrophil emigration which occurs remarkably early in the evolution of the acute inflammatory response represents one of the earliest events in the resolution process. Clearly there is much work to be done on the underlying mechanisms. These are likely to be more accessible with the recent molecular characterization of chemotactic cytokines and surface molecules involved in neutrophil-endothelial adhesion transmigration events. It is also clear that apoptosis, which represents an alternative tissue injury-limiting fate to necrosis in situ, may be important in limiting tissue injury and determining whether inflammation persists or resolves. It is also possible that there are circumstances in which the macrophage recognition and clearance of apoptotic neutrophils is impaired.

However, a second glance at the events which must occur during the resolution of even the simplest model of acute inflammation (Fig. 1) is sufficient reminder that the 'surface has barely been scratched'. There remain many obscure areas, not the least of which is the fate of the inflammatory macrophage after it has completed its scavenging functions.

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