0014-4754/96/110950-07$1.50 + 0.20

Recognition of apoptotic cells by phagocytes

S. P. Hart, C. Haslett and I. Dransfield*

Respiratory Medicine Unit, The Rayne Laboratory, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG (United Kingdom), Fax +44 131 650 4384

Abstract. Effective removal of dying cells is crucial to a variety of processes in health and disease. Cells undergoing apoptosis are recognized and ingested intact by phagocytes, which are not stimulated to release inflammatory mediators. The alternative uncontrolled form of cell death, necrosis, is associated with release of cell contents with the potential to cause tissue damage and inflammation. Four distinct molecular mechanisms have been identified to date which mediate recognition by phagocytes of mammalian cells undergoing apoptosis, but further mechanisms remain to be discovered. The capacity for phagocyte removal of cells undergoing apoptosis may be closely regulated, for example by local cytokines.
Key words. Apoptosis; integrins; lectins; macrophage; phagocytosis; scavenger receptors.

Introduction

Although apoptotic cell death occurs continuously within tissues, isolated apoptotic cells with their characteristic appearance are rarely visualized in tissue sections by light microscopy. It is now well established that cells undergoing apoptosis are recognized and rapidly ingested by neighbouring phagocytes. In contrast to phagocytosis of microbes and other foreign material, phagocytosis of apoptotic cells occurs independently of antibody or complement and does not induce tissue injury or inflammation [1]. Removal of senescent cells plays a vital role in many fundamental biological processes, including normal tissue turnover [2], remodelling of embryological tissues [3], development of the immune system [4] and resolution of inflammation [5, 6]. For example, pneumococcal pneumonia in humans and experimental animals is associated with massive emigration of inflammatory leukocytes, particularly polymorphonuclear neutrophils, from the blood into the lungs, such that the alveolar air spaces become almost completely filled with neutrophils. Neutrophils contain many toxic molecules (e.g. oxidants, enzymes, cationic proteins and inflammatory mediators) with the potential for causing tissue damage, propagation of inflammation, and subsequent fibrosis should the cells undergo necrosis, lose membrane integrity and disintegrate [7]. Despite this, during the resolution of pneumococcal pneumonia billions of neutrophils which die in situ are removed intact from the lungs by macrophages, resulting in preservation of the normal delicate lung architecture [8] (fig. 1).

Phagocytosis of senescent cells was first reported in the late 19th century by the Russian biologist Elie Metchnikoff who, using a simple light microscope, observed that 'microphages' (neutrophils) were 'englobed' by macrophages in injured tadpole fins. Light microscopy of human pathology specimen sections may also reveal apoptotic cells inside other cells (e.g. Reiter's cells are macrophages containing apoptotic neutrophils in joint aspirates from patients with inflammatory arthritis). It is thought that in mammals tissue macrophages are the key cells that remove apoptotic cells. However, cells of other lineages (e.g. hepatocytes [9], endothelial cells [10], glomerular mesangial cells [11], and fibroblasts [12]) may also act as 'nonprofessional' phagocytes and remove apoptotic cells, contributing to the regulation of apoptotic cell load within tissues.

An in vitro assay of phagocyte recognition of apoptotic cells

In an attempt to understand how inflammation resolves, Newman and colleagues developed an in vitro model of the interaction between human monocyte-derived macrophages and neutrophils [13] which has served as the basis for many subsequent studies of phagocytosis of apoptotic cells. Monocyte-derived macrophages, grown adherent to culture plastic wells, were overlaid with a suspension of either freshly isolated neutrophils or neutrophils aged for 24 h in culture. Non-ingested neutrophils were washed away, the ingested intact neutrophils were selectively stained for myeloperoxidase, and the proportion of macrophages ingesting neutrophils was quantified by light microscopy (fig. 2). This study demonstrated that macrophages recognized and ingested intact neutrophils that had been aged in culture, but that fresh neutrophils were not recognized. A limitation of methods based on direct counting of cells by light microscopy is that it is difficult (and often impossible) to distinguish between

* Corresponding author.

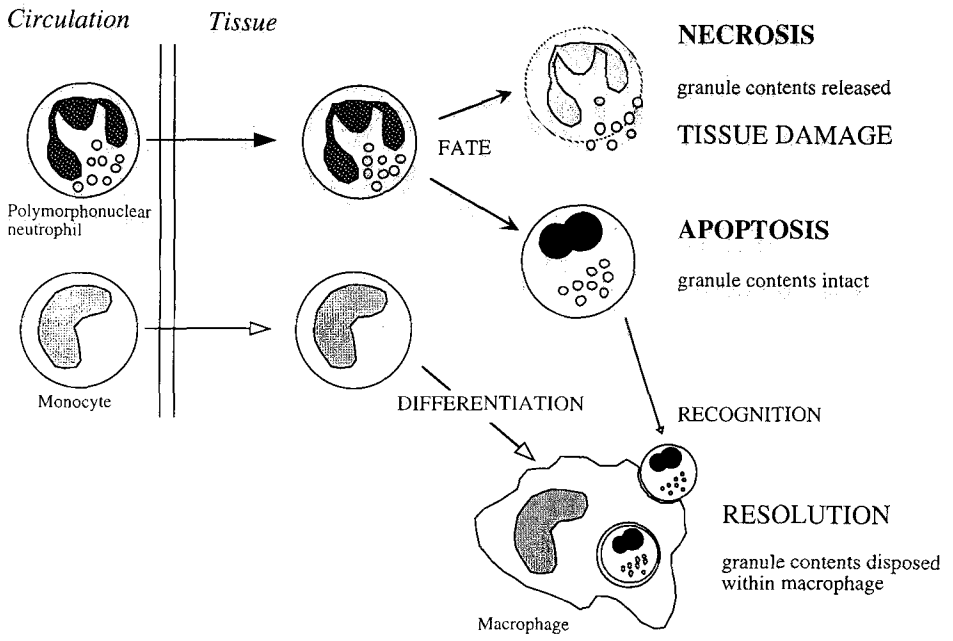

Figure 1. Schematic representation of the fate of neutrophils. Polymorphonuclear neutrophils and monocytes are recruited from the blood to an inflammatory site. Neutrophils may undergo necrosis, which is associated with loss of membrane integrity and release of damaging granule contents. Alternatively, neutrophil apoptosis results in recognition and ingestion of the intact leukocyte by monocyte-derived macrophages, so favouring resolution of inflammation [8].

binding and ingestion of the apoptotic cell by the phagocyte (although attempts have been made to dissect the relative prevalence of the two processes; see fig. 3). In this review we use the term 'recognition' to describe the interaction between apoptotic cells and phagocytes.

Phagocytes ingest apoptotic cells but not healthy cells

In 1989 Savill and colleagues extended the studies of Newman et al. and demonstrated that it was the process of apoptosis in the aging human neutrophil that leads to its recognition in vitro. Neutrophils isolated from the blood of healthy human subjects were found to undergo apoptosis spontaneously over a period of up to 24 h in culture. Separation of aged neutrophils by elutriation into fractions containing different proportions of apoptotic cells demonstrated that macrophage recognition was directly correlated with the proportion of apoptotic cells present [6]. Similarly, human joint-derived inflammatory macrophages preferentially recognize apoptotic

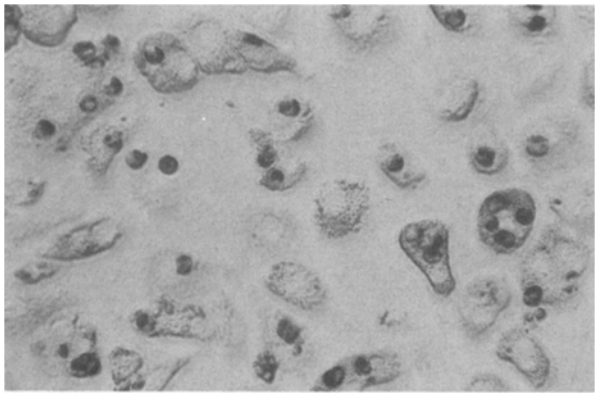

Figure 2. Photomicrograph of the in vitro interaction between macrophages and apoptotic neutrophils [13]. Six-day-old human monocyte-derived macrophages have been overlaid with a suspension of apoptotic neutrophils, and the interaction allowed to proceed for 30 min. Ingested neutrophils have been stained for myeloperoxidase. Note that macrophages are myeloperoxidase-negative.

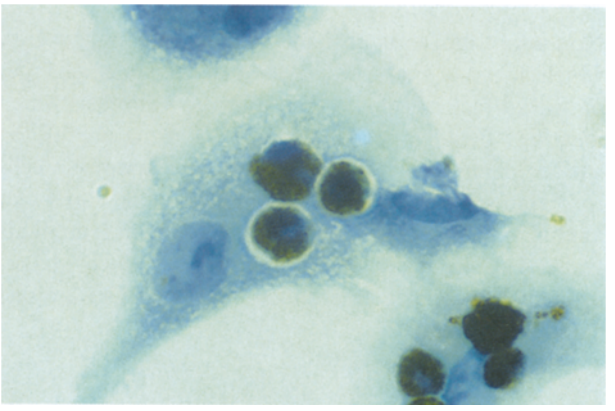

Figure 3. Three intact apoptotic neutrophils (stained for myeloperoxidase) are demonstrated within phagocytic vacuoles inside a macrophage (counterstained with Diff-Quik). Following the interaction the macrophages were detached from the plastic wells and cytocentrifuged onto a glass slide before staining.

Table 1. Molecular mechanisms of recognition of apoptotic cells by phagocytes.

Mechanism	Animal species	Apoptotic cell	Phagocyte
Lectin-like molecule [14]	mouse	thymocyte	peritoneal macrophage
Asialoglycoprotein receptor/ mannose receptor [9, 10]	rat	liver cell	liver cell liver sinusoid endothelial cell
A mannose/fucose receptor [12]	human	neutrophil	fibroblast
Vitronectin receptor, CD36, TSP [23, 12, 16]	human	neutrophil	monocyte-derived macrophage fibroblast
	mouse	lymphocyte	bone marrow-derived macrophage
Phosphatidylserine receptor [22]	mouse	lymphocyte	peritoneal macrophage bone marrow-derived macrophage (simulated with digestible particles)
	human		THP-1 cell
Scavenger receptor [26]	mouse	thymocyte	peritoneal macrophage thymic macrophage
61D3 antigen [19]	human	lymphocyte neutrophil	monocyte-derived macrophage

rather than non-apoptotic neutrophils, suggesting that this mechanism can function in vivo.

Phagocytosis of apoptotic cells does not cause tissue damage or inflammation

Ingestion of antibody-coated particles by macrophages results in the acquisition of an inflammatory phenotype and release of a variety of mediators that potentiate inflammation (e.g. cytokines and arachidonic acid metabolites). Phagocytosis of apoptotic cells does not provoke this response [1], which is consistent with the existence of a distinct mechanism for identification and removal of senescent cells.

Phagocyte mechanisms for recognition of cells undergoing apoptosis

Non-apoptotic cells are not ingested by phagocytes, which suggests that phagocytes can recognize changes on the surface of cells as they undergo apoptosis which identifies them as 'senescent self'. The nature of the surface changes involved in identifying apoptotic cells for removal has been investigated using a variety of animal species, cell types and experimental methods (table 1). The different mechanisms by which phagocytes may recognize cells undergoing apoptosis are discussed below.

Lectin-like receptors

Mouse thymocytes can be induced to undergo apoptosis by exposure to corticosteroids, and they are then recognized by isologous peritoneal macrophages. This interaction was specifically inhibited by the carbohydrates *N*-acetylglucosamine and *N*,*N*-diacetylchitibiose [14]. In contrast, macrophage recognition of immunoglobulin-opsonized erythrocytes was unaffected, excluding a non-specific inhibition of phagocytosis. These experiments led to the first suggestion that lectin-like molecules may mediate macrophage recognition of changes in the surface carbohydrates on apoptotic cells. In support of this suggestion, neonatal rat liver cells form apoptotic envelopes which react with labelled lectins indicating exposure of surface carbohydrates that have lost sialic acid residues. Apoptotic rat liver cells are ingested by neighbouring liver cells which express the asialoglycoprotein receptor (ASGPR), an interaction that can be inhibited by antibodies against the ASGPR and by sugars (*N*-acetylgalactosamine and D-galactose) and glycoproteins (lactosylated bovine serum albumin and asialofetuin) that are ligands for the ASGPR [9]. Inhibition by *N*-acetylglucosamine and mannose suggests additional participation by the mannose receptor. Both in vivo and in vitro studies have shown that recognition of labelled rat liver apoptotic bodies by rat hepatic sinusoid endothelial cells is inhibited by pre-exposure of the endothelial cells to a mixture of sugars which bind to the ASGPR and the mannose receptor [10]. Mannan (a mannose-rich glycoprotein) and fucoidin (a sulphated fucose polysaccharide) were found to inhibit recognition of apoptotic neutrophils by human fibroblasts, suggesting that a novel lectin-like molecule may be involved, distinct from the mannosyl-fucosyl receptor which is not expressed by fibroblasts or neutrophils [12].

The vitronectin receptor/CD36

The integrin $\alpha_v\beta_3$ (vitronectin receptor, VnR) is one of a family of heterodimeric cell surface receptors involved

in adhesion of cells to extracellular matrix components (e.g. laminin, fibronectin, vitronectin). A possible role for integrins in the recognition of apoptotic cells by phagocytes was suggested by the observation that aminosugars and basic amino acids, which inhibit ligand binding to integrins, inhibited recognition of apoptotic cells [15]. Further evidence for the role of an integrin was demonstrated by the dependence of recognition on divalent cations (Ca^{2+} and Mg^{2+}). In experiments using human monocyte-derived macrophages and human neutrophils which undergo apoptosis in culture, Savill and colleagues demonstrated that macrophage recognition of apoptotic neutrophils was specifically inhibited by monoclonal antibodies against either the α or β subunits of the VnR [16]. Ligand binding by $\alpha_v\beta_3$ is dependent on the sequence of amino acids arginine-glycine-aspartic acid (RGD) in the ligand. The RGD-bearing proteins vitronectin and fibronectin and the synthetic tetrapeptide RGDS were found to inhibit macrophage recognition of apoptotic neutrophils, whereas the control peptide RGES had no effect. These agents were shown to act on the macrophage rather than on the apoptotic neutrophil.

It was subsequently demonstrated that thrombospondin (TSP), an adhesive RGD-bearing glycoprotein which binds to the macrophage surface receptor CD36, cooperates with the VnR in the recognition of human neutrophils undergoing apoptosis [17]. TSP could be measured in the supernatant from the macrophage/apoptotic neutrophil interaction, and recognition was inhibited by pretreatment of macrophages with the protein synthesis inhibitor cycloheximide or by the addition of anti-TSP antibodies, and 'rescued' by addition of exogenous TSP. Exogenous TSP also enhanced the basal level of recognition if preincubated with either the macrophages or the apoptotic neutrophils, suggesting that it may function as a 'molecular bridge' between the two cell types, comparable to its role in platelet aggregation. A role for the TSP receptor CD36 was suggested by inhibition of recognition by anti-CD36 antibodies [17], and in recent studies in which transfection of melanoma cells with CD36 conferred capacity for phagocytosis of a variety of cell types undergoing apoptosis [18]. Recognition of neutrophils by human fibroblasts in culture may also be mediated by the VnR, with the possible additional involvement of a lectin-like receptor (see above). However, the VnR mechanism for human macrophage recognition of apoptotic cells does not appear to be universal. In some subjects the VnR mechanism cannot be demonstrated, although an alternative mechanism involving the macrophage 61D3 antigen appears to be involved (see below), in contrast with other subjects in whom both VnR and 61D3-ligand mechanisms can be shown to be involved [19].

The phosphatidylserine receptor and the scavenger receptor

Phosphatidylserine is a negatively charged membrane phospholipid which is normally confined to the inner half of the plasma membrane bilayer. Recent evidence has shown that phosphatidylserine appears on the surface of cells at an early stage during apoptosis. Loss of phospholipid asymmetry of the intact plasma membrane is independent of cell type or stimulus to apoptosis, and altered activity of an adenosine triphosphate-dependent phospholipid translocase or a non-specific lipid scramblase have been postulated to be responsible [20, 21]. Following the observation that macrophages bind to resealed red blood cell ghosts which have increased amounts of phosphatidylserine on the outer surface of the plasma membrane, it was shown that thioglycollate-elicited peritoneal murine macrophages recognize apoptotic cells by a phosphatidylserine receptor (PSR)-dependent mechanism. In an in vitro model, macrophage binding of apoptotic lymphocytes was stereospecifically inhibited by L-isoforms of phosphatidylserine, and inhibited by liposomes containing phosphatidylserine but not by liposomes containing other anionic phospholipids [22]. Fadok and colleagues subsequently demonstrated that the mechanism used by phagocytes for recognizing cells undergoing apoptosis depends upon the subpopulation of macrophages, and is independent of the type of apoptotic cell. Whereas murine elicited peritoneal macrophages and phorbol ester-stimulated THP-1 cells (a human monocyte-like cell line) utilize the PSR mechanism, murine bone marrow-derived macrophages utilize the VnR mechanism, as demonstrated for human monocyte-derived macrophages [23, 16]. Exposure of murine bone marrow-derived macrophages to β1,3 glucan (a digestible particle which induces an inflammatory phenotype) resulted in a switch from VnR-mediated to PSR-mediated recognition of apoptotic cells, with no associated change in VnR expression on the plasma membrane [24]. Expression of the PSR was mediated by macrophage secretion of transforming growth factor-β (TGF-β), a cytokine which acts in an autocrine and paracrine fashion to prime the macrophage for development of the inflammatory phenotype.

One possible candidate for the PSR is a member of the scavenger receptor family. The term 'scavenger receptor' was initially used to describe the macrophage surface binding site for chemically modified (acetylated or oxidized) forms of low density lipoprotein (LDL), a package of cholesteryl ester, phospholipid and protein that allows hydrophobic lipids to circulate in the blood. The original scavenger receptor (class A scavenger receptor, previously called the acetyl-LDL receptor) was demonstrated to mediate endocytosis of modified LDL independently of specific LDL receptors, and was implicated in the pathogenesis of atherosclerosis [25]. Unlike

most cell surface receptors, class A scavenger receptors bind with high affinity to a range of different ligands, such as modified LDL and other proteins, polyribonucleotides, polysaccharides, anionic phospholipids (such as phosphatidylserine) and a variety of synthetic molecules [25]. The monoclonal antibody 2F8, which binds specifically to murine class A scavenger receptors, partially blocks ingestion of apoptotic thymocytes by murine thymic macrophages or peritoneal macrophages [26]. Almost complete inhibition of recognition can be achieved when macrophages are preincubated with the scavenger receptor ligand polyinosinic acid, but not by related polyribonucleotides. Interestingly, CD36, which has been shown to cooperate with the VnR and TSP in recognition of apoptotic cells, may also bind to anionic phospholipids [27]. Scavenger receptors may have a role in atherosclerosis, cell adhesion, host defence and possibly recognition of apoptotic cells [28, 25]. Oxidatively damaged human red blood cells (a model for ageing red cells in vivo) are recognized and ingested by murine peritoneal macrophages in an antibody-independent fashion using a scavenger receptor which binds oxidized LDL but not acetylated or native LDL [29]. Oxidized LDL also inhibits macrophage binding of eythrocytes damaged by glutaraldehyde or malondialdehyde, which cause cross-linking of plasma membrane proteins. Red blood cells are enucleate and do not undergo apoptosis, but further studies demonstrated that macrophage binding of apoptotic mouse thymocytes (and oxidized erythrocytes) is specifically but incompletely inhibited by oxidized LDL and phosphatidylserine liposomes, and that the VnR is not involved [30]. Ligand blotting studies demonstrated that both phosphatidylserine and oxidized LDL bound to the same 94–97 kDa membrane protein which is identical to macrosialin, the mouse homologue of human CD68 [31]. However, when compared with murine resident peritoneal macrophages, elicited macrophages have higher macrosialin expression and bind oxidized LDL more avidly, but they bind oxidatively damaged erythrocytes less well. In addition, the mouse macrophage cell line RAW264.7 expresses macrosialin and binds phosphatidylserine liposomes and oxidized LDL, but does not bind oxidatively damaged red blood cells. These findings suggest that although macrosialin may be involved in recognition of phosphatidylserine on murine apoptotic cells, there may be additional complexity in receptor specificity.

The 61D3 antigen

Recognition of a variety of apoptotic cell types by human monocyte-derived macrophages can be specifically inhibited by the monoclonal antibody 61D3 [19]. The 61D3 antigen has not been fully characterized, but preliminary results suggest it is a 75-kD single-chain polypeptide (i.e. distinct from the VnR), expression of which is restricted to cells of the mononuclear phagocyte lineage. Pretreatment of macrophages with intact 61D3 or $F(ab')_2$ fragments inhibited recognition of apoptotic BL cells (a human lymphoma cell line), neutrophils, T lymphocytes and B lymphocytes, but pretreatment of the apoptotic cells with 61D3 had no effect on the interaction. Although some human subjects utilize both the VnR and the 61D3 antigen for macrophage recognition of apoptotic cells, total inhibition of recognition cannot be demonstrated even when both anti-VnR and 61D3 antibodies are used in combination. The implication of this observation is that further unidentified molecular recognition pathways may be involved in the removal of apoptotic cells.

Control of removal of apoptotic cells

Removal of effete neurophils from an inflamed site would be expected to be subject to control mechanisms. VnR-mediated phagocytosis of cells undergoing apoptosis is specifically augmented in vitro by pro-inflammatory cytokines (GM-CSF, interleukin-1β interferon-γ, tumour necrosis factor-α) and by TGF-1β (which may exhibit pro-inflammatory and anti-inflammatory effects in different situations). Preincubation of macrophages with any of these agents for 6 h increased the proportion of macrophages that recognized apoptotic neutrophils, and also increased the phagocytic capacity of individual macrophages. However, there was no associated change in expression of the VnR or CD36 on the surface of the macrophage, no change in macrophage expression or secretion of TSP, and no recruitment of a PSR-dependent recognition mechanism [32]. During inflammation the effect of proinflammatory cytokines to potentiate resolution by hastening removal of apoptotic neutrophils may seem paradoxical, but it may be a way of exerting negative feedback to control the inflammatory response. GM-CSF also plays a role in monocyte-macrophage maturation, and may prolong the life span of neutrophils at a site of inflammation by inhibiting the rate of apoptosis, and so regulate the number of neutrophils available for recognition by macrophages.

Recent experiments in our laboratory suggest that the adhesive state of the macrophage regulates the capacity for removal of apoptotic cells by an $\alpha_v\beta_3$-independent mechanism. Elevation of intracellular cyclic adenosine monophosphate in macrophages rapidly and specifically inhibits recognition of apoptotic neutrophils, and is associated with changes in the localization of the cytoskeletal proteins actin and talin within the macrophages suggestive of altered adhesion (unpublished observations).

Conclusion

Removal of apoptotic cells by phagocytes is crucial to many processes in health and disease, but the mechanism by which cells undergoing apoptosis are recognized as 'senescent self' are only just beginning to be understood. In *Caenorhabditis elegans* (a nematode worm which has become a principal model for developmental genetics) seven different genes have been identified which are involved in removal of dying cells by phagocytes [33]. In mammals, too, there appears to be significant redundancy in the mechanisms used by phagocytes to recognize cells undergoing apoptosis. Four distinct molecular mechanisms have been identified thus far in humans and rodents ($\alpha_v\beta_3$/CD36, 61D3 antigen, lectin-like receptors, and the PSR). Whilst removal of apoptotic neutrophils by human monocyte-derived macrophages failed to induce release of proinflammatory mediators (e.g. thromboxane B_2), similar studies have yet to be performed for other recognition mechanisms. The phenotype of the phagocyte population may determine which mechanism is utilized for removal of apoptotic cells. Modulation of macrophage phenotype by inflammatory cytokines may result in recruitment of previously unresponsive cells, or alter which recognition mechanism is used.

Furthermore, although all human monocyte-derived macrophages express $\alpha_v\beta_3$ and CD36, under normal conditions less than half of the macrophage population are able to ingest apoptotic cells. Moreover, simultaneous blockade of both $\alpha_v\beta_3$ and CD36 fails to inhibit recognition completely. In all experimental systems examined to date, no single mechanism can account for all the observed recognition of apoptotic cells, raising the possibility of the existence of further recognition mechanisms.

It is possible that multiple recognition mechanisms operating in parallel may provide a fail-safe to ensure that clearance of apoptotic cells occurs effectively. The capacity for phagocytic removal of apoptotic cells was apparently overwhelmed in mice treated with anti-Fas antibody, which resulted in necrotic cell death and severe tissue damage [34]. The recognition mechanism that is employed may be determined by changes associated with progression of the inflammatory response. This might account for the observed switching between apparently mutually exclusive removal mechanisms. In addition, different removal pathways may be employed depending upon the apoptotic cell stimulus. Thus, early membrane alterations associated with apoptosis such as exposure of phosphatidylserine may signal phagocytosis via the PSR pathway, whereas changes that occur later in the apoptotic process, for example altered acetylation or glycosylation of membrane proteins, would recruit scavenger receptor-dependent removal mechanisms. Thus, there would be multiple opportunities for apoptotic cell recognition by phagocytes that would ensure their complete removal prior to cellular necrosis and associated tissue damage. In support of this suggestion, we have recently demonstrated that apoptotic cells defined by exposure of an antigen as undergoing 'late' apoptosis are recognized and removed by a different mechanism than that used during 'early' apoptosis (unpublished observations).

During the host defence response any perturbation in the balance between neutrophil recruitment from the circulation, their life span at the site of inflammation, and their removal intact by macrophages may determine whether inflammation terminates by resolution or progresses to chronic inflammation and scarring. Neutrophil-mediated tissue damage has been implicated in the pathogenesis of a variety of diseases, such as rheumatoid arthritis, idiopathic pulmonary fibrosis, and adult respiratory distress syndrome [35]. More information about the mechanisms controlling removal of apoptotic cells by phagocytes may lead to development of therapies that modulate the inflammatory response such that resolution is favoured rather than tissue destruction and scarring.

Acknowledgements. S.H. is a University of Edinburgh Medical Faculty Research Fellow; C.H. is supported by a Medical Research Council programme grant.

1 Meagher L. C., Savill J. S., Baker A., Fuller R. W. and Haslett, C. (1992) Phagocytosis of apoptotic neutrophils does not induce macrophage release of thromboxane B_2. J. Leuk. Biol. **52:** 269–273
2 Han H., Iwanaga T., Uchiyama Y. and Fujuti, T. (1993) Aggregation of macrophages in the tips of intestinal villi in guinea pigs: their possible role in the phagocytosis of effete epithelial cells. Cell Tissue Res. **271:** 407–416
3 Hopkinson-Woolley J., Hughes D., Gordon S., and Martin P. (1994) Macrophage recruitment during limb development and wound healing in the embryonic and foetal mouse. J. Cell Science **107:** 1159–1167
4 Cohen J. J. (1991) Programmed cell death in the immune system. Adv. Immunol. **50:** 55–85.
5 Akbar A. N., Savill J. S., Gombert W., Bofill M., Borthwick N. J., Whitelaw F., Grundy J., Janossy G. and Salmon M. (1994) The specific recognition by macrophages of CD8+, CD45RO+ T cells undergoing apoptosis: a mechanism for T cell clearance during resolution of viral infections. J. Exp. Med. **180:** 1943–1947
6 Savill J. S., Wyllie A. H., Henson J. E., Walport M. J., Henson P. M. and Haslett C. (1989) Macrophage phagocytosis of aging neutrophils in inflammation: programmed cell death in the neutrophil leads to its recognition by macrophages. J. Clin. Invest. **83:** 865–875
7 Hurley J. V. (1983) Terminations of acute inflammation I. Resolution. In: Acute Inflammation, 2nd edition, pp. 109–117, Hurley. J. V. (ed.), Churchill Livingstone, London
8 Savill J. S. and Haslett C. (1994) Fate of neutrophils. In: Immunopharmacology of Neutrophils, pp. 295–314, Hellewell P. G. and Williams T. J. (eds), Academic Press, London
9 Dini L., Autuori F., Lentini A., Oliverio S. and Piacentini M. (1992) The clearance of apoptotic cells in the liver is mediated by the asialoglycoprotein receptor. FEBS Lett. **296:** 174–178
10 Dini L., Lentini A., Diez Diez G., Rocha M., Flasca L., Serafino L. and Vidal-Vanaclocha F. (1995) Phagocytosis of apoptotic bodies by liver endothelial cells. J. Cell Science **108:** 967–973

11 Savill J. S., Smith J., Sarraf C., Ren Y., Abbot, F. and Rees A. (1992) Glomerular mesangial cells and inflammatory macrophages ingest neutrophils undergoing apoptosis. Kidney Int. **42:** 924–936
12 Hall S. E., Savill J. S., Henson P. M. and Haslett C. (1994) Apoptotic neutrophils are phagocytosed by fibroblasts with participation of the fibroblast vitronectin receptor and involvement of a mannose/fucose-specific lectin. J. Immunol. **153:** 3218–3227
13 Newman S. L., Henson J. E. and Henson P. M. (1982) Phagocytosis of senescent neutrophils by human monocyte-derived macrophages and rabbit inflammatory macrophages. J. Exp. Med. **156:** 430–442
14 Duvall E., Wyllie A. H. and Morris R. G. (1985) Macrophage recognition of cells undergoing programmed cell death (apoptosis). Immunology **56:** 351–358
15 Savill J. S., Henson P. M. and Haslett C. (1989) Phagocytosis of aged human neutrophils by macrophages is mediated by a novel "charge-sensitive" recognition mechanism. J. Clin. Invest. **84:** 1518–1527
16 Savill J. S., Dransfield I., Hogg N. and Haslett C. (1990) Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. Nature **342:** 170–173
17 Savill J. S., Hogg N., Ren Y. and Haslett C. (1992) Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. J. Clin. Invest. **90:** 1513–1522
18 Ren Y., Silverstein R. L., Allen J. and Savill, J. S. (1995) CD36 gene transfer confers capacity for phagocytosis of cells undergoing apoptosis. J. Exp. Med. **181:** 1857–1862
19 Flora P. K. and Gregory G. D. (1994) Recognition of apoptotic cells by human macrophages: inhibition by a monocyte/macrophage-specific monoclonal antibody. Eur. J. Immunol. **24:** 2625–2632
20 Martin S. J., Reutelingsperger C. P. M., McGahon, A. J., Rader J. A. van Schie R. C. A. A., LaFace D. M. and Green D. R. (1995) Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of bcl-2 and Abl. J. Exp. Med. **182:** 1545–1556
21 Verhoven B., Schlegel R. A. and Williamson P. (1995) Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. J. Exp. Med. **182:** 1597–1601
22 Fadok V. A., Voelker D. R., Campbell P. A., Cohen, J. J., Bratton D. L. and Henson P. M. (1992) Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J. Immunol. **148:** 2207–2216
23 Fadok V. A., Savill J. S., Haslett C., Bratton, D. L., Doherty D., Campbell P. A. and Henson P. M. (1992) Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. J. Immunol. **149:** 4029–4035
24 Fadok V. A., Laszlo D. J., Noble P. W., Weinstein L., Riches D. W. H. and Henson P. M. (1993) Particle digestibility is required for induction of the phosphatidylserine recognition mechanism used by murine macrophages to phagocytose apoptotic cells. J. Immunol. **151:** 4274–4285
25 Kreiger M. and Herz J. (1994) Structure and function of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). Annu. Rev. Biochem. **63:** 601–637
26 Platt N. and Gordon S. (1995) Role of murine scavenger receptor in the recognition of apoptotic thymocytes by macrophages. J. Cell. Biochem. (Suppl.) **19B:** 300 (abstract)
27 Rigotti A., Acton S. L. and Krieger M. (1995) The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. J. Biol. Chem. **270:** 16221–16224
28 Fraser I., Hughes D. and Gordon S. (1993) Divalent cation-independent macrophage adhesion inhibited by monoclonal antibody to murine scavenger receptor. Nature **364:** 343–346
29 Sambrano G. R., Parsatharathy S. and Steinberg D. (1994) Recognition of oxidatively damaged erythrocytes by a macrophage receptor with specificity for oxidized low density lipoprotein. Proc. Natl Acad. Sci. USA **91:** 3265–3269
30 Sambrano G. R. and Steinberg D. (1995) Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine. Proc. Natl Acad. Sci. USA **92:** 1396–1400
31 Ramprasad M. P., Fischer W., Witztum J. L., Sambrano G. R., Quehenberger O. and Steinberg D. (1995) The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosialin, the mouse homologue of human CD68 Proc. Natl Acad. Sci. USA **92:** 9580–9584
32 Ren Y. and Savill J. S. (1995) Proinflammatory cytokines potentiate thrombospondin-mediated phagocytosis of neutrophils undergoing apoptosis. J. Immunol. **154:** 2366–2374
33 Ellis R. E., Yuan J. and Horvitz H. R. (1991) Mechanisms and functions of cell death. Annu. Rev. Cell Biol. **7:** 663–698
34 Ogasawara J., Watanabe-Fukunaga R., Adachi M., Matsuzawa A., Kasugai T., Kitamura Y., Itoh N., Suda T. and Nagata S. (1993) Lethal effect of the anti-Fas antibody in mice. Nature **364:** 806–809
35 Weiss S. J. (1989) Mechanisms of disease: tissue destruction by neutrophils. N. Eng. J. Med. **320:** 365–376