Editorial

Phagocytic clearance of apoptotic cells: food for thought

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Apoptosis aficionados readily appreciate the importance of the swift and (phlogistically speaking) quiet phagocytosis of apoptotic cells that is observed *in situ*. The 'melting away' of the apoptotic cell into its surroundings confounded many a histologist and the efficiency of the clearance process played a large part in shielding the significance of apoptosis from the biomedical research community (after all, if evidence of apoptosis is not easy to spot *in vivo*, how important could the process possibly be?). Happily, the days of profound scepticism over the significance of programmed cell death, and apoptosis in particular, are over. We now even have scientific journals devoted to the subject! But how many papers of the tens of thousands published each year in the cell-death field are devoted to understanding the clearance process? The answer is only a handful. Does this reflect the passive acceptance by the scientific community in apoptosis of the importance of clearance and a lack of real need to understand the details? The following reviews by Valerie Fadok and colleagues and by Yi Ren and John Savill explain why we should be studying the clearance process and what we know about it so far.

How important to apoptosis, then, is the ability to be eaten? Intuitively, it is difficult to appreciate that a cell-death process that allowed cells to cause damage to their neighbours could be in any way useful. This would, we perceive, be the logical consequence of the apoptotic cell not being eaten. Of course, as any student of cell death knows, such a tissue-damaging physiologically useless process is manifest in necrosis, accidental cell death that is widespread and, incidentally, much more readily appreciated histologically than apoptosis. As Ren and Savill point out, we assume, quite rationally, that the apoptotic cell, if it remains uncleared, becomes secondarily necrotic and, as such is a potentially injurious cell. This assumption has yet to be proven: formal proof awaits *in vivo* studies of clearance-blockade. Apart from the genetic studies in *Caenorhabditis elegans*, modulation of clearance *in vivo* has not been investigated, not least because of practical problems caused by the apparent complexity of the clearance mechanisms. However, there is much circumstantial evidence that an uncleared apoptotic cell is a potentially dangerous cell and both reviews highlight the likelihood that, in addition to the dangers caused by the intracellular constituents of the leaky secondarily-necrotic cell, early alteration of the apoptotic cell surface could itself be problematic if the cell were to remain uncleared. Thus, the early exposure of phosphatidylserine (PS) on the

apoptotic cell surface would be expected to be a source of danger if the cell were to persist in the vascular spaces since the exposed PSS has pro-coagulant activity.

The systematic phagocytosis of apoptotic cells has a further non-injurious feature: it is appropriately non-inflammatory. Indeed, evidence is beginning to emerge that phagocytosis of apoptotic cells is not simply passively non-inflammatory but rather is actively anti-inflammatory (Voll *et al*, 1997; Fadok *et al*, 1998). It is particularly intriguing that a macrophage receptor, CD14, that is involved in the recognition and non-phlogistic removal of apoptotic cells (Devitt *et al*, 1998) achieved prior fame as a receptor for the bacterial endotoxin, lipopolysaccharide, after binding which the macrophage elicits pro-inflammatory responses. Apart from raising the important question of how this receptor directs opposite macrophage responses, these results lead us to speculate on the chicken-and-egg question of the evolution of receptors for apoptotic cells: were receptors for 'apoptotic self' fortuitously useful as innate receptors for potentially infectious non-self, or *vice versa*?

As well as preventing tissue damage by exposure to apoptotic cell components – be they intracellular or surface-exposed – phagocytosis of apoptotic cells serves another useful purpose: it disposes efficiently of potentially immunologically active cellular constituents that might otherwise promote autoimmune phenomena and autoimmune disease pathogenesis. Or does it? Again we rely on intuition, since little experimental data on the many immunological implications of apoptotic cell clearance are yet available. Logically, removal of apoptotic cells before exposure of intracellular antigens to the humoral arm of the immune system would be expected to militate against autoantibody production. Similarly, presentation of peptides derived from 'apoptotic self' to T cells would be expected to lead to tolerogenic signals. It is particularly interesting that, in addition to macrophages, dendritic cells, antigen-presenting cells par excellence that are capable of eliciting primary immune responses are able to phagocytose apoptotic cells and present apoptotic cell-derived antigens to T cells (Rubartelli *et al*, 1997; Albert *et al*, 1998). Again, rational thought would suggest that such presentation should normally be tolerogenic, but is this always so? Again, the field begs more experimental data.

Accepting that phagocytic clearance of apoptotic cells really *is* important, how is it achieved? The excellent review by Fadok and colleagues considers this in depth. Amateur phagocytes such as fibroblasts, epithelial cells, smooth muscle cells and Sertoli cells are capable of engulfing their apoptotic neighbours. However, macrophages are the professional apoptotic – cell-eaters, infiltrating sites of high-rate apoptosis. Virtually all our present knowledge of the molecular mechanisms that

underlie apoptotic-cell clearance is derived from *in vitro* studies of macrophages. The story so far is a complex one involving multiple macrophage receptors: the integrin $\alpha_v\beta_3$ (vitronectin receptor), CD36, class A scavenger receptors, CD14, the ATP-binding cassette transporter ABC-1, and putative receptors for PS and sugars. On the surface of the apoptotic cell, fewer varieties of potential ligand have so far emerged, the leading contender being PS, closely followed by carbohydrate changes; other possibilities remain, for the present, on the sidelines. What is most disappointing at the time of writing is that no macrophage receptor has yet been linked definitively to a ligand on apoptotic cells. Fadok and colleagues consider carefully the identity of the PS-receptor and conclude that it is unlikely to be any of the presently identified panel of macrophage receptors implicated in apoptotic-cell clearance. Importantly, the way in which apoptotic-cell-associated PS is presented to the macrophage PS-receptor that mediates apoptotic-cell clearance is crucial. For example, it is probable that PS exposed on lipid-symmetric erythrocytes is seen by macrophages in a different way to the PS exposed on apoptotic cells.

The lucid and logical consideration of the 'importance of being eaten', as Ren and Savill put it, leads to thoughts on the contribution of defective clearance as a factor in the pathogenesis of inflammatory and autoimmune diseases. Their conclusion that, for an apoptotic cell, being removed is a good thing, and that being phagocytosed non-phlogistically is the right and proper way to be removed leads naturally to discussion of whether disease pathogenesis may be dependent upon ineffectual clearance or inappropriate, pro-inflammatory clearance. There are tantalising suggestions that defective clearance may be contributory to disease. For example, the anti-phospholipid antibodies that bind to apoptotic cells in systemic lupus erythematosus patients may interfere with the quiet removal of apoptotic cells either by blocking the recognition by phagocytes or by converting the normally inert apoptotic body into an opsonized particle with pro-inflammatory potential. Again, more hard experimental evidence is called for. If, as anticipated, the link between apoptotic cell clearance mechanisms and disease pathogenesis can be proven, then, as Ren and Savill point out, novel therapies should follow. Indeed, since the apoptotic – cell surface appears to be utterly appetising to phagocytes, we might anticipate that the changes in an apoptotic cell that make it appear apoptotic at its surface and make it irresistible to phagocytes would be attractive targets for novel therapeutic agents, since whether a cell is truly apoptotic or not, its destruction is guaranteed once it is phagocytosed. Philosophically we may consider that the single most important facet of the apoptosis program is the marking of a cell for phagocytosis: degradation in phagolysosomes follows whether or not apoptosis-driven degradative changes have occurred beforehand. Further work is required to determine how the machinery for

apoptosis leads to the marking of the cell surface for phagocytosis.

As our phagocytes continue to feed on our apoptotic cells, we are provided with much food for thought, perhaps not so much on why they, the phagocytes, do it *per se*, but on how they do it and why they do it in the way they do it – and what happens if they do not make a good job of it or do not do it in quite the way they should. We appear to possess many of the pieces of the jigsaw of 'close encounters' between apoptotic cells and phagocytes, but we have yet to get to grips with how they really fit together. Is the apparent complexity of receptor-ligand interactions in apoptotic-cell clearance an indication of redundancy in recognition mechanisms – a necessary failsafe to ensure engulfment – or, as Ren and Savill suggest, does it represent a series of first-line and back-up mechanisms that are required for clearance of cells at different stages of apoptosis? Most importantly, what intracellular signalling pathways subserve phagocytosis-without-inflammation? Recent work implicates DOCK 180, a protein that interacts with the cytoskeleton through the SH2/SH3 domain-containing adaptor CRK, in a signalling pathway that leads to phagocytosis of apoptotic cells (Wu and Horvitz, 1998). Significantly, DOCK 180 is a mammalian homolog of CED-5, a protein involved in cell-corpse engulfment in *C. elegans*.

Answers to the multitude of questions that still remain at the 'clearance end' of programmed cell death and apoptosis are being actively sought. Perhaps we will observe in the near future an upsurge in interest in the clearance of apoptotic cells and a more respectable representation of this process in the scientific literature – a representation that more accurately reflects "the importance of being eaten".

References

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