

## Commentary

# Phosphatidylserine receptor and apoptosis: consequences of a non-ingested meal

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Received: 8 Mar 2004 Revisions requested: 5 Apr 2004 Revisions received: 19 Apr 2004 Accepted: 28 Apr 2004 Published: 4 Jun 2004

*Arthritis Res Ther* 2004, **6**:147-150 (DOI 10.1186/ar1191)

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### Abstract

Apoptosis, a physiological process of controlled cell death, is essential during embryonic development and for the maintenance of tissue homeostasis. In recent years the view has emerged that dying cells can provide specific signals that enable recruitment and recognition by phagocytes. Exposure of phosphatidylserine, the best characterized of such signals, allows safe clearance of apoptotic waste without induction of inflammation. Here I re-examine some of the arguments that underpin the importance of these clearance mechanisms in light of recent observations from an animal model that lacks the receptor specific for phosphatidylserine.

**Keywords:** apoptosis, autoimmunity, inflammation, phagocytosis

### Introduction

Apoptosis is an active process of cell suicide that leads to ordered destruction of the cells and their safe disposal by professional (macrophages and immature dendritic cells) and nonprofessional (such as fibroblasts and epithelial cells) phagocytes [1]. The removal of apoptotic cells is the final step and perhaps the ultimate objective of the apoptotic programme. When apoptosis was initially described by Kerr and coworkers [2], the phenomenon of programmed cell death was greeted with a striking lack of interest. It has now become apparent that the process is ubiquitous and plays a key role in many fundamental biological events, including embryonic development, normal tissue homeostasis, development of the immune system and resolution of inflammation. In addition, apoptotic cells are a potential source of self-antigens [3], and defective clearance of cell corpses has recently been implicated in the pathogenesis of autoimmune diseases [4].

Although enormous progress has been made in our understanding of the molecular mechanisms of apoptosis, the events that lead to clearance of apoptotic cells are still undefined. However, it has become increasingly clear that *in vivo* apoptosis and engulfment are not distinct events,

but rather are two linked stages in the same process. Cells dying by apoptosis provide both 'recruitment' and 'eat me' signals to scavenger cells, facilitating their own uptake [5]. The best studied of these signals is exposure of phosphatidylserine (PS), a phospholipid that is normally limited to the inner leaflet of the plasma membrane bilayer. Although it has been well established that PS exposure on apoptotic cells is critically required for their proper uptake, the identification of a single dominant receptor that is capable of recognizing and removing the apoptotic cells has remained controversial.

### Is a phosphatidylserine-specific receptor required for safe clearance of dying cells?

Many receptors and soluble ligands have been proposed to mediate the recognition and uptake of apoptotic cells. These include lectin-like molecules, scavenger receptors, CD14, the thrombospondin receptor CD36, the vitronectin receptor CD51/CD61, the oxidized low-density lipoprotein receptor CD68, the low-density lipoprotein receptor related protein (LRP1, known as CD91) and annexins [6]. Some receptors recognize apoptotic cells in the early phase of cell death, whereas others, which recognize later stages of the process, act as backup systems. Some

recognition pathways distinguish between apoptosis, necrosis and cellular debris, whereas others do not.

Consistent with the idea that apoptotic cell recognition/engulfment may require a coordinated engagement of multiple receptors, inhibition studies conducted *in vitro* have failed to block phagocytosis completely, even when inhibitory antibodies or ligands have been used in combination. In addition, mice engineered to carry deletions in any single one of these multiple receptors exhibited no defective clearance or minor defects in embryonic development. Thus, it has been suggested that different phagocytic receptors may cooperate with each other and function as a team. Some receptors may simply play a role in tethering of phagocyte to apoptotic cells without generating a signal, whereas others would engage a signal pathway leading to cytoskeleton rearrangements and engulfment. Many of the receptors implicated in the recognition of apoptotic cells have been shown to bind PS liposomes. However, strong evidence for an *in vivo* stereo-specific recognition of PS exists only for the phosphatidylserine receptor (PSR) [7], indicating that this receptor may play a dominant role.

The PSR, which remained elusive for a long time because of lack of specific reagents [7], was postulated to be a prerequisite for uptake of apoptotic cells by macrophages but *in vivo* evidence was lacking. Reassuringly, the knockout model described in the paper by Li and colleagues [8] provided such proof. The PSR-deficient animals were found to be unable to breathe and died within 24 hours of birth. Histological examination of the lung revealed a severe reduction in the number of airspaces formed and accumulation of noningested dying cells, suggesting that PSR is crucial for clearing apoptotic cells from the developing lungs. The role of PSR in this process was also confirmed by *in vitro* phagocytic experiments showing that the engulfment of apoptotic cells by PSR-deficient macrophages was significantly impaired and the defect was specific for PS liposomes. In addition, a parallel study conducted by Wang and coworkers [9], which demonstrated that *psr-1*, the *Caenorhabditis elegans* homologue of PSR, is important for cell corpse engulfment, provided further strong support to the idea that this receptor plays a critical role in recognizing PS during phagocytosis. Indeed, the clearance defect in the *psr-1* mutant was rescued by overexpression of human PSR. Furthermore, in that study the intracellular signalling pathways engaged by PSR-1 to promote the cell corpse engulfment were also identified, providing important clues regarding how the PSR may act to transduce the engulfment signal in mammalian phagocytes.

Although the *in vivo* observations reported by Li and coworkers [8] would be consistent with the idea that

different tissues may use different clearance mechanisms, the report did not provide a detailed examination of other organs, apart from the malformations observed in the brain. Hence, the potential role of PSR in embryogenesis of other organs remains undefined. In this context it is of note that Kunisaki and coworkers [10] recently generated a second strain of PSR-deficient mice. They found that although these animals died within 24 hours from birth (like those generated by Li and coworkers [8]), they exhibited severe defects in erythroid and T-lymphoid cell differentiation. Nevertheless, in that second study the lung tissue was not examined, and the abnormalities described in erythropoiesis and T-lymphopoiesis might not have caused the lethal phenotype. Interestingly, Kunisaki and coworkers [10] also found that the lack of PSR caused repression of apoptosis in several tissues, including the foetal liver and thymus, whereas Li and colleagues [8] observed hyperplastic brain malformations associated with an increased number of noningested apoptotic bodies in a small proportion of PSR-deficient mice (~15%).

Can these observations be reconciled with each other? One could speculate that the PSR-mediated uptake of dying cells may trigger feedback mechanisms in which macrophages regulate the fate of developing cells, as previously described in the worm [11–13] and in humans [14], and these signals may be tissue specific. Alternatively, the different abnormalities in the PSR-deficient mice may be related to the loss of a still unknown nonphagocytic function of the PSR. Further research will be required to test these hypotheses.

### **Disposing of dying cell: a fine balance between proinflammatory and anti-inflammatory signals**

Phagocytosis of apoptotic cells is known to be an anti-inflammatory [15] and immunologically silent process [16]. Micropinocytosis of apoptotic bodies triggers the production of transforming growth factor- $\beta$  – a cytokine that suppresses inflammatory processes – whereas the release of granule enzymes and proinflammatory cytokines is inhibited [15,17,18]. Strikingly, cross-linking the PSR or adding apoptotic cells to the inflamed milieu can reverse the inflammatory response induced by potent stimuli such as lipopolysaccharide [7,19,20]. This would suggest that the engagement of PSR, without the engulfment of dying cells, can mediate this anti-inflammatory process and thus the uptake process, and cytokine production may be functionally separable [7]. In this context it is of note that in the lung of the PSR-deficient animals there was evidence of inflammation associated with cell necrosis. This observation is consistent with the hypothesis that when cells undergoing apoptosis are not properly cleared, they may enter late stages of apoptosis and secondary necrosis consecutively. The membranes of the dying cells became leaky, and the usually anti-inflammatory clearance

becomes proinflammatory. However, Kunisaki and coworkers [10] found no evidence of an inflammatory response in thymus and foetal liver of the PSR-deficient mice, perhaps reflecting the lack of an increased number of apoptotic cells in these tissues. Currently, the signal events following [15] PS recognition by PSR that would induce the anti-inflammatory response are very poorly characterized. Hopefully, analysis of cells lacking PSR will provide important insights into the mechanisms that mediate these effects.

The idea that PS exposed on the cell surface may serve as a single and direct target for recognition/uptake by phagocytic receptors such as the PSR, although very attractive, does not reflect the complexity of the process implicated in the safe clearance of dying cells. There is now accumulating evidence that well defined serum opsonins such as antibodies and complement components can bind to apoptotic cells and mediate phagocytosis by traditional phagocytic mechanisms [21]. With the abundance of these bridging molecules, one cannot avoid wondering whether there is any unbound PS left on apoptotic cells and what would be the role of the PSR *in vivo*. Clearly, the report by Li and coworkers [8] has demonstrated that PSR plays a crucial role in the development of the lung and brain. However, like many studies that uncover new insights, the findings of Li and coworkers could not fully demonstrate that these abnormalities were only due to the impaired PSR-mediated phagocytosis of dying cells.

## Conclusion

The recent surge of interest in apoptosis is not without reason, least of all for those involved in the care of patients with systemic lupus erythematosus. Recent studies have focused on the possibility that an inability to clear dying cells may lead to inappropriate processing and presentation of self-antigens, which in turn could lead to activation of self-reactive lymphoid cells [4]. Among the observations underlying such arguments are the findings that mice deficient in receptor tyrosine kinases such as Mer [22] or lacking serum opsonin such as C1q [23,24] have defective clearance of apoptotic cells and develop a lupus-like disease. Although the mechanisms by which defects in the scavenging process of apoptotic cells lead to the wide spectrum of autoreactivity that is seen in patients with systemic lupus erythematosus remain unclear, exposure of PS and its recognition by the PSR remains one of the best characterized signals that results in anti-inflammatory clearance of debris without induction of an immune response. By generating and analyzing a mouse that lacks the PSR, Li and colleagues [8] have provided strong evidence that this molecule is essential for development of lung and brain, and it may play a key role in controlling inflammatory events in these organs. Nevertheless, dissecting the PSR-mediated pathways in

viable conditional knockout animals (engineered to lack the PSR only in certain tissue) promises to be an even more fascinating and exciting story, which may shed new lights into the anti-inflammatory signalling pathways that are implicated in the resolution of inflammation and in the efficient disposal of 'apoptotic waste', preventing it from inducing an immune response.

## Competing interests

None declared.

## References

1. Gregory CD: **CD14-dependent clearance of apoptotic cells: relevance to the immune system.** *Curr Opin Immunol* 2000, **12**:27-34.
2. Kerr JF, Wyllie AH, Currie AR: **Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics.** *Br J Cancer* 1972, **26**:239-257.
3. Casciola-Rosen LA, Anhalt G, Rosen A: **Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes.** *J Exp Med* 1994, **179**:1317-1330.
4. Pickering MC, Botto M, Taylor PR, Lachmann PJ, Walport MJ: **Systemic lupus erythematosus, complement deficiency, and apoptosis.** *Adv Immunol* 2000, **76**:227-324.
5. Savill J, Fadok V: **Corpse clearance defines the meaning of cell death.** *Nature* 2000, **407**:784-788.
6. Fadok VA, Bratton DL, Henson PM: **Phagocyte receptors for apoptotic cells: recognition, uptake, and consequences.** *J Clin Invest* 2001, **108**:957-962.
7. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM: **A receptor for phosphatidylserine-specific clearance of apoptotic cells.** *Nature* 2000, **405**:85-90.
8. Li MO, Sarkisian MR, Mehal WZ, Rakic P, Flavell RA: **Phosphatidylserine receptor is required for clearance of apoptotic cells.** *Science* 2003, **302**:1560-1563.
9. Wang X, Wu YC, Fadok VA, Lee MC, Gengyo-Ando K, Cheng LC, Ledwich D, Hsu PK, Chen JY, Chou BK, Henson P, Mitani S, Xue D: **Cell corpse engulfment mediated by *C. elegans* phosphatidylserine receptor through CED-5 and CED-12.** *Science* 2003, **302**:1563-1566.
10. Kunisaki Y, Masuko S, Noda M, Inayoshi A, Sanui T, Harada M, Sasazuki T, Fukui Y: **Defective fetal liver erythropoiesis and T-lymphopoiesis in mice lacking phosphatidylserine receptor.** *Blood* 2004, **103**:3362-3364.
11. Hengartner MO: **Apoptosis: corralling the corpses.** *Cell* 2001, **104**:325-328.
12. Hoepfner DJ, Hengartner MO, Schnabel R: **Engulfment genes cooperate with *ced-3* to promote cell death in *Caenorhabditis elegans*.** *Nature* 2001, **412**:202-206.
13. Reddihem PW, Cameron S, Horvitz HR: **Phagocytosis promotes programmed cell death in *C. elegans*.** *Nature* 2001, **412**:198-202.
14. Brown SB, Savill J: **Phagocytosis triggers macrophage release of Fas ligand and induces apoptosis of bystander leukocytes.** *J Immunol* 1999, **162**:480-485.
15. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM: **Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF.** *J Clin Invest* 1998, **101**:890-898.
16. Voll RE, Herrmann M, Roth EA, Stach C, Kalten JR, Girkontaite I: **Immunosuppressive effects of apoptotic cells.** *Nature* 1997, **390**:350-351.
17. McDonald PP, Fadok VA, Bratton D, Henson PM: **Transcriptional and translational regulation of inflammatory mediator production by endogenous TGF-beta in macrophages that have ingested apoptotic cells.** *J Immunol* 1999, **163**:6164-6172.
18. Huynh ML, Fadok VA, Henson PM: **Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation.** *J Clin Invest* 2002, **109**:41-50.
19. Henson PM, Bratton DL, Fadok VA: **Apoptotic cell removal.** *Curr Biol* 2001, **11**:R795-R805.

20. Henson PM, Bratton DL, Fadok VA: **The phosphatidylserine receptor: a crucial molecular switch?** *Nat Rev Mol Cell Biol* 2001, **2**:627-633.
21. Hart SP, Smith JR, Dransfield I: **Phagocytosis of opsonized apoptotic cells: roles for 'old-fashioned' receptors for antibody and complement.** *Clin Exp Immunol* 2004, **135**:181-185.
22. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK: **Phagocytosis and clearance of apoptotic cells is mediated by MER.** *Nature* 2001, **411**:207-211.
23. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, Loos M, Pandolfi PP, Walport MJ: **Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies.** *Nat Genet* 1998, **19**:56-59.
24. Taylor PR, Carugati A, Fadok VA, Cook HT, Andrews M, Carroll MC, Savill JS, Henson PM, Botto M, Walport MJ: **A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo.** *J Exp Med* 2000, **192**:359-366.