

Tethering and tickling: a new role for the phosphatidylserine receptor

Selin Somersan and Nina Bhardwaj

The Laboratory of Molecular Neuro-Oncology, The Rockefeller University, New York, NY, 10021

Several receptors are implicated in apoptotic cell (AC) uptake by phagocytic cells; however, their relative dominance in mammalian systems remains to be established. New studies shed light on the role of the phosphatidyl serine (PS) receptor (PSR). Ligation of PSR by PS on AC surfaces is considered essential for signaling uptake of ACs that are tethered to phagocytes via other receptors.

Contribution of phosphatidylserine receptor to apoptotic cell uptake

Apoptosis or programmed cell death occurs during embryogenesis, normal cell turnover, and as a consequence of immune mediated, infectious, or inflammatory effects. Clearance of intact apoptotic cells (ACs)* by phagocytes protects surrounding tissues from intracellular factors and reduces the likelihood of tissue damage caused by inappropriate autoimmune responses. ACs exhibit numerous changes including the surface exposure of phosphatidylserine (PS) and alteration of membrane carbohydrates. Multiple ligands and receptors have been implicated in the recognition and uptake of ACs (Fig. 1), but attempts to assign function to discrete receptors has proven difficult. In this issue, Hoffmann et al. (2001) assess the relative role of various receptors through AC surrogates that ligate individual receptors. These surrogates consist of biotinylated human erythrocytes coated with avidin (Eba), which are bound to a biotinylated protein ligand or antireceptor antibody, creating Eba-X. Binding to and internalization of Eba-X by macrophages and other cells is evaluated in the absence of serum (to prevent interference of serum proteins) and is distinguishable by microscopy.

Surprisingly, individual or multiple engagement of the AC receptors CD36, the $\alpha\text{v}\beta\text{3}$ and $\alpha\text{v}\beta\text{5}$ integrins, CD14, and CD68 caused tethering of erythrocytes but little internalization. In contrast, engagement of the PS receptor (PSR) alone through PS-coated erythrocytes induced neither tethering nor uptake. However, ligation of both PSR and other

receptors (including receptors not normally involved in phagocytosis) converted the adhesion mediated by the latter to ingestion. Direct ligation of PSR produced TGF β , supporting earlier data that PSR modulation is critical for inducing immunosuppressive cytokines. Based upon these studies, in this issue Hoffmann et al. (2001) propose a new paradigm for AC uptake by phagocytes. Ligation of PSR on phagocytes delivers a “tickle” signal, which stimulates the internalization of ACs, including bystander cells, that are “tethered” through other recognition receptors. Simultaneously, the immune response is modulated through secretion of immunosuppressive cytokines (Fig. 2). The observation that ACs which do not express PS are poorly phagocytosed (Fadok et al., 2001) suggests that PS–PSR interactions play a crucial regulatory role in dead cell clearance.

The “tether and tickle” mechanism is attractive for many reasons. The tethering step makes it feasible for signaling to proceed through low avidity PS–PSR interactions. Specificity and regulation is provided to the multiple recognition mechanisms for AC uptake uncovered in mammals. Because cells can transiently express PS during their activation, the two step model protects against accidental uptake (Henson et al., 2001b). Finally, clearance of apoptotic cells would suppress any potential autoimmune responses directed toward self antigens through inhibition of monocyte and T cell activation and maturation of professional antigen-presenting cells like dendritic cells (DCs).

New paradigms generate a host of new questions. For instance, what is the role of receptors which potentially bind PS directly (CD14, scavenger receptors) or through bridging molecules such as thrombospondin, lactadherin, iC3b, and $\beta\text{2glycoprotein I}$? Do they simply provide tethering signals or do they deliver both signals? Since PSR expression may be a function of the activation status, geographic location and nature of the phagocyte (absent on fresh monocytes but upregulated on activated macrophages [Fadok et al., 2000] and present on immature DCs [unpublished data]), it remains to be established when this PSR-dependent mechanism is dominant. Is it only involved in the removal of ACs during normal cell homeostasis, or does it also play a role in clearing cells generated during immune or inflammatory responses when responses must be subsequently downregulated?

PSR-independent pathways clearly exist (Fig. 1). Defense collagens such as the collectins (surfactant binding protein

Address correspondence to Nina Bhardwaj, The Laboratory of Molecular Neuro-Oncology, The Rockefeller University, Room 41, Box #176, 1230 York Ave., New York, NY 10021. Tel.: (212) 327-8332. Fax: (212) 327-7232. E-mail bhardwn@mail.rockefeller.edu

*Abbreviations used in this paper: AC, apoptotic cell; MBL, mannose-binding lectin; NC, necrotic cell; PS, phosphatidylserine; PSR, PS receptor; SP, surfactant binding protein.

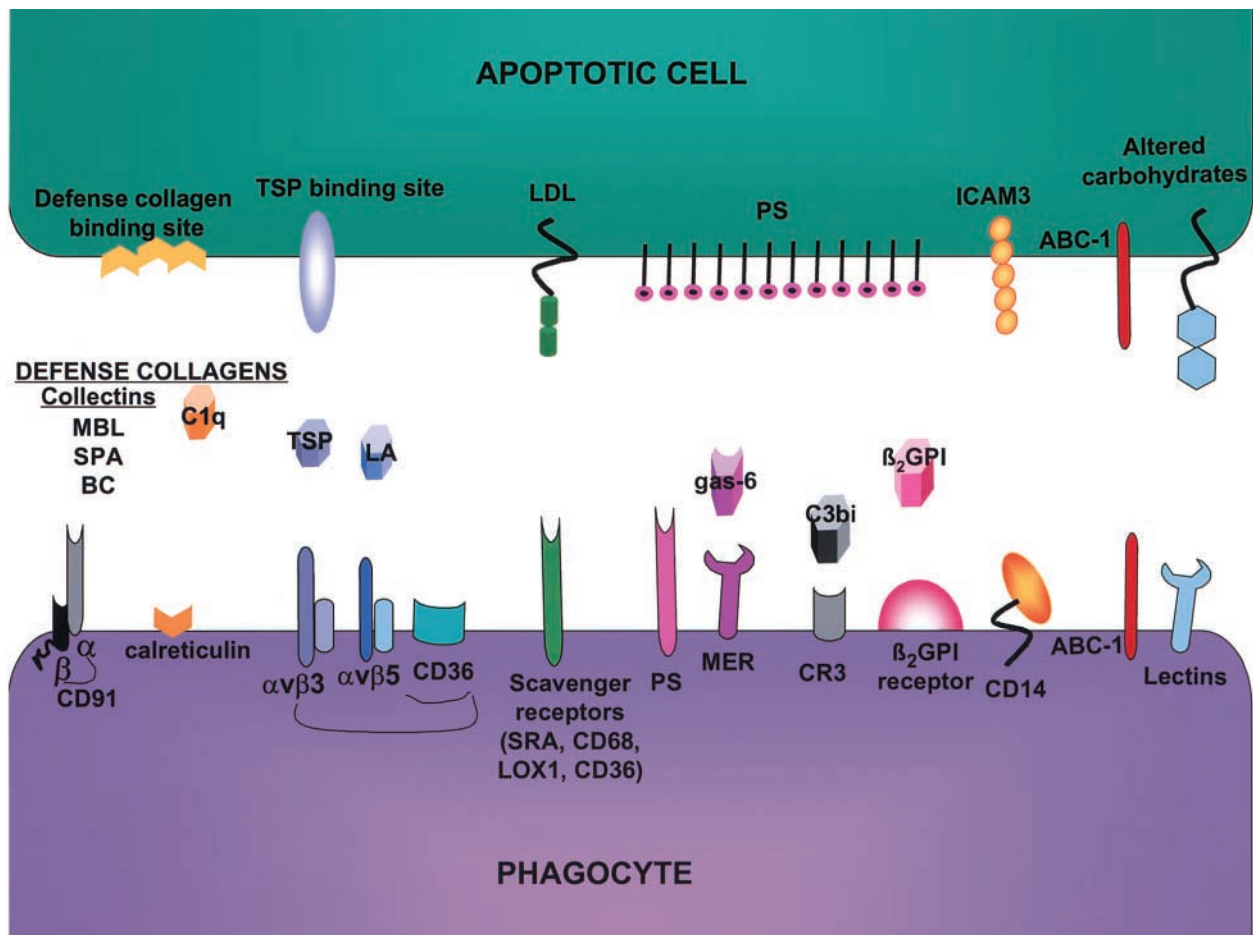


Figure 1. **Phagocyte apoptotic cell interactions.** Several receptors are implicated in the uptake of ACs by phagocytes. These receptors interact with their ligands on the AC either directly or via bridging proteins. The dominance, cooperation, and redundancy amongst these receptors are discussed in the text. BC, bovine conglutinin; LA, lactadherin; TSP, thrombospondin. MBL, SPA, BC, and C1q are defense collagens, which bridge AC to phagocytes via calreticulin and CD91. LA and TSP are putative bridging molecules for the $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins, respectively. The latter are thought to cooperate with CD36 in AC uptake. Gas6, a product of growth arrest-specific gene 6, appears to bridge PS with receptor tyrosine kinases such as Mer.

[SP]-A; mannose-binding lectin [MBL]) and the complement component C1q, coat ACs via their globular heads and initiate uptake by interacting with phagocyte receptors through their structurally homologous collagenous tail groups (Ogden et al., 2001). This interaction requires the recognition of tail groups by calreticulin (cC1qR) and CD91. The latter is a receptor for $\alpha 2$ macroglobulin and heat shock proteins in addition to calreticulin (Basu et al., 2001). Importantly, defective AC clearance in $C1q^{-/-}$ animals implicates this pathway in removal of dead cells under noninflammatory conditions (Taylor et al., 2000).

The elegant AC surrogate model described here will be useful to address the hierarchical roles of recognition receptors in various circumstances and their relative dominance amongst professional and nonprofessional phagocytes.

Signaling mechanisms in apoptotic cell uptake

Particles and cells may be internalized through receptor-mediated endocytosis, macropinocytosis, or phagocytosis. Uptake via the tether and tickle mechanism occurs through macropinocytosis, since it is accompanied by membrane ruffling, formation of fluid filled phagosomes, and requires

Rac-1 and Cdc42, members of the Rho family of GTPases that link cell surface receptors to actin cytoskeletal organization. A general role of these GTPases in AC uptake is supported by studies showing that murine bone marrow-derived macrophages are dependent on Rac-1, Cdc42, and Wiskott-Aldrich syndrome protein (WASp). WASp is activated by Cdc42 to stimulate actin polymerization through the Arp2/3 complex (Leverrier et al., 2001). Interestingly, immature DCs, which are constitutively macropinocytic and phagocytic, express activated Cdc42. As they mature, endocytic downregulation is accompanied by loss of activated Cdc42. In this context, one would predict that DCs would be less dependent on a "tickling" mechanism to internalize tethered particles.

The signaling mechanisms that link AC receptors to the GTPases remain to be defined. PSR may associate with other surface molecules, since its intracellular tail composition provides few clues regarding uptake. Association of calreticulin with CD91 after exposure to MBL or C1q-opsonized AC may induce aggregation and signaling via the latter's tail, which contains two NPXY endocytosis signal sequences (Gliemann et al., 1994). Signaling through

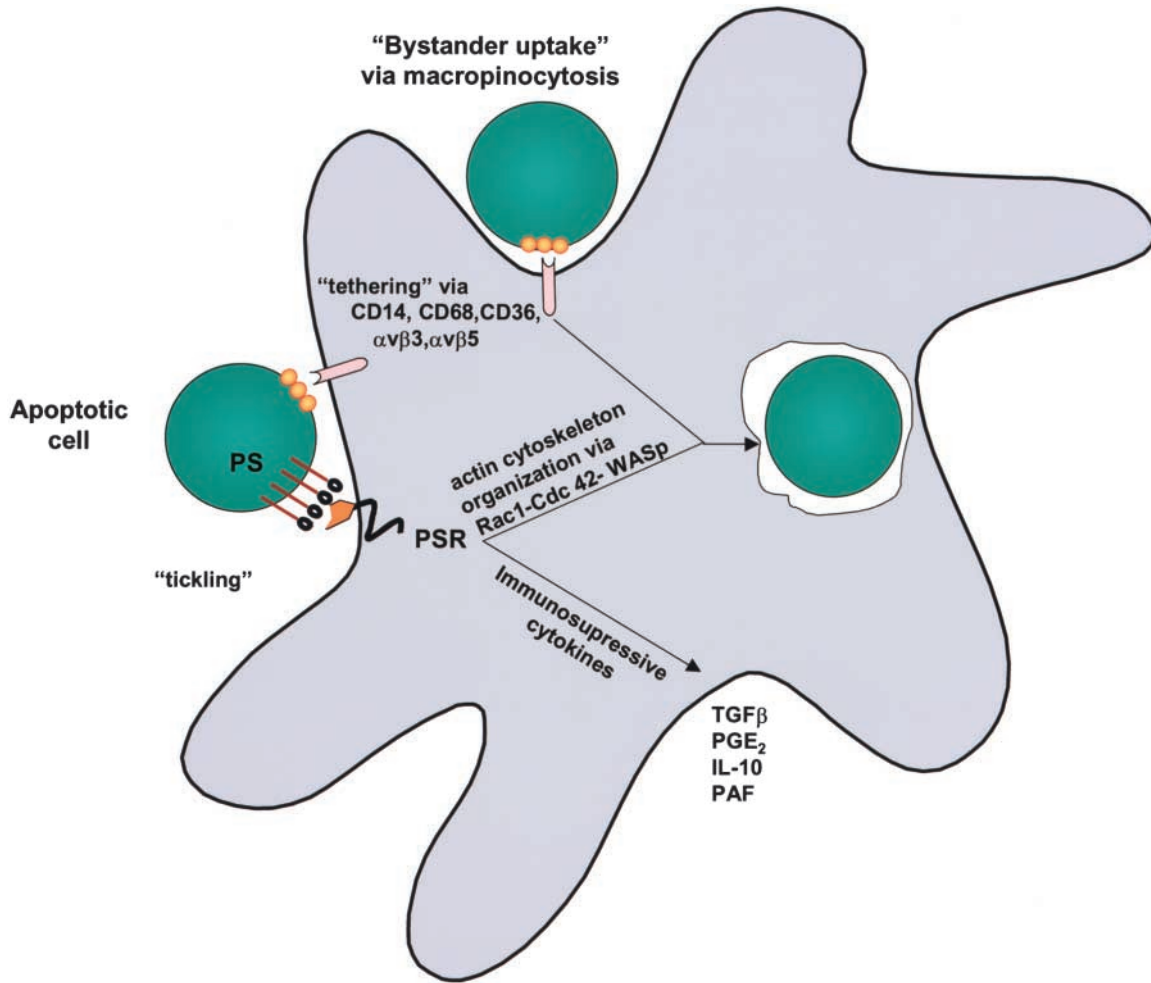


Figure 2. **The dual role of PSR.** PSR engagement tickles the phagocyte to take up tethered apoptotic cells, including bystander cells, by macropinocytosis. Internalization requires reorganization of the actin cytoskeleton through activation of the Rho GTPases Rac1, and Cdc42, and the WASp. In this scenario, PSR also has an immunoregulatory role. Signaling through this receptor leads to secretion of immunosuppressive cytokines such as TGF- β and possibly PGE₂, IL-10, and platelet-activating factor (PAF).

the integrin $\alpha v \beta 5$ on nonprofessional phagocytes recruits p130cas–CrkII–Dock180 molecular complexes and triggers Rac-1, a process analogous to the homologous CED2–CED5–CED10 complex of engulfment genes defined in the nematode *Caenorhabditis elegans* (Albert et al., 2000). The tyrosine protein kinases or phosphatidylinositol 3-kinases may link surface receptors to these downstream signaling molecules. How do these various signals translate into biological events associated with AC clearance? Rac-1 is an essential regulatory component of the NADPH oxidase enzyme complex, and both Rac and Cdc42 activate the JNK and p38 MAPK pathways. Interestingly, these two molecular switches were found to be essential for Fc gamma R–mediated uptake, which is associated with a respiratory burst and inflammatory responses in professional phagocytes (Caron and Hall, 1998). Presumably, the nature of the initial recognition receptor influences the subsequent responses. Further biochemical and genetic analyses of the signaling pathways controlling AC uptake will lead to a better understanding of these fundamental processes.

Modulation of the immune response

Under noninflammatory conditions, uptake of ACs by macrophages is thought to suppress autoimmune responses through production of IL-10, TGF β , PAF, and inhibition of TNF α , GM-CSF, IL-12, IL-1 β , and IL-8 release (Voll et al., 1997; Fadok et al., 1998). Ligand of PSR has been proposed to be the primary mechanism through which these responses are initiated (Henson et al., 2001a). Under inflammatory conditions or in circumstances where PS or PSR levels are reduced, Henson et al. (2001b) propose that other recognition systems dominate in clearance of dead cells (e.g., defense collagens) and skew immune responses in a proinflammatory direction. However, this model of PSR function as a molecular switch for immunity must take into account that ligation of other receptors can suppress autoimmune responses. For instance, direct engagement of CD36 (Voll et al., 1997; Urban et al., 2001) blocks the release of proinflammatory cytokines upon stimulation with lipopolysaccharide. In addition, C1q^{-/-} mice which are defective in AC clearance, develop a syndrome resembling systemic lupus erythematosus (Taylor et al., 2000).

Uptake of ACs by DCs is also significant to the immune response. It has been suggested that immature DCs promote tolerogenic responses through ligation of PSR and CD36 (Henson et al., 2001a). In some studies, this interaction has been shown to inhibit DC maturation and production of IL-12, while inducing IL-10 (Urban et al., 2001). This is a point of contention, since others have failed to show inhibition of maturation or of inflammatory cytokine production upon AC uptake (Sauter et al., 2000). Furthermore, it is clear that immature DCs phagocytose multiple sources of ACs (including bacteria- or virus-infected cells and cancer cells) and upon maturation “cross present” (i.e., process and present) antigens from these sources and activate CD4⁺ and CD8⁺ T cells (Albert et al., 1998). DCs use $\alpha\beta 5$ preferentially to $\alpha\beta 3$ as an AC receptor. This difference may explain the disparity in fates of ACs in DCs versus macrophages.

Studies of receptor recognition systems have shed light on how antigen-presenting cells react to different types of dead cells. Exposure of macrophages and DCs to necrotic cells (NCs) induces their activation, likely via heat shock proteins and calreticulin within cell lysates. Interestingly, through binding to CD91 these same proteins promote cross presentation of chaperoned antigenic peptides (Basu et al., 2001). Since defense collagens bridge AC to macrophages via calreticulin and CD91 (Ogden et al., 2001), NC and C1q, SPA, or MBL-opsonized AC may share a common receptor for their uptake (Chung et al., 2000). This pathway could account for the ability of DCs to crosspresent antigens from AC and NC. Indeed, a common set of engulfment genes mediates removal of both apoptotic and necrotic cell corpses in the nematode (Chung et al., 2000).

Further studies of dead cell receptor recognition proteins will lead to a better understanding of how immunity can be modulated to minimize autoimmune responses while maximizing the host response against infection.

Submitted: 15 October 2001

Revised: 15 October 2001

Accepted: 18 October 2001

References

- Albert, M.L., B. Sauter, and N. Bhardwaj. 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature*. 392:86–89.
- Albert, M.L., J.I. Kim, and R.B. Birge. 2000. Alphavbeta5 integrin recruits the CrkII-Dock180-rac1 complex for phagocytosis of apoptotic cells. *Nat. Cell Biol.* 2:899–905.
- Basu, S., R.J. Binder, T. Ramalingam, and P.K. Srivastava. 2001. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity*. 14:303–313.
- Caron, E., and A. Hall. 1998. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science*. 282:1717–1720.
- Chung, S., T.L. Gumienny, M.O. Hengartner, and M. Driscoll. 2000. A common set of engulfment genes mediates removal of both apoptotic and necrotic cell corpses in *C. elegans*. *Nat. Cell Biol.* 2:931–937.
- Fadok, V.A., D.L. Bratton, A. Konowal, P.W. Freed, J.Y. Westcott, and P.M. Henson. 1998. Macrophages that have ingested apoptosis cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF- β , PGE₂, and PAF. *J. Clin. Invest.* 101:890–898.
- Fadok, V.A., D.L. Bratton, D.M. Rose, A. Pearson, R.A. Ezekewitz, and P.M. Henson. 2000. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature*. 405:85–90.
- Fadok, V.A., A. de Cathelineau, D.L. Daleke, P.M. Henson, and D.L. Bratton. 2001. Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. *J. Biol. Chem.* 276:1071–1077.
- Gliemann, J., A. Nykjaer, C.M. Petersen, K.E. Jorgensen, M. Nielsen, P.A. Andreasen, E.I. Christensen, A. Lookene, G. Olivecrona, and S.K. Moestrup. 1994. The multiligand alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein (alpha 2MR/LRP). Binding and endocytosis of fluid phase and membrane-associated ligands. *Annu. NY Acad. Sci.* 737:20–38.
- Henson, P.M., D.L. Bratton, and V.A. Fadok. 2001a. Apoptotic cell removal. *Curr. Biol.* 11:R795–R805.
- Henson, P.M., D.L. Bratton, and V.A. Fadok. 2001b. The phosphatidylserine receptor: a crucial molecular switch? *Nat. Rev. Mol. Cell Biol.* 2:627–633.
- Hoffmann, P.R., A.M. deCathelineau, C.A. Ogden, Y. Leverrier, D.L. Bratton, D.L. Daleke, A.J. Ridley, V.A. Fadok, and P.M. Henson. 2001. Phosphatidylserine (PS) induces PS receptor mediated macropinocytosis and promotes clearance of apoptotic cells. *J. Cell Biol.* 155:649–659.
- Leverrier, Y., R. Lorenzi, M.P. Blundell, P. Brickell, C. Kinnon, A.J. Ridley, and A.J. Thrasher. 2001. Cutting edge: the Wiskott-Aldrich syndrome protein is required for efficient phagocytosis of apoptotic cells. *J. Immunol.* 166:4831–4834.
- Ogden, C.A., A. deCathelineau, P.R. Hoffmann, D. Bratton, B. Ghebrehiwet, V.A. Fadok, and P.M. Henson. 2001. C1q and mannose binding lectin engagement of cell surface calreticulin and cd91 initiates macropinocytosis and uptake of apoptotic cells. *J. Exp. Med.* 194:781–796.
- Sauter, B., M.L. Albert, L. Francisco, M. Larsson, S. Somersan, and N. Bhardwaj. 2000. Consequences of cell death. Exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J. Exp. Med.* 191:423–434.
- Taylor, P.R., A. Carugati, V.A. Fadok, H.T. Cook, M. Andrews, M.C. Carroll, J.S. Savill, P.M. Henson, M. Botto, and M.J. Walport. 2000. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. *J. Exp. Med.* 192:359–366.
- Urban, B.C., N. Willcox, and D.J. Roberts. 2001. A role for CD36 in the regulation of dendritic cell function. *Proc. Natl. Acad. Sci. USA*. 98:8750–8755.
- Voll, R.E., M. Herrmann, E.A. Roth, C. Stach, and J.R. Kalden. 1997. Immunosuppressive effects of apoptotic cells. *Nature*. 390:350–351.