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"Eat me" imaging and therapy☆

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ABSTRACT

Clearance of apoptotic debris is a vital role of the innate immune system. Drawing upon principles of apoptotic clearance, convenient delivery vehicles including intrinsic anti-inflammatory characteristics and specificity to immune cells can be engineered to aid in drug delivery. In this article, we examine the use of phosphatidylserine (PtdSer), the well-known "eat-me" signal, in nanoparticle-based therapeutics making them highly desirable "meals" for phagocytic immune cells. Use of PtdSer facilitates engulfment of nanoparticles allowing for imaging and therapy in various pathologies and may result in immunomodulation. Furthermore, we discuss the targeting of the macrophages and other cells at sites of inflammation in disease. A thorough understanding of the immunobiology of "eat-me" signals is requisite for the successful application of "eat-me"-bearing materials in biomedical applications.

Contents

Abbreviations: PtdSer, phosphatidylserine; OVA, ovalbumin; PSL, phosphatidylserine liposomes; ABL, apoptotic body like liposomes; PC, phosphatidylcholine; OxLDL, oxidized low density lipoprotein; PA, phosphatidicacid; PE, phosphoethanolamine; AA, adjuvant arthritis; IL6, interleukin 6; IL12, interleukin 12; IL 10, interleukin 10; IL1β, interleukin 1β; PPAR-γ, peroxisome proliferator activated receptor; TGFβ, transforming growth factor β; TNFα, Tumor necrosis factor α; Ccl5, Chemokine (C-C motif) ligand 5; Ccl2, Chemokine (C-C motif) ligand 2\; Cx_3cl1, fractalkine chemokine (C-X3-C motif) ligand 1; NO, nitric oxide; EMS, eat me signal; NPs, nanoparticles; DNCB, 2,4-dinitro, 1-chlorobenzene; $CD4^+$ T cells, T helper cells expressing cluster of differentiation 4; ROS, reactive oxygen species; PLD, phospholipase D; MTB, Mycobacterium tuberculosis; PGE_2, prostaglandin E2; SapC-DOPS, Saponin C dioleoylphosphatidylserine; LPS, lipopolysaccharide; LXR, Liver X receptor; ABCA1, ATP-binding cassette transporter; SREBP-1c, Sterol regulatory element-binding transcription factor 1; PLGA, poly(lactic-co-glycolic acid); GW3965, LXR agonist; HepG2, hepatocarcinoma cell line; CD68, Cluster of Differentiation 68 expressed on macrophage; SAC, splenic adherent cells; HIV-1 virus, human immunodeficiency 1 virus; GFP, green fluorescent protein; MRI, magnetic resonance imaging; SPECT, single photon emission computed tomography; CT, computed tomography; PEG, polyethylene glycol; RES, reticuloendothelial system; 9-CCN, cholesterol-9-carboxynonanoate; MRP 8/14, myeloid related protein 8/14; ApoE, apolipoprotein E; WHHL, Watanabe hereditary hyperlipidemic; NZW, New Zealand white; MCE, myocardial contrast echocardiography; VCAM-1, vascular adhesion molecule.

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1. Introduction

Efficient clearance of pathogenic invaders, dead and dying cells (apoptotic and necrotic), and cellular debris is vitally important for long-term maintenance of tissue homeostasis in living systems [1–3]. The immune system plays a large role in tissue homeostasis as it is finely tuned to recognize and rapidly process both endogenous and exogenous particles [4,5]. Innate immune cells recognize and often engulf exogenous particles via recognition of "danger-associated" moieties; see Table 1. Canonically, recognition of danger-associated patterns by innate immune cells results in cellular activation and the release of chemoattractant proteins, which recruit additional inflammatory cells to the area [6–9]. In contrast, clearance of dead cells as part of constant cell deletion and renewal is by and large "immunologically silent" [10]. For instance, it is estimated that the human immune system clears about one million dead or dying cells every second without causing immune activation [11]. Understanding and adapting the mechanisms involved in these processes offer a paradigm for advanced drug delivery. Approaches that mimic apoptotic cells, utilize apoptotic cells themselves as therapeutic agents, or interfere with receptors that recognize "danger-associated" species have been shown to be effective in delivery strategies in various disease states.

We will discuss therapeutic strategies that harness apoptotic clearance machinery by incorporation of "eat-me" signals (EMS). An "eat-me" signal is a molecular signal expressed on the surface of a cell or a particle that is recognized by a corresponding receptor on a phagocytic cell (innate immune cell) to initiate phagocytosis (cell), endocytosis, or pinocytosis (particle) [12]. This action is usually concomitant with repressed immune activation, and recapitulates the clearance of endogenous apoptotic debris. Probably the most widely described EMS — phosphatidyserine (PtdSer) [13,14] is the main focus of this review. Although other EMS have been described (calreticulin, phosphoethanolamine, oxidized lipids and recently discovered cardiolipin), reports describing the therapeutic utility or use in *in vivo* imaging are scarce (Table 1). In the first part of this article we discuss various applications of nanomaterial-based vehicles decorated with PtdSer that have been used for standalone therapy or for delivery of therapeutic agents. In the second part of this article, we review imaging probe delivery, and utility of PtdSer-targeted contrast agents in various pathological conditions employing numerous imaging modalities.

We limit our discussion to nanoparticulate- based vehicles bearing EMS serving as substrates for immune cell engulfment. We will not discuss the work defining those mechanisms behind the detection of cell death by innate immune cells (Annexin V [15–18] and other PtdSer-targeted molecules [19–22]). We refer the reader to a series of excellent reviews on the biology of EMS and apoptosis [13,14,23,24]. Likewise, the approaches that take advantage of "danger-associated" molecules for drug delivery have been reviewed elsewhere [25–27].

2. Therapy with phosphatidylserine carriers

Phosphatidylserine (PtdSer) is a phospholipid component of cell membranes in eukaryotes. Under normal physiological conditions, PtdSer serves primarily as a component of the inner cell membrane of the phospholipid bilayer and is retained there by the enzyme aminophospholipid translocase [14,28]. When a cell undergoes apoptosis, however, the activity of aminophospholipid translocase is lost and phospholipid scramblases serve to both disturb the membrane's aminophospholipid asymmetry and expose a significant amount of PtdSer to the outer leaflet [14,29]. This exposed PtdSer is a specific recognition signal for phagocytic digestion of apoptotic cells [30], with several ligand-receptor mechanisms having been elucidated for the recognition of PtdSer on the surface of apoptotic cells [13,14].

Engineered nanomaterials bearing synthetic PtdSer have been designed to mimic apoptotic cells and serve as a great resource for nanoparticle targeting and drug delivery. We highlight two approaches utilizing PtdSer nanoparticles. First, nanoparticles that mimic apoptotic cells via surface-decorated PtdSer have been shown to exhibit immunoregulatory functions in multiple models of inflammation. Second, PtdSer decorated nanocontainers loaded with drugs and therapeutic oligonucleotides, with PtdSer, directing these agents to sites of abundant phagocytic cell accumulation and activity. The utilization of PtdSer as a targeting ligand is based on the intrinsic capacity of phagocytic cells to clear PtdSer-containing vehicles more efficiently than the bare drug or imaging probe, enhancing agent delivery to phagocytic cell types in various diseases, such as sickle cell anemia, thalassemia, and various neoplastic cells in leukemia, melanoma, and colon cancer [31–33]. Thus, there is obvious utility in applying PtdSer-containing nanoparticle in both investigative and therapeutic settings.

2.1. Anti-inflammatory therapy via PtdSer signaling

Nanoparticles (NPs) such as liposomes containing PtdSer in their bilayer have been used to mimic cellular debris as a therapeutic strategy for inflammation resolution, these are often referred to as "synthetic apoptotic cells". Synthetic apoptotic cells leverage signaling cascades initiated in innate immune cells during endogenous cellular apoptosis, a process that is fundamentally anti-inflammatory and "immunologically silent". While receptors that recognize PtdSer are increasingly better-defined, the signaling through these receptors is not completely understood. It is known, however, that the engulfment of PtdSer-containing nanoparticles such as liposomes (PSL) has a direct effect on anti-

Table 1
Some of the well-described molecules that facilitate immune clearance and their proposed use in drug delivery and imaging.

Recognition molecule		Description/mechanism of action	Therapy or imaging applications
Danger-associated molecules	Urate crystals	[93,94]	
	Cholesterol crystals	[95]	
	HMGB1	[96,97]	
	HSP90	[98]	
	Plasma DNA	[99,100]	
	Formylated peptides and Mitochondrial DNA	[101]	
	Neutrophil extracellular traps	[102]	
	Purine metabolites: ATP	[103]	
	IL-33	[104]	
	S100 calcium binding proteins	[63,105]	[63]
	Extracellular matrix proteins: Hyaluronate	[106]	A number of reports, see, for example [107–109]
	Exhaustive review list of other DAMPs	[5,12]	
"Eat-me" signals (EMS)	Phosphatidylserine (PtdSer) and oxidized PtdSer	[12,30,110–112] [113]	See specific references further in this review
	Calreticulin (CRT)	[114,115]	
	Phophoethanolamine (PE) and oxPE	[116] [117]	[118,119]
	Cardiolipin (CL) and oxCL	[120,121]	
	Other oxidized lipids	[122]	[62,123–126]