Apoptotic cell clearance by macrophages and dendritic cells : immunoregulation in the context of innate immunity

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General Introduction

1. Introduction

Systemic lupus erythematosus (SLE) is an incurable autoimmune disease characterized by a wide array of clinical manifestations and involvement of multiple organs, such as skin, kidneys, and the central nervous system [1]. Despite genetic susceptibility, the actual pathogenesis of SLE remains elusive [2]. To note, SLE is a systemic autoimmune disease and it differs from other autoimmune diseases in such a way that no particular cell type seems to be targeted rather, the response seems to be directed against antigens that are widely expressed [3]. Among these antigens, nuclear components (DNA, histones, ribonucleoproteins) are the major targets [1,3]. Dying cells serve as potential reservoirs of modified forms of autoantigens that may trigger autoantibody responses in susceptible individuals [4]. Therefore, it has been proposed that defective clearance of dying cells breaks peripheral tolerance and predisposes to the development of SLE [5-7]. Several *in vitro* and *in vivo* studies have provided evidence for a link between inappropriate clearance of dying cells and SLE [8,9]. However, it remains unclear how apoptotic cell clearance is regulated by different phagocytes and soluble factors from the innate immune system, and how dying cells ultimately initiate a break of peripheral tolerance.

2. The many modes of cell death

Cell death is an essential and highly orchestrated process, which contributes significantly to normal homeostasis and tissue turnover. There are at least three major modes of cell death: apoptosis, necrosis and autophagy [10]. Apoptosis, coined in 1972 by Kerr *et al.* [11], comes from two Greek words, apo- and -ptosis. “Apo" means "separate from" and "ptosis" means "fall from"--a description of cells that naturally die as part of normal development without any inflammatory flare (cited from Wikipedia). Apoptosis is an active molecular “programmed” process [12-14]. It was called “programmed”, owing to the significant findings by Sulston and Horvitz who elegantly showed that in each worm (*C. elegans*), out of 1090 newborn cells, the same 131 cells die during development, resulting in a nematode of exact 959 cells [15]. During apoptosis, dramatic biochemical and morphological changes take place, including the redistribution of membrane lipids such as phosphatidylserine (PS), and fragmentation of the nucleus [16]. Importantly, the membrane of apoptotic cells remains intact until relatively late in the process [12]. Thus based on the permeability of cell membranes, apoptotic cells can be further divided into two categories, namely early apoptotic and late apoptotic cells.

In contrast to apoptosis, necrosis refers to a distinct mode of death where a cell swells and ruptures during its accidental demise [10], and that sometimes occurs as an alternative form of programmed cell death when apoptosis is blocked [17]. To note,

late apoptotic cells are sometimes referred as post-apoptotic or secondary necrotic cells, behaving like necrotic cells as both of them release intracellular contents [18]. In experimental settings, difference between apoptosis and necrosis can be appreciated at the changes of their sizes and granularities based on the dot plots of forward and side scatter by flow cytometry (Fig. 1).

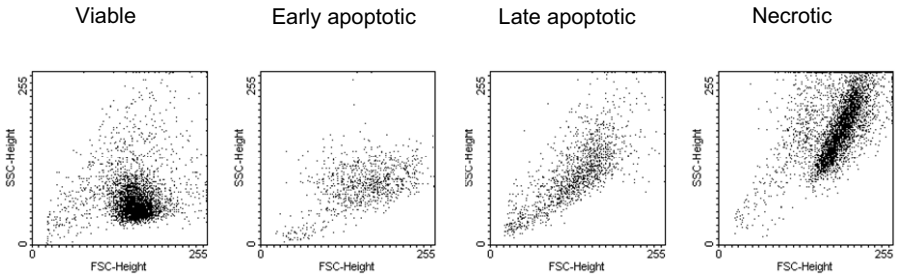

Figure 1. Distinct characteristics of cells at different stages of cell death. Jurkat T cells were treated with UV-C at a dose of 50J/m^2, and cultured in serum-free RPMI culture medium for 3 hours or 30 hours to obtain early apoptotic cells and late apoptotic cells, respectively. Necrosis was induced by incubating cells at 56°C for 1 hour.

As a third major form of cell death, autophagy is a relatively new term and it is a process that a cell recycles cellular products such as cytoplasma and defective organelles [19]. The major difference between apoptosis and autophagy is that apopotic cells are degraded by phagocytic cell lysosomes while autophagic cells do it by their endogeneous lysosomal machinery. It remains unclear whether autophagy directly executes cell death or that it is a secondary effect of apoptosis [19].

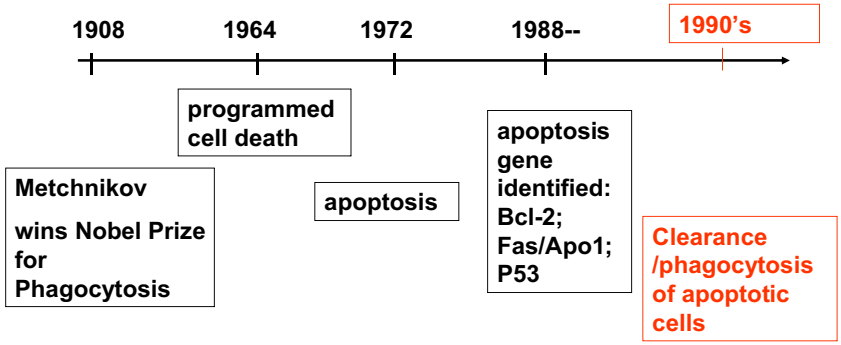

Figure 2. A brief history of apoptosis.

3. The clearance machinery

The theory of "phagocytosis" was formulated by the pioneering biologist Elie Metchnikov in 1880's, who won a Nobel Prize in 1908 (www.nobelprizes.com). Phagocytosis was initially recognized as the first line of internal defence when foreign particles that enter our bodies. Although apoptosis or programmed cell death was discovered in 1960's, not much attention has been paid by scientists to unravel the mechanisms of phagocytosis of apoptotic cells until the 1990's [5,6,20,21] (Fig. 2).

Investigators in the last 15 years have made clear that once a cell undergoes apoptosis, phosphatidyserine (PS) is redistributed onto the outer layer of the cell membrane and serves as the very first "eat me" signal to attract phagocytes and initiation of phagocytosis (Fig.3). There are many cell types that can be involved in the phagocytic process, including professional phagocytes, i.e. immature dendritic cells (iDCs) and macrophages (Mφ), but also non-professional phagocytes, i.e. epithelial cells, fibroblasts or mesangial cells [22]. Even within the family of professional phagocytes, both DCs and Mφ consist of heterogeneous subsets of cells with different functional characteristics [23,24]. Therefore, the nature of a phagocyte defines the complexity and the consequence of phagocytosis. Furthermore, since both DCs and Mφ are professional antigen presenting cells (APCs), processing of self-antigens derived from dying cells by these cells becomes an essential issue in understanding how these dying cells control and regulate immunity.

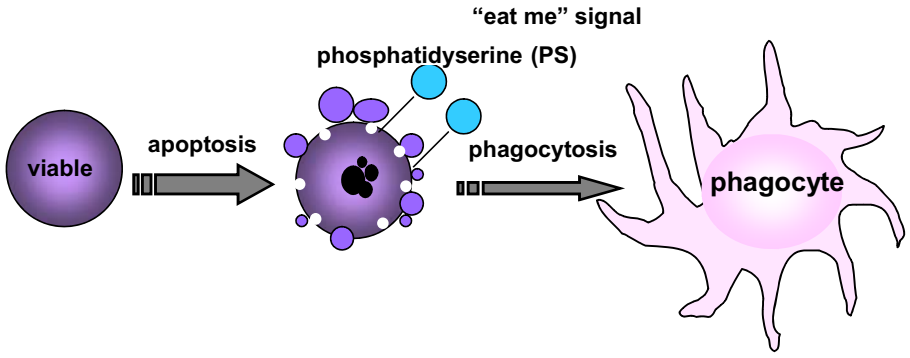

Figure 3. Recognition of apoptotic cells by phagocytes. Viable cells provide "don't eat me" signals, therefore are not recognized by phagocytes. Once cells undergo apoptosis, phosphatidyserine (PS) is redistributed onto the outer layer of the cell membrane, which then serve as "eat me" signals. Phagocytes such as DCs and Mφ are recruited by chemotactic stimuli to phagocytose the dying cells.

4. Linking cell death to autoimmune lupus

Apoptotic cells are a rich source of autoantigens [25]. During daily life, billions of cells undergo apoptosis and then are promptly phagocytosed by different phagocytes and/or APCs and the self-antigens are processed by these APCs or transferred to other professional APCs. Therefore, it is generally thought that presentation of antigens derived from apoptotic cells will contribute to the induction and maintenance of peripheral tolerance or autoimmunity [5,6]. Emerging evidence indicates that the uptake of apoptotic cells is immunosuppressive, as documented by the fact that anti-inflammatory cytokines such as TGF-β are induced whereas pro-inflammatory cytokines are inhibited, by phagocytes that have ingested apoptotic cells [26-29]. This contrasts with the effect of late apoptotic or necrotic cell uptake, which leads to the activation of phagocytes and release of pro-inflammatory cytokines [30,31]. This is most likely due to "danger signals" such as heat shock proteins [32], HMGB1 [33] and uric acid [34], that are released by necrotic cells. Each of these mediators is essential to induce DC maturation and activation of the immune system.

Another challenging recent view proposes that also stimuli for induction of apoptosis may define the immune response upon uptake by phagocytes [35]. Several studies have shown that apoptosis triggered via death receptors results in release of bio-active lipids such as sphingosine-1-phosphate (S1P) [36], or lysophosphatidylcholine (LPC) [37], which then signal through endothelial-derived G-protein-coupled (EDG) receptors. As a consequence, EDG receptors activate nuclear factor-κB (NF-κB), leading to a pro-inflammatory response [35].

A central question is then how apoptotic /necrotic cells ultimately lead to aberrant autoimmunity? The very first evidence from animal studies has shown that injection of large amounts of apoptotic cells into mice [38] or rats [39] led to the production of autoantibodies. In SLE patients, a decreased clearance of apoptotic cells has been documented [9,40], suggesting a strong link of defective clearance to autoimmunity. Furthermore, elevated levels of apoptotic cells have been found in the peripheral blood of SLE patients [41,42]. Together, there is a prevailing belief that delayed or defective clearance of apoptotic cells ultimately leads to a break in self-tolerance and induction of autoimmunity [5,9].

During phagocytosis, soluble factors from the innate immune system enhance the interaction between dying cells and phagocytes and play an important role in this process. Both complement and other innate molecules such as pentraxin family members can opsonize apoptotic cells, and thereby promote their removal by phagocytes [16,43]. In humans, homozygous deficiency of any of the early components of the classical pathway of complement (C1q, C1r, C1s, C4, and C2) predispose to the development of SLE [43], implying that complement is involved in

removal of dying cells. These observations are also supported by animal models showing that C1q knockout mice on certain genetic background develop lupus like disease and exhibit accumulation of apoptotic bodies in the glomeruli [8]. Complement-mediated clearance of apoptotic cells has been well documented both *in vitro* [44] and *in vivo* [45]. Activation of complement by the classical pathway (via C1q) and lectin pathway (via MBL and ficolin) on dying cells seems to be a favorable process [44-48], although complement activation may cause tissue damage and inflammation, suggesting that a balance between the two processes is desirable. Nevertheless, the main product of complement activation, iC3b, was suggested not only to facilitate the removal of dead material, but also to mediate peripheral tolerance [44,49,50].

Therefore, clearance of apoptotic cells is a complex process, involving many factors as discussed so far. Firstly, death stimuli may be important to determine which signal is going to be delivered to phagocytes. Secondly, appropriate opsonization by soluble factors from the innate immune system contributes enormously to the removal of dying cells and the subsequent consequence on the immune response. Thirdly, as discussed earlier, the phagocyte system is largely heterogeneous, therefore the nature of a specific phagocyte that encounters a dying cell, defines the consequences of being eaten. In conclusion, the link between defective clearance of dying cells and autoimmune lupus has a reasonable solid scientific basis, but the exact immunological mechanisms involved remain to be defined.

5. Scope of this thesis

The current thesis was dedicated to understand how different components of the innate immune system contribute to the clearance of apoptotic cells and to the immunological response involved in this process. In **part I** of this thesis, we focus on the biology of phagocyte subsets and their role in the handling of dying cells. This part consists of 4 chapters: **Chapter 2** is a review discussing the latest knowledge on how different subsets of DCs and Mφ handle dying cells, and particularly the immune response evoked by APCs that have eaten dying cells. **Chapter 3** describes the differential contribution of pro-inflammatory Mφ (GM-CSF-driven Mφ1) and anti-inflammatory Mφ (M-CSF-driven Mφ2) in the phagocytosis of early apoptotic, late apoptotic and necrotic cells and the mechanisms that are involved in this process. **Chapter 4** is a follow-up of **Chapter 3,** describing the finding that *in vitro*-polarized pro-inflammatory and anti-inflammatory Mφ seem to have an *in vivo* counterpart as well. We analyzed human peritoneal Mφ (pMφ) freshly isolated from patients on peritoneal dialysis and performed functional comparisons among pMφ, Mφ1 and Mφ2. In **Chapter 5**, it is demonstrated that polarized pro-inflammatory and anti-inflammatory Mφ can be re-differentiated

towards anti-inflammatory and pro-inflammatory cells by switching lineage-determining factors GM-CSF and M-CSF, respectively.

In the latter part of this thesis (**Part II),** the role of serum factors in the handling of dying cells and its association to pathogenesis of SLE are described. **Chapter 6** discusses the importance of the innate immune system, particularly complement, in the clearance of apoptotic cells. **Chapter 7** reports on the role of properdin (an important positive complement regulator) on binding to dying cells and physiological consequences such as complement activation and immune regulation by DCs and Mφ.

Finally, in **Chapter 8** general conclusions are drawn and topics of interest are discussed. We also describe several ongoing studies in the direction of: 1.) elucidation of the role of serum factors in the processing of dying cells in SLE patients; 2.) dissection of how DCs, that are loaded with early, late or necrotic cells, process and present antigens to T cells; 3.) immuno-modulation of Mφ subsets.

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