Developmental Cell
Review

The Dynamics of Apoptotic Cell Clearance

Michael R. Elliott[1,2,*] and Kodi S. Ravichandran[3,4,5,*]
[1]Department of Microbiology and Immunology
[2]David H. Smith Center for Vaccine Biology and Immunology
University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA
[3]Department of Microbiology, Immunology, Cancer Biology
[4]Beirne B. Carter Center for Immunology Research
[5]Center for Cell Clearance
University of Virginia, Charlottesville, VA 22908, USA
*Correspondence: michael_elliott@urmc.rochester.edu (M.R.E.), ravi@virginia.edu (K.S.R.)
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The phagocytic clearance of dying cells in a tissue is a highly orchestrated series of intercellular events coordinated by a complex signaling network. Recent data from genetic, biochemical, and live-imaging approaches have greatly enhanced our understanding of the dynamics of cell clearance and how the process is orchestrated at the cellular and tissue levels. We discuss how networks regulating apoptotic cell clearance are integrated to enable a rapid, efficient, and high-capacity clearance system within tissues.

Introduction

The maintenance of tissue health and function requires the constant replacement of damaged or aged cells with new ones. In fact, on a daily basis the typical healthy adult is estimated to turn over via apoptosis approximately ~150 billion cells (out of the estimated 37.2 trillion cells in the human body), or roughly 0.4% of a body's cellular mass (Bianconi et al., 2013). Removal of these apoptotic cell corpses without damaging the healthy neighboring cells via phagocytosis thus plays a decisive role in cellular homeostasis. The phagocytic clearance of apoptotic cells (a process referred to as efferocytosis) plays key roles in embryonic development, organogenesis, tissue repair, and immunity. Moreover, aberrant interstitial cell clearance is increasingly being viewed as both a cause and consequence of the pathobiology of many diseases (Elliott and Ravichandran, 2010; Green et al., 2016; Nagata et al., 2010; Poon et al., 2014b; Saas et al., 2013). Accordingly, there is growing interest in understanding the regulatory networks that control this dynamic and ubiquitous biophysical process.

Even in healthy tissues where cellular turnover rates are high (e.g., intestine, lung, bone marrow, and thymus), uncleared apoptotic cells are relatively rare, suggesting that homeostatic efferocytosis operates at an impressive level of efficiency (De Paepe, 2004; Elliott et al., 2009, 2010; Lee et al., 2016; Sunaga et al., 2013; Surh and Sprent, 1994). The mechanistic underpinnings of this efficiency are the focal point of a number of important unanswered questions in the field: How is cell clearance orchestrated such that diverse signaling pathways facilitate efferocytosis? What is the capacity of the clearance system? What are the biophysical and spatiotemporal constraints for corpse recognition and removal? What are the constraints and unique features of cell clearance in specific tissue/disease contexts? What are the consequences of cell clearance on the metabolomics of cells and tissues? Over the past 20 years, we have gained an impressive amount of knowledge regarding the key molecular players and signaling pathways that regulate both cell death and efferocytosis. Moreover, we have recently begun to gain insight into how this complex and seamless biological process is carried out in real time at the cellular and tissue levels in mammals. Increasingly sensitive, tractable intravital and in vitro microscopy techniques together with new tissue-specific genetic probes have led to a number of recent advances in our understanding of apoptotic cell clearance in vivo. This has allowed us to begin to develop an integrated view of the physical and temporal constraints on cell clearance in a tissue context, which will have implications for cell clearance under both physiological and pathological conditions. This review highlights some of these recent advances. As there are a number of excellent reviews of the relevant technical advances (Liu and Keller, 2016; Pittet and Weissleder, 2011), we specifically focus here on findings that have helped reframe our conceptual understanding of this dynamic intercellular process and discuss how newer approaches can be applied to key unanswered questions in the field. While this review is focused primarily on cell clearance in the mammalian context, we acknowledge the invaluable contribution of many elegant genetic and intravital imaging studies from the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the zebrafish *Danio rerio* models in developing our understanding of cell clearance in vivo.

Meeting up: How Phagocytes Find Dying Cells

The first and most obvious requirement for efficient apoptotic cell clearance is to bring the apoptotic cell and phagocyte near enough to facilitate physical interaction between the cells. This proximity is facilitated in three different ways: adjacency, phagocyte migration, and the more recently recognized concept of apoptotic cell motility. Although useful for categorization, these mechanisms are not mutually exclusive, but rather likely act in concert to influence efficient cell clearance in the interstitium (Desch et al., 2011; Fujimori et al., 2015; Larson et al., 2016; Lee et al., 2016; Lu et al., 2011; Yang et al., 2015). Interstitial cell clearance is frequently carried out by neighboring or adjacent phagocytes that are of non-hematopoietic origin, such as epithelial cells in the lung and gut, and mesenchymal cells in the developing embryo (Juncadella et al., 2012; Lee et al., 2016; Wood et al., 2000). The efficiency and capacity of these so-called "non-professional" phagocytes to clear dying cells is typically much less than that of "professional" phagocytes of

hematopoietic origin such as macrophages and dendritic cells. The roles of professional versus non-professional phagocytes in the clearance of dying cells has been discussed at length in several recent reviews (Arandjelovic and Ravichandran, 2015; Green et al., 2016). Here we focus on spatiotemporal features related to motile, professional phagocytes that are important to the establishment of the phagocyte-apoptotic cell interactions required for highly efficient removal of dead cells.

Possible Relevance of Phagocyte Positioning within the Interstitium for Apoptotic Cell Clearance

Most tissues are interspersed with networks of hematopoietic phagocytes, including macrophages, monocytes, and dendritic cells (Davies et al., 2013; Dzhagalov et al., 2013; Kim et al., 2010; Okabe and Medzhitov, 2015; Perdiguero and Geissmann, 2015; Westphalen et al., 2014). These cells act as immune sentinels for infection and tissue damage and are also key mediators of dead cell clearance. However, in most tissues, professional phagocytes are greatly outnumbered by the non-phagocytic cells in the organ. Therefore, the positioning of these phagocytes within a tissue is likely important for maximizing their opportunity for interaction with dying cells. For example, in sinusoidal tissues such as bone marrow, spleen, and liver, the tissue-resident macrophages are positioned either within or just exterior to the arterial sinus. While these macrophages can engulf apoptotic cells (e.g., aged neutrophils in the bone marrow and hepatocyte corpses in the liver [Arandjelovic and Ravichandran, 2015; Casanova-Acebes et al., 2013; Furze and Rankin, 2008; Juncadella et al., 2012; Suratt et al., 2004]), their primary function is thought to be the clearance of damaged or effete red blood cells (RBC). By contrast, interstitial positioning of macrophages and dendritic cells (DC) for engulfment of nucleated cells appears to be highly dependent on the nature of the cellular environment and function of the tissue. This is particularly true for lymphoid organs, where lymphocyte development, activation, and subsequent contraction of immune effector cells lead to large numbers of apoptotic leukocytes (Garrod et al., 2012; Klein et al., 2014; LeBien and Tedder, 2008). In these tissues, macrophages and dendritic cells appear to be pre-positioned at locations where apoptotic cells accumulate or are likely to occur based on the nature of death stimuli in the tissue. For example, during an adaptive immune response, tingible body macrophages are located at the light/dark border of the germinal centers in the spleen and lymph nodes where they capture proliferating B cells undergoing apoptosis due to low affinity or self-reactivity (Gray and Cyster, 2012; Hanayama et al., 2004; Vinuesa et al., 2009). T lymphocyte development in the thymus results in large numbers of apoptotic T cells, where thymic macrophages, and to a lesser extent dendritic cells, are sparse in number (~1% of total thymic cells) but are positioned in small clusters throughout the organ, providing widespread efferocytic coverage through the tissue (Dzhagalov et al., 2013; Kim et al., 2010; Tacke et al., 2015). The $CD169^{+}$ macrophages, the predominant efferocytes in the bone marrow, are located within dense cellular regions adjacent to the sinuses (Morrison and Scadden, 2014). These macrophages appear optimally located to multitask in the engulfment of apoptotic B cells, aged neutrophils, and erythrocytes.

In some non-lymphoid tissues where moderate to high rates of apoptosis are normal, "specialized" phagocytes appear positioned to maximize the opportunity for encountering apoptotic cells. In this context, "specialized" refers to a population of tissue-resident phagocytes (of either hematopoietic or non-hematopoietic origin) that have evolved highly unique gene expression and functional characteristics that support the function of one type of tissue. For example, in the seminiferous tubules of the testes, approximately 75% of developing germ cells will undergo apoptosis before ever becoming mature sperm (Bailey et al., 2002; Braun, 1998). Sertoli cells are specialized phagocytes attached to the basal lamina of the seminiferous tubules that extend their processes toward the lumen, forming a network of membrane structures that intertwine with spermatogonia and can recognize and rapidly engulf specifically those germ cells undergoing apoptosis (Elliott et al., 2010; Griswold, 1998; Nakanishi and Shiratsuchi, 2004; Park et al., 2011; Shiratsuchi et al., 1997). Similarly, in the brain, ongoing neurogenesis in the hippocampus features moderate rates of apoptosis, involving hundreds of developing neurons. Microglia, a specialized brain phagocyte, line the dentate gyrus and mediate engulfment of developing neurons that undergo apoptosis (Fourgeaud et al., 2016; Lu et al., 2011; Mattocks and Tropepe, 2010; Sierra et al., 2010). In contrast, in the pleural cavities, sentinel, tissue-resident macrophages can move freely or are loosely adhered to the membrane surfaces, enabling rapid response to pathogens outside the lung and visceral organs (Jenkins et al., 2011; Okabe and Medzhitov, 2014; Rosas et al., 2014). Within the lung and airways, specialized tissue-resident macrophages and dendritic cells are integrated into the epithelial layer that lines the airway. A subset of migratory lung DC ($CD103^{+}$/$CD11c^{+}$/$MHCII^{hi}$) appear to be the dominant DC subtype that captures apoptotic cells in the lung and can traffic to the draining lymph node for antigen presentation to naive T cells (Desch et al., 2011). Recently, Bhattacharya and colleagues used intravital microscopy to demonstrate that a subset of alveolar macrophages forms gap junction channels with epithelial cells, enabling calcium-dependent signaling between macrophages and across many cell widths (Westphalen et al., 2014). This is important as, unlike many tissue-resident macrophages, alveolar macrophages are mostly sessile. Considering the critical role of airway epithelial cells in the clearance of apoptotic cells during inflammation and allergic responses, it is possible that both professional and non-professional phagocytes operate in concert to mediate efficient cell clearance and immune responses (Juncadella et al., 2012). Although emerging evidence strongly suggests that tissue-resident phagocytes receive interstitial localization and positioning cues, the source and identity of these cues remains poorly understood. In light of the recent flood of information regarding the ontogeny and tissue-specific development of macrophages and dendritic cells (Gautier et al., 2012; Okabe and Medzhitov, 2015; Perdiguero and Geissmann, 2015), it seems likely that we are poised to apply new, sophisticated genetic models and gene-expression analyses to understand how the positioning of tissue-resident interstitial phagocytes occurs and how the environmental, phenotypic, and functional heterogeneity are interconnected (Guilliams and van de Laar, 2015; van de Laar et al., 2016).

In contrast to homeostatic cell clearance, disease-associated tissue damage can lead to multiple waves of cell death, and the importance of cell clearance in these tissues is increasingly