New Lives Given by Cell Death: Macrophage Differentiation Following Their Encounter with Apoptotic Leukocytes during the Resolution of Inflammation

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New lives given by cell death: macrophage differentiation following their encounter with apoptotic leukocytes during the resolution of inflammation

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INTRODUCTION

Macrophages are highly plastic monocyte-derived cells that acquire different molecular and functional phenotypes following exposure to different bioactive molecules and environments. The early studies on the interactions of macrophages and lymphocytes in battling bacterial infections revealed the Th1 (IL-12, IL-23, TNFα, IL-10) and Th2 (IL-4 and IL-13) lymphocytes activated macrophages were previously classified as classically (M1) or alternatively (M2) activated macrophages, based on their exposure to different fate-determining mediators. These macrophage subsets display distinct molecular markers and differential functions. At the same time, studies from recent years found that the encounter of apoptotic leukocytes with macrophages leads to the clearance of this cellular “debris” by the macrophages, which concomitantly reprograming/immune-silencing the macrophages. While some of the features of M2 differentiation, such as arginase-1 (murine) and 15-lipoxygenases (human and murine) expression, were also displayed by macrophages following the engulfment of apoptotic cells, it was not clear whether apoptotic cells can be regarded as an M2-like differentiating signal. In this manuscript, we review the recent information regarding the impact of apoptotic cells on macrophage phenotype changes in molecular terms. We will focus on recent evidence for the in vivo existence of distinct pro-resolving macrophages and the role of apoptotic cells, specialized lipid mediators, and glucocorticoids in their generation. Consequently, we will suggest that these pro-resolving CD11b+M2W macrophages have morphed from M2-like macrophages, and modulated their protein profile to accommodate the changes in their function.

Keywords: resolution of inflammation, macrophage differentiation, efferocytosis, pro-resolving lipid mediators
differentiation and transcriptional events activated by early effero-
cytosis. In addition, we will discuss recent results that support the
notion that efferoctyosis can eventually transform macrophages to
another phenotype that is postulated to limit tissue repair/fibrosis
and promote macrophage regulatory properties at remote sites. In
this regard, it is important to note the early studies that indicated
“non-phlogistic” activation of monocytes by the pro-resolving
“eat me” signals (and the absence of “do not eat me” signals)
(Ariel and Serhan, 1996; Maddox et al., 1997, 1998) hence prompting the
notion that resolution-driven monocyte/macrophage activation
promotes tissue repair and wound healing.

EFFEROCYTOSIS AS AN ALTERNATIVE MODE OF
MACROPHAGE ACTIVATION

The recognition, engulfment, and responsiveness to apoptotic cells
are cardinal properties of resident and inflammatory macrophages
and play a role in processes, such as tissue morphogenesis and
homeostasis, embryonic development, hematopoiesis, immunity,
and the resolution of inflammation (Savill et al., 2002; Erwig
and Henson, 2007; Ravichandran and Lorenz, 2007). The recog-
nition and uptake of apoptotic cells by macrophages through
“eat me” signals (and the absence of “do not eat me” signals)
expressed on their surface and their cognate receptors have been
extensively studied and reviewed (Ravichandran, 2011). However,
apoptotic cells also transduce signals to the engulfing macrophages
that result in significant molecular and functional adjustments
that address physiological needs consequent to the identified
cell death. During the resolution of inflammation, macrophages
engulf apoptotic cells and consequently, apoptotic cell recognition
evokes distinct signaling events (Patel et al., 2006) that block the
release of pro-inflammatory mediators from macrophages. This
release is activated by bacterial moieties, and its blockage, which is
termed immune-silencing (Voll et al., 1997; Fadok et al., 1998;
Kim et al., 2004), is accompanied by the production of TGFβ
and IL-10 (Byrne and Reen, 2002; Huynh et al., 2002; Mitchell
et al., 2002), cytokines that can promote resolution and wound
repair. The engulfment of apoptotic leukocytes by macrophages
also leads to inhibition of iNOS expression and stimulates the
expression of arginase-1 in the RA W 264 macrophage cell line
(Freire-De-Lima et al., 2006) thereby preventing reactive NO pro-
duction. In addition, the production of angiogenic growth factors
(Golpon et al., 2004) by macrophages is consequent to the uptake
of apoptotic cells. Elucidation of the signaling pathways activated
by efferoctyosis revealed significant roles for nuclear transcrip-
tional regulators, such as peroxisome proliferator activated recep-
tor (PPAR)-γ (Freire-De-Lima et al., 2006; Johann et al., 2006)
and -β (Mukundan et al., 2009) as well as the liver X receptor
(LXR; A-Gonzalez et al., 2009) in promoting anti-inflammatory
properties.

It is important to note that while macrophages engulf tissue-
infiltrating apoptotic PMN during the resolution of inflamma-
tion, different experimental models used different sources of
apoptotic cells, including Jurkat T cells, mouse thymocytes, or
human peripheral blood neutrophils. All types of apoptotic cells
express phosphatidylserine on the outer leaflet of their cytoplas-
mic membrane, and this is apparently the major signaling module
used by these cells to communicate their mortal status with phago-
cytic cells (Ravichandran, 2011). Nevertheless, it is conceivable that
other molecules (“eat me signals”) are expressed on apoptotic cells
different sources to give a more detailed “report” as to the con-
sequences of their demise. Thus, the interpretation of the results
obtained following incubations of macrophages with apoptotic
cells of different sources should be evaluated carefully depending
on the source of apoptotic cells used.

The prototypic Th2 cytokines IL-4, IL-13, and IL-10, as well as
immune responses to parasites were found to promote many of the
outcomes of efferoctyosis in macrophages. These cytokines are
well appreciated antagonists of the M1 response and macrophage
pro-inflammatory properties (Martinez et al., 2009) while IL-4
and IL-13 can also promote fibrosis through TGFβ production
(Fichtner-Feigl et al., 2006; Wynn, 2008). IL-13 was also found to
promote vascular endothelial growth factor production during
lung injury (Corne et al., 2000). Importantly, IL-4 and IL-13
also activate PPAR-γ (Huang et al., 1999; Berry et al., 2007) and
PPAR-δ (Kang et al., 2008) to promote monocyte/macrophage
alternative activation. LXR was recently found to synergize with
IL-4 in the induction of arginase-1 expression and promotion of
an M2 phenotype in regressive atherosclerotic lesions (Pourett
et al., 2011). Thus, efferoctyosis induces phenotypic and molecu-
lar switches and activates signaling pathways in macrophages that
resemble M2 polarization. Moreover, M2 polarization promotes
efferoctyosis through induction of different molecular modules,
whereas M1 macrophages exert reduced uptake of apoptotic cells.
Along these lines, recent studies also found that efferoctyosis is a
self-promoting process, and that M2 pathways play key roles in
mediating this feature of macrophage function. These aspects of
efferoctyosis are covered by Korns et al. (2011) in this research
topic and will not be elaborated on here. Nevertheless, while
macrophages are paradoxically involved in both the generation
of fibrosis and its resolution (Wynn and Barron, 2010) and effe-
roctyosis and M2 polarization generate a positive feedback loop
during resolution of inflammation, it is much less clear what are
the events and mediators that stop M2 differentiation and tis-
sue repair/remodeling short of excessive, fibrotic outcomes. Such
events and mediators are inevitably required to complete the res-
olution of inflammation and restore homeostasis rather than end
every infection with a debilitating scar.

15-LIPOXYGENASE AND ITS PRODUCTS

A major enzymatic pathway that mediates key events in
the resolution of inflammation involves the expression and
activation of 12/15-lipoxygenase (LO) in mice and 15-LO-
1 in humans. 15-LO expression and activity are upregu-
lated by IL-4 and IL-13 in murine and human monocytes,
macrophages, and peripheral blood mononuclear cells
(Levy et al., 1993; Nassar et al., 1994; Heydeck et al., 1998;
Huang et al., 1999; Ariel et al., 2005). This upregulation leads to
the production of 15-LO products from eicosatetraenoic and
docosahexaenoic acids (ETA and DHA, respectively), such as
15-hydroxyeicosatetraenoic acid (15-HETE), lipoxin (LX) A4 and
5(S,6S,15S-trihydroxy-7E,9E,11Z,13E-EPA, and 5S,14R,15S-
trihydroxy-6E,8Z,10E,12E-EPA, respectively), 17S-hydroxy-DHA
(17S-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-DHA), and protectin D1
While 15-HETE binds PPARα and Serhan Efferocytosis modulates macrophage phenotypes (DHA) and maresin 1 (7,14-dihydroxy-4Z,8,10,12,16Z,19Z-DHA), in addition to LXA4 and PD1 (Merched et al., 2008; Serhan et al., 2009). The expression of 12/15-LO was also found to be upregulated in mouse macrophages following their incubation with apoptotic cells (Freire-De-Lima et al., 2006; Schif-Zuck et al., 2011) and resulted in the production of 15-HETE and LXA4 (Freire-De-Lima et al., 2006). Macrophages from chronic granulomatous disease (CGD) mice display impaired efferocytosis that could be repaired by IL-4 through the expression of 12/15-LO and activation of PPAR-γ (Fernandez-Boyanapalli et al., 2009). Hence, 15-LO-mediated signaling seems to be a major convergence point for efferocytosis and M2 polarization, and its down-stream signaling pathways could play a paramount role in deciphering whether macrophages will become pro-fibrotic or will finalize the resolution sequel to restore tissue homeostasis.

Hence, 15-LO products can be generated by macrophages following their interaction with apoptotic cells and/or polarization to at least three distinct populations based on F4/80+ macrophages from resolving peritoneal exudates (Schif-Zuck et al., 2011). Recent reports have indicated the co-existence of various macrophage phenotypes in resolving peritoneal cavities (Bystrom et al., 2008; Schif-Zuck et al., 2011). Macrophages from resolving murine peritonitis expressed an alternatively activated phenotype albeit with increase expression of M1 markers, such as cyclooxygenase 2 (COX 2) and iNOS (Bystrom et al., 2008). Thus, these macrophages were termed resolution-phase macrophages (rMs) and were postulated to have a hybrid phenotype of classically and alternatively activated macrophages (Bystrom et al., 2008). A recent report from the same group has indicated that rMs could be divided to at least three distinct populations based on F4/80 and Ly-6C expression, with varying expression of pro-inflammatory and anti-inflammatory markers as well as CD11b (Stables et al., 2011). Along these lines, we have recently characterized F4/80+ macrophages from resolving peritoneal exudates into two distinct macrophage subtypes: CD11bhigh and CD11blow (Schif-Zuck et al., 2011). CD11bhighb macrophages were found to express low to intermediate levels of the M1 markers iNOS, COX 2, and matrix metalloproteinase (MMP)-9 and high levels of the M2 marker arginase-1. These cells also expressed very low levels of 12/15-LO. In addition, these macrophages secrete high levels of inflammatory cytokines and chemokines, as well as IL-10, in response to TLR ligands, are highly phagocytic, and do not migrate to lymphoid tissues. CD11blow macrophages express even lower levels of iNOS, COX 2, and MMP-9 than CD11bhigh ones, but they also do not express arginase-1. In addition, these macrophages secrete very low levels of inflammatory cytokines and chemokines, and IL-10, but higher amounts of TGFβ. Moreover, CD11blow macrophages, despite containing higher numbers of apoptotic PMN, are no longer phagocytic and are prone to emigrate to remote sites. Hence, CD11blow macrophages were termed “satiated” (Schif-Zuck et al., 2011). A seminal report from Ravichandran and colleagues (Park et al., 2011) has recently revealed that the mitochondrial membrane protein UCP2 controls satiation vs. continued clearance of apoptotic cells, and it would be interesting to examine its role in the generation of CD11blow macrophages. The integration of the results from Schif-Zuck et al., Bystrom et al., and Stables et al. suggests rM/CD11bhigh macrophages are a mixed macrophage population with dominant M2-like characteristics, and some low-grade M1 activity and that early efferocytosis promotes the conversion of the M1-like population to an M2-like phenotype (Fadok et al., 1998; Freire-De-Lima et al., 2006; Korns et al., 2011) as well as enhanced phagocytosis/efferocytosis. However, the CD11blow subset of macrophages, although converting from the CD11bhigh subset ex vivo and in vivo (following late, threshold-meeting, efferocytosis; Schif-Zuck et al., 2011), are not M2-like, but rather display a distinct phenotype with its own molecular and functional characteristic (Figure 1). Of interest, a similar series of macrophage phenotype switches was found to take place.
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**Efferocytosis modulates macrophage phenotypes**

**FIGURE 1 | Macrophenome phenotype conversions induced by efferocytosis.** A monocyte that infiltrates an inflamed tissue differentiates to a macrophage and adopts an M1-like phenotype previous to encounter with apoptotic PMNs (A). Once it encounters apoptotic PMN and starts to engulf them (early efferocytosis), the macrophage switches to an M2-like phenotype that is anti-inflammatory, highly efferocytic, and involved in tissue repair and return to homeostasis, but can also promote fibrosis and scar formation (B). As the engulfment of apoptotic PMN by the macrophage continues and reaches a threshold level determined by the resolving milieu (satiating efferocytosis), the macrophage undergoes another switch to the Mres phenotype (C). These macrophages reduce the expression of pro-fibrotic arginase-1 and display reduced phagocytosis of extracellular particulate including apoptotic cells. Consequently, rapid Mres departure of the resolving tissue and emigration to remote sites takes place. At these target organs Mres macrophages presumably produce 12/15-LO-derived pro-resolving lipid mediators, and deliver homeostatic signals to antigen presenting cells and lymphocytes. Moreover, Mres that stay in the resolving tissue might express higher levels of anti-inflammatory, anti-fibrotic, and anti-oxidant proteins to limit tissue damage and fibrosis. 12/15-LO-derived lipid mediators probably also contribute to the anti-inflammatory and anti-fibrotic properties of Mres in the resolving tissue. Early and satiating efferocytosis can be modulated by pro-resolving and anti-inflammatory mediators, such as lipoxins, resolvins, protectins, maresin, GC, IL-4, TGFβ, IL-10, and PPARγ ligands (D). This modulation can enhance the immune-silencing and departure of Mres to the lymphatics, where they can contribute to the termination of acquired immune responses.

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Conflict of Interest Statement: Charles N. Serhan is an inventor on patents (resolvins) assigned to BWH and licensed to Resolvyx Pharmaceuticals. Charles N. Serhan is a scientific advisor of Resolvyx Pharmaceuticals and owns equity in the company. Charles N. Ser- han’s interests were reviewed and are managed by the Brigham and Women’s Hospital and Partners HealthCare in accordance with their conflict of interest policies.

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