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Immunomodulatory properties of apoptotic cells

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INTRODUCTION

Chronic inflammation usually originates from a dysfunction in tolerance processes implicating a variety of soluble factors and immune cells. In homeostatic conditions, an inflammatory event triggering by, for instance, pathogen invasion, necrotic damaged cells or irritants is a natural process initiatedto protect the target tissue, remove the injurious stimulus,and then initiate the healing process. Such a process detects and engages immune cells that are able to restore toleranceconsequently. A defect in that process could lead to a continuous stimulation of immune cells, overpassing tolerogenic mechanisms andthus leading to chronic inflammation. Such a defect can implicate one or many factors and/or effector cells that can sustain inflammatory signals overwhelming inflammation. A way to break the inflammatory process is to reintroduce in the damaging loop factors that will favor the production of anti-inflammatory elements and desensitize immune responsesorchestrated byantigen presenting cells, thusallowing immune cells to take control all over again. One strategy is to use apoptotic cell injection in order to benefit from their direct and indirect immunomodulatory properties to favor tolerance induction. This has been already evaluated in many experimental models and proposedin a fewclinical trials.

APOPTOTIC CELLS

One of the key elements allowing restoration of homeostasis is apoptotic cell death. First, after doing their job such as elimination of pathogens, reactive T cells need to graciously disappear mainly through apoptotic cell death. Apoptotic cell death, i.e., apoptosis, is a physiological mechanism that allows the elimination of cells inexcess or unwantedcells preventing an inflammatory process (1, 2). The lack of inflammation associated with apoptosis is attributed to the fact that professional phagocytes (mainly macrophages and subpopulations of dendritic cells)–but also neighbor cells− efficiently engulf apoptotic cells and apoptotic residues called

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apoptotic bodies. This prevents the secondary necrosis of apoptotic cells, a pro-inflammatory cell death (3),and so, the release of proteases and other inflammatory mediators as alarmines(4, 5)by late apoptotic/necrotic cells (3, 6, 7). Efficient apoptotic cell removal is governed by multiple signals delivered by apoptotic cells including: "find me" signals responsible for professional phagocyte attraction, the expression of "eat me" signals and the repression of "don't eat me" signals to avoid the elimination of viable cells (Fig. 1)(8, 9). Regulation is also provided by "keep out" signals such as lactoferrinthat prevent neutrophil migration(10).

The mechanisms associated with the efficient elimination of cells entering apoptosis are also associated with thoseallowing the preventionof the immune response initiation. These mechanisms are critical and redundant as they should prevent the occurrence of autoimmune diseases (11, 12). It is possible to distinguish two types of mechanisms: those directly related to apoptotic cell death and the others dependent on their elimination by phagocytic cells. Thus, phagocytes will shape a new microenvironment through the secretion of soluble factors affecting themselves as well as all the neighboring cells,preventing in concert the appearance of unwanted immune response deleterious to the host.

DIRECT EFFECT OF APOPTOTIC CELLS

Several studies report that during the process of apoptosis,apoptotic cells secrete immunosuppressive cytokines such as IL-10 and TGF-β (13, 14). TGF-β, stored in a latent form in intracellular compartments, is released during apoptosis (13). Cytokine release allows to generate immunosuppressive microenvironment, inhibits the secretion of pro-inflammatory cytokines (TNF-α or IL-1β) by macrophages (15, 16)and neutralize the development of an

effective immune response. This also prevents the initiation of an immune response targeting antigenspresent on –or expressed by– apoptotic cells, and thus prevents autoimmune responses. Indeed, apoptotic bodies escaping from removal have been reported to cluster clinical relevant auto-antigens at their surface(17). These auto-antigens are exposed at cell surface or translocated from internal compartments to cell membrane during early stages of apoptosis(18, 19). Apoptotic cells arealso able to clear or neutralize inflammatory chemokines, such as CCL3 and CCL5 *via* CCR5 expression, thus preventing the migration of other leukocytes (20). All these immunosuppressive effects are time-limited until the cell dislocation occurs through secondary necrosis and so, other mechanisms need to take place to prevent chronic inflammation.

INDIRECT EFFECT OF APOPTOTIC CELLS

Apoptotic cells also allow the development of an immunomodulatory environment indirectly through phagocytic cells. Indeed, professional phagocytic cells such as macrophages can release or express immunosuppressive molecules (IL-10, TGF-β, prostaglandin E2 or PGE-2, Fas ligand) in the clearance of apoptotic cells (21-23). Thus, apoptotic cells have immunosuppressive properties *in vitro* notably through the secretion of IL-10 which induces*in vivo* immune deviation to type 2cytokine secretion (14, 21, 22). The production of TGF-β1 is observed during phagocytosis by macrophages or immature dendritic cells(15, 23). In addition, it has been shown that phagocytosis of apoptotic cells induce a down-regulation of IL-12 secretion as well as TNF by macrophages and could also block the synthesis of pro-inflammatory cytokines by interfering with NF-kappaB(24-26). The elimination of apoptotic neutrophils inhibits the synthesis of cytokines called "Th17", such as IL-23 and IL-17 by phagocytic cells (27). Altogether, this suggests that pro-inflammatory Th1 and Th17 responses are prevented by professional phagocytes participating in apoptotic cell removal.

Macrophages appear to be the main phagocytic cells which remove most effectively apoptotic cells (15, 28, 29). Indeed, they express a large number of membrane receptors involved in this elimination (Fig.1). The stimulation of these receptors favors induction of animmunomodulatoryphenotype of the phagocytes. Many immunomodulatory mechanisms have been reported. They are represented mainly by the release of soluble factors such as cytokines IL-10 or TGF-β. Macrophage functions will be limited after removal of apoptotic cells. In addition, some subpopulations of dendritic cells have been involved also in the capture of apoptotic cells (23, 30-34). One study conducted on rats showed that a subpopulation of circulating dendritic cells would be responsible for capturing continuously apoptotic cells and bodies from intestinal epithelial cells removed every day after desquamation. Then, dendritic cells migrate to the mesenteric lymph nodeswhere they inactivate the naïve autoreactiveT cells(30, 32,35). Other experimental studies also suggest that the capture of apoptotic cells by dendritic cell subsets leads to tolerance (23, 31, 33, 34). In addition, an *in vitro* study showeddendritic cells that have captured apoptotic cells did not respond to lipopolysaccharide (36).This is also true for macrophages (26). Thus, professional phagocytes that encountered apoptotic cells become refractory to Danger signal triggering.

A consequence of the interaction ofapoptotic bodies and phagocytic cells: induction of regulatory T cells

The consequences of apoptotic cell-phagocyte interactions influence the differentiation of naïve CD4^{+} T cells. Although contact with apoptotic cells blocks the ability of maturation and cytokine production of conventional dendritic cells, their migration capabilities are not affected or at least

redefined. So, dendritic cells can acquire the expression of CCR7 and migrate in response to gradients of CCL19 and CCL21 to the lymph nodes closest to the site where the cells died (37, 38). In the lymph nodes, such dendritic cells can interact with naive CD4^{+} T cells and deliver a "tolerogenic" signal to the T cellfavoring T cell commitment to a regulatory phenotype, such as induced Foxp3^{+} regulatory T cells (Treg) or IL-10^{+} Tr1 cells (23, 39-41). Whether natural Treg commitment in the thymus also respects such mechanism is still uncertain. However, several dendritic cell subsets are able to migrate from the periphery to the thymus to transport peripheral antigens(42). This may occur *via* the capture of auto-antigens from apoptotic cells. A main feature of Treg generated by dendritic cells is their ability to increase production of IL-10 (43, 44). It was also suggested that plasmacytoid dendritic cells are the dendritic cell subtype favoring Tr1 commitment(45). In addition, plasmacytoid dendritic cells can also promote the differentiation of inducible Foxp3^{+}Treg(46-48).Immature plasmacytoid dendritic cells transport antigens from the periphery to the thymus*via* the expression of CCR9, α4-integrin and functional binding sites for P-selectin(49), and human thymicplasmacytoid dendritic cells favor natural Treg generation(50, 51).

Apoptotic cells are endowed with immunomodulatory properties by targeting innate and adaptive immunity at different levels like at the microenvironment level, the polarization of antigen presenting cells,T and B cells. Generation of regulatory B cells (Breg) has also been reported(52). The immunomodulatory microenvironment created through apoptotic cell elimination suggests that apoptotic cell injection might be a powerful tool to control inflammation and restore tolerance *in vivo*. Thus, our group and others have demonstrated in various experimental models that indeed apoptotic cell injection can control inflammation allowing the restoration of homeostasis and in some settings, tolerance induction (Table 1).

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These accumulated data also favored the initiation of clinical trials, the first one demonstrating the safety of the approach and the second one the efficacy of the approach.

CONCLUSION: TRANSLATION TO ANTI-NEUTROPHIL CYTOPLASMIC AUTO-ANTIBODY (ANCA)-ASSOCIATED VASCULITIS

Neutrophil apoptosis may play a role in the pathogenesis of anti-neutrophil cytoplasmic auto-antibody (ANCA)-associated systemic vasculitis(53). Several tracks have been proposed to explain its role in the pathophysiological process of ANCA-associated vasculitis. Apoptotic neutrophils can be considered as a Danger signal since they contain a lot of proteases, including serine proteases and elastase(54). However, repeated infusion of apoptotic neutrophils in brown Norway rats has been shown to induce ANCA, but did not lead to systemic vasculitis(55). This suggests that apoptotic neutrophils by themselves are not sufficient to trigger the complete clinical features of ANCA-associated vasculitis. Recent works by Witko-Sarsat and colleagues (56, 57)suggest that proteinase-3, the target of ANCA in granulomatosis with polyangiitis, delays apoptotic neutrophil removal when expresses at the cell membrane and leads to enhanced secretion of pro-inflammatory cytokines, including: TNF, IL-8 and MIP-1β.So, restoring efficient apoptotic neutrophil uptake can be considered as a potential therapeutic approach to treat ANCA-associated vasculitis.

Table 1.Therapeutic applications of apoptotic cell administration

	References
Chronic inflammatory autoimmune diseases	
Diabetes	Xia, 2007 (41); Mougel, 2012 (58)
Experimental Autoimmune Encephalomyelitis	Miyake, 2007 (28); Qiu, 2009 (59)
Arthritis	Gray, 2007 (52); Perruche, 2009 (26); Notley, 2011 (60)
Acute inflammatory diseases	
Sepsis	Huynh, 2002 (61); Ren, 2008 (62)
Fulminant hepatitis	Zhang, 2011 (63)
Contact hypersensitivity	Griffith, 2007 (64)
Transplantation	
Cardiac allograft	
Acute rejection	Sun, 2004 (65); Wang, 2006 (66)
Chronic rejection	Wang, 2009 (67)
Islets allograft	Mougel, 2012(68)
Hematopoietic cell transplantation	
Hematopoietic engraftment	Bittencourt, 2001 (69); Kleinclauss, 2006 (39); Perruche, 2004 (70); Bonnefoy, 2011 (48)
Graft-versus-host disease	Kleinclauss, 2006 (39)
Allo-antibodies after graft rejection	Perruche, 2004 (70)
Acute myocardial infarction	Ankersmith, 2009 (71); Lichtenauer, 2011 (72)

Adapted from (73)

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Figure 1.Different signals involved in apoptotic cell removal. Different signals orchestrate apoptotic cell removal by neighbor cells or professional phagocytes such as macrophages. This includes: 1) the loss of "don't eat me" signals; 2) the secretion of "find me" signals that can be counterbalanced by "keep out" signals(74, 75); 3) the acquisition of "eat me"signals.Adapted from Ref.and updated with the following reviews(8, 9) and original publications(76, 77). Abbreviations used:BAI-1, Brain Angiogenesis Inhibitor-1; CRP, C reactive protein; CRT, calreticulin; Gas6, Growth Arrest 6; MBP, Mannose Binding Protein; MFG-E8, lactadherin; PS, phosphatidylserines; PSR, phosphatidylserine receptors; PR3, proteinase-3; RAGE, receptor for advanced glycation end products; SRA, Scavenger Receptor A; TSP1, thrombospondin-1.