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SPOTLIGHT

When aging gets on the way of disposal: Senescent cell suppression of efferocytosis

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Chronic senescence can trigger pathological inflammation. In this issue, Schloesser et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202207097) demonstrate that senescent cells employ "don't eat me" signals that inhibit the ability of macrophages to engulf them and additionally prevent macrophages from removing neighboring corpses, revealing a new mechanism by which senescence may contribute to triggering inflammation.

Cellular senescence is a persistent cell-cycle arrest of proliferation competent cells, often but not always induced by stress (1). Transient or acute senescence, such as during embryogenesis or even in adulthood, is considered beneficial and allows for changes in cellularity, wound healing, or tumor suppression (1, 2). By contrast, chronic senescence is pathological and leads to tissue dysfunction or tumorigenesis (1, 2). There is a bidirectional crosstalk between senescent cells and cells of the immune system. On one hand, chronic senescence is avoided by immunosurveillance and immune cell-mediated clearance of senescent cells (3). Senescent cells, on the other hand, influence their microenvironment, including immune cells, through the secretion of pro-inflammatory and pro-proliferative mediators, collectively known as senescence-associated secretory phenotype or SASP (1, 4, 5). This is, at least, the prevalent view. In this issue, Schloesser et al. (6) characterize a new mechanism by which senescent cells may disarm immune cells from engulfing them and also impede their ability to clear cellular corpses (6). Collectively, these activities may contribute to chronic inflammation (Fig. 1).

Macrophages are professional phagocytes that express a panoply of discrete receptors for recognition and phagocytosis of specific cargo, including apoptotic cells (7). In studying the response of macrophages to senescent cells, Schloesser et al. (6) reported that senescent cells are not only resistant to macrophage engulfment but are also able to significantly suppress the ability of macrophages to remove bystander apoptotic corpses or efferocytosis. Thus, senescent cell-mediated efferocytosis suppression (SCES) could result in the build-up of cell debris and kindling of inflammation. To test the interaction of senescent cells with macrophages, the authors made use of a battery of human and murine cells and rendered them senescent through various mechanisms, including cell cycle inhibition, induction of DNA damage, and serial passage to Hayflick limit. Next, they employed live imaging of co-cultured senescent cells and macrophages labeled with distinct fluorescent markers to reveal that macrophages swarm around senescent cells but neither kill them nor engulf them. The resistance to being eliminated was independent from the polarization state of the macrophage as it was also observed when LPS-, IL-4-, or IL-13-polarized macrophages were tested. Overall, this resistance is in keeping with the notion that live cells are refractory to macrophage-dependent phagocytosis. What was probably unexpected was the extension of this resistant mechanism to the engulfment of dead cells when they were added to the macrophage-senescent cell co-cultures. Using a tripartite cell culture, the authors detected a significant inhibition in the uptake of UV-irradiated apoptotic cells by macrophages in the presence of senescent cells. This inhibitory mechanism was long-lasting and still detectable when macrophages were re-challenged with apoptotic cells even 24 h after their first encounter with corpses. Schloesser et al. (6) also reported that senescent but not proliferating cells suppressed the ability of macrophages to phagocytose necrotic corpses, bacteria, or silicon beads.

What is the mechanism by which senescent cells mediate such a broad-spectrum, paralytic effect on macrophages? Exposure of macrophages to conditioned media from senescent cells did not induce SCES, and the ability of senescent cells to mediate this inhibitory effect was overcome when they were separated from macrophages by a trans-well. Hence, the inhibitory effect was not mediated by a soluble factor produced by these prominently secretory cells. Rather, it appeared to require contact between the senescent cell and the macrophage. This finding led the authors on a hypothesis-based search for the identity of the cell-surface receptor/s that may mediate the inhibitor effect. They turned their focus to the CD47-SIRPα

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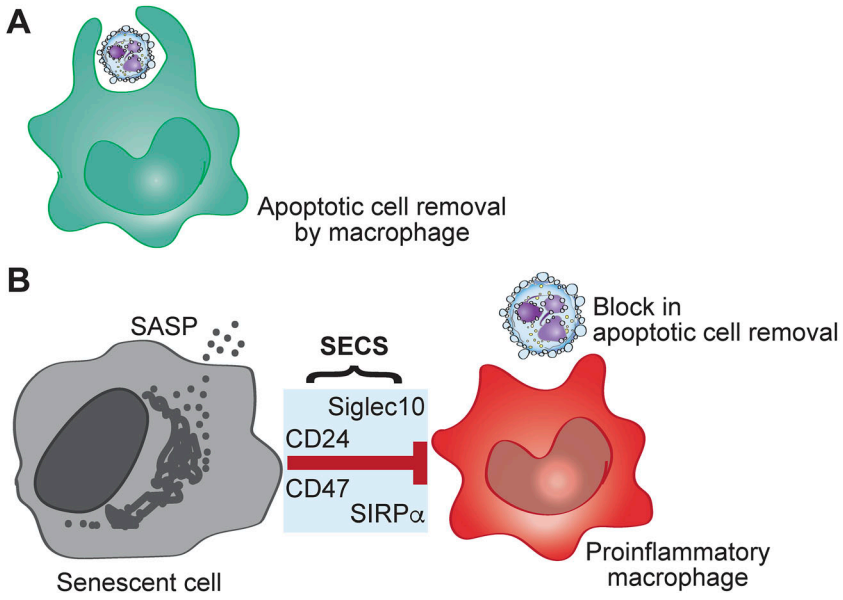

Figure 1. **Schematic of homeostatic phagocytosis of apoptotic cells versus inhibition of efferocytosis in the presence of senescent cells. (A)** Cartoon depicting apoptotic cell removal or efferocytosis by macrophages during homeostasis. **(B)** Presence of senescence cells leads to the engagement of the "don't eat me" signaling pathways CD47-SIRPα and/or CD24-Siglec10, resulting in the inhibition of the removal of the senescent cell as well as bystander apoptotic corpses. This paralytic effect, or SCES, is predicted to result in inflammatory responses.

axis, a well-established signaling pair that mediates "don't eat me" signals and impairment of phagocytosis (8). In support for their hypothesis, CD47 was found to be upregulated in a multitude of stromal and epithelial senescent cells by comparison to proliferating cells. Additionally, genetic ablation of *Cd47* or *Qpct/l*—a glutaminyl cyclase required for CD47 bioactivity—in 3T3 senescent fibroblasts, abrogated SCES. Surprisingly, this was not the case when CD47 $^{-/-}$ senescent pancreatic epithelial cells were tested, leading Schloesser et al. (6) to identify a parallel CD24-Siglec10 inhibitory axis that mediates SCES and is able to compensate for CD47 loss in senescent epithelial cells. This study broadens our understanding of the biological settings in which suppression of efferocytosis may contribute to disease progression and sets the spotlight on the CD47-SIRPα and CD24-Siglec10 signaling pathways as potential therapeutic targets for the inhibition of SCES. It remains to be investigated if quiescence—a different form of replicative dormancy—also results in a similar upregulation of CD47-SIRPα and CD24-Siglec10 signaling pathways and inhibition of phagocytosis.

A natural advance that should follow this interesting original report would be the demonstration of SCES in vivo. The results from Schloesser et al. (6), if proven in vivo, could have significant implications in settings characterized by accumulation of senescent cells, from aging to chronic inflammatory diseases. Could SCES break the cycle of homeostatic removal of apoptotic cells by tissue-resident macrophages during tissue renewal as we age? Tissue-resident macrophages express different types and amounts of phagocytic receptors as well as SIRPα (7; www.immgen.org). Thus, it would be interesting to compare the ability of senescent cells to induce SCES in tissue-resident in addition to the monocyte-derived macrophages tested in this study. Efferocytosis is not limited to tissue renewal. Phagocytosis of apoptotic neutrophils together with IL-4 signaling induces tissue repair responses in macrophages (9). It is tempting to speculate that the presence of senescent cells in chronic diseases, such as idiopathic pulmonary fibrosis or liver fibrosis, and the associated SCES may contribute to the non-resolving nature of these pathologies. Another interesting notion that arises from this study is the implication of SCES in tumorigenesis and/or tumor progression. While senescence is a cell-intrinsic mechanism that arrests cells with oncogenic signals (oncogene-induced senescence), SASP can be a cell-extrinsic signal for tumorigenesis (10). It remains unknown whether SCES deters tumorigenesis by accumulation of corpses and enhanced antigen availability or promotes inflammation-associated carcinogenesis. Finally, apoptosis and senescence are typically at opposite ends of a spectrum of cell fates (2). Senescent cells are characterized by resistance to apoptosis (2). The pro-apoptotic drug navitoclax—an inhibitor of anti-apoptotic BCL2 and BCL-xL proteins—functions as a potent senolytic agent (11). The discovery of SECS and its influence on removal of apoptotic cells adds a new dimension to this complex interrelationship.

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