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SPOTLIGHT

Macrophage Nrf 2 the rescue

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The exuberant phagocytosis of apoptotic cell corpses by macrophages in *Drosophila* embryos creates highly oxidative environments. Stow and Sweet discuss work from Clemente and Weavers (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202203062) showing for the first time how macrophage Nrf2 is primed to help sustain immune function and mitigate bystander oxidative damage.

In their role defending the body against pathogen invasion and infection, innate immune cells produce potent antimicrobial weapons, including reactive oxygen species (ROS) via the phagocyte NADPH oxidase NOX2 (1). The macrophage phagosome, responsible for engulfing pathogens, is a well-known site for assembly and activation of the NOX2-containing phagocyte oxidase complex. Even in the absence of infection, ROS is deployed for many other cellular roles, including redox signaling in metabolic regulation and stress responses (2). Releasing harmful oxidants like hydrogen peroxide and superoxide, either acutely or over the longer term, can come at significant cost to the immune cells themselves and to the surrounding tissues, which are vulnerable to bystander damage. Moreover, ROS-induced damage accumulates in cells and tissues over time, contributing to functional decline and aging, positioning ROS and redox pathways as targets for therapeutic intervention in cancer and in cardiovascular, mitochondrial, developmental, and inflammatory diseases (2, 3). Cytoprotective mechanisms are exigent for mitigating ROS-mediated oxidative damage in order to safeguard cells and tissues. The nuclear factor erythroid derived-2 (Nrf2) transcription factor is a well-established cytoprotective regulator, with a leading role in responding to oxidative stress by upregulating antioxidant gene expression and restoring redox balance (4). Nrf2 offers cytoprotection and self-preservation for innate immune cells during infection (5) and in aging and inflammation. Weavers et al. (6) previously used the tractable *Drosophila* model to demonstrate how cytoprotective pathways, including Nrf2, are invoked by ROS during inflammation and repair at wound sites populated by innate immune cells.

In this issue, Clemente and Weavers (7) now address the damage caused by and to macrophages in a different setting: during *Drosophila* embryogenesis. In addition to pathogen-mediated phagocytosis, macrophages are responsible for extraordinary and sustained levels of phagocytosis throughout embryogenesis in order to dispose of the apoptotic cell corpses produced during tissue sculpting. In this sterile environment, it is development (rather than host defense), that triggers and perpetuates an oxidative storm. Macrophage phagocytosis of apoptotic cell corpses, with the attendant production of ROS, including hydrogen peroxide and superoxide, can clearly be demonstrated in this embryonic environment (7). Cytoprotection in phagocytic macrophages during embryogenesis is a less well understood, but much-needed accompaniment. Nrf2 was thus found to be activated downstream of corpse phagocytosis in the fly embryos. Using several approaches, including RNAi-mediated *Nrf2* depletion in fly lines, macrophage Nrf2 was implicated in cytoprotection, acting as a buffer to phagosomal ROS and importantly, reducing damage to the embryonic macrophages themselves (7). Macrophage Nox is also shown to mediate ROS production downstream of cell corpse phagocytosis, initiating subsequent Nrf2 activation. Deeper investigation into how Nrf2 is triggered, focused on the phospholipid transitions inherent to phagosomal maturation, demarked in this study by enrichment of phosphatidylinositol-3,4,5-triphosphate (PIP_3) on the phagosomes by means of PI3kinase (PI3K) activation. Depletion or inhibition of *Drosophila* PI3K subunits dramatically reduced superoxide, linking the enrichment of PIP_3 to phagocytic Nox activation. Phagocytosis is also accompanied by calcium signaling and new findings further demonstrate that calcium is required for Nox activity and phagosomal ROS generation. Thus, integral to apoptotic corpse phagocytosis, the release of calcium and the activation of PI3K and Nox, serve in turn to initiate Nrf2 (7). Furthermore, the authors observed fine-tuning of Nrf2 to match levels of phagocytic ROS, signifying coordination of phagocytic function and cytoprotection.

The in vivo impact of Nrf2 cytoprotection on macrophage behaviour was addressed elegantly by employing live imaging to track leukocyte migration in staged *Drosophila* embryos (7). In later stage embryos,

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the random migration of embryonic macrophages for immune surveillance was found to be Nrf2 dependent. Nrf2-deficient macrophages were also less efficient at navigating to sterile epithelial wounds. ROS can also be generated for roles in migration, which may then represent an extra requirement for cytoprotection in migrating cells. Interestingly, it was noted that Nrf2-deficient macrophages with impaired migration became hypervacuolated, which could be due to half-finished phagocytosis, but it could also reflect an accumulation of the fluid-filled macropinosomes that are used by leukocytes for surveillance and to facilitate migration (8, 9). Indeed, in cancer cells where macropinocytosis is a pro-survival and migration mechanism (9), Nrf2 has a critical and direct role in promoting macropinocytosis (10). In immune cells that migrate to sites of epithelial damage, ROS can once again be deployed for phagocytic clearing of cell debris, creating another oxidative onslaught. Clemente and Weavers accordingly found locally elevated levels of both ROS and Nrf2 in macrophages at these sites, ensuring extra capacity for execution and resilience in this immune response (7). Taken together, their findings make a comprehensive case for Nrf2 as a locally responsive, tunable means of cytoprotection throughout successive stages of macrophage inflammatory responses. Featured also during *Nrf2* deficiency in *Drosophila* macrophages, were prominent triggers and signs of early senescence, implying that Nrf2 contributes to the progression of these macrophages towards senescence, controlling acquisition of their mature pro-inflammatory capacity over time.

Finally, phagocytic ROS associated with clearing cell debris is an indiscriminate weapon with messy consequences, including causing damage to neighboring cells. Fittingly, bystander damage of epithelial cells can be demonstrated in *Drosophila* at wound margins (6). Furthermore, the phagocytosis of apoptotic cell corpses is also associated with bystander damage in epithelial cells, measured here by DNA oxidation, which was evident in normal embryos and further elevated in those with Nrf2-deficient macrophages (7). With the evidence presented here it appears that non-autonomous protection by macrophage Nrf2 contributes measurably to shielding epithelial cells, even in the presence of their endogenous Nrf2. Thus, in addition to offering self-protection, macrophage Nrf2 emerges with a new and exciting role in offering cytoprotection to surrounding epithelial cells. Embryonic macrophages are bestowed with the capacity for more fastidious "housekeeping" when Nrf2 is available to demarcate the oxidative range of phagocytic ROS, ensuring it can be effectively deployed with limited risk to the macrophages and other cells in developing tissues.

Existing knowledge of phagocytic ROS and Nrf2 cytoprotection has traditionally come from the perspective of host defense (5). Underscoring the need for oxidative killing of pathogens and coordinated cytoprotection in immunity, are the recurrent infections and excessive inflammation experienced in chronic granulomatous disease, where a genetic deficiency of NADPH oxidase is coupled with loss of Nrf2 activation (11). The new study (7) by Clemente and Weavers adds an important new dimension to the field by highlighting the roles of phagocytic ROS and macrophage Nrf2 in a completely different and persistent physiological context in embryonic development. Their findings strongly endorse the potential for mitigating tissue damage in chronic disease and aging by specifically boosting leukocyte Nrf2 for cytoprotection, which we now see can have influence at a tissue level through autonomous and non-autonomous shielding of immune cells and surrounding cells (7). In macrophages, Nrf2 is also regulated by anti-inflammatory molecules like the metabolite itaconate (12), which is pursued for therapeutic potential to enhance anti-oxidative and anti-inflammatory biases in inflammatory disease and cancer (12, 13). Harnessing knowledge from this new study by Clemente and Weavers could be used to boost Nrf2 pathways to combat chronic disease and for cancer immunotherapy.

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