A peaceful death orchestrates immune balance in a chaotic environment

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Immunity evolved as an impossibly elegant, yet devastatingly destructive force to combat pathogens, environmental insults, and rogue malignant cellular agents arising from within. The immunologic arsenal developed in a veritable coevolutionary arms race with the world's pathogens, culminating in lymphocytic weapons of mass destruction. Indeed, T cells and B cells endowed with antigen specificity, the capacity for clonal expansion, and most importantly, long-lived memory, represent the pinnacle of such evolution. Together with the innate immune response, the adaptive immune system holds the power to mediate sustained inflammatory responses with such voracity that tissues, organs, or the host itself may endure critical collateral damage. To preserve balance, adaptive immunity has developed under the guiding principle of *primum non nocere*, or "first, to do no harm," limiting the aggression of the innate immune response (e.g., septic shock, penumbra of cerebrovascular and brain infarcts). Herein, redundant mechanisms to preclude aberrant deleterious immunity have evolved as the predominant state of being, establishing a significant molecular and cellular threshold to initiate and maintain inflammation. Often overlooked, following the excitement of the active immune response, are the critical means by which the host resolves the inflammatory process, restoring local and systemic balance. The findings by Liu et al. (1) provide further description of molecular processes and cellular mediators of the resolution process, shedding light on mechanistic aspects of immune homeostasis.

The inflammatory process is characterized by 3 phases of active, overlapping response, including the initiation, resolution, and a postresolution period. During onset, local, tissue-resident cellular mediators respond to noxious stimuli through the release of soluble factors including chemotractants, cytokines, and eicosanoids, establishing a microenvironmental milieu to recruit, differentiate, and polarize leukocytes. Local vascular endothelium provides a quiescent barrier, regulating homeostatic interchanges between tissues and the circulation. During acute inflammation, complement anaphylatoxins C3a and C5a mediate microvascular flow, permeability, and leukocyte extravasation, serving as a gatekeeper of cell-mediated inflammation. Coordinate signaling through a myriad of agonists/antagonists, receptors, and transcription factors, and interpreted through metabolic and epigenetic states of effector cells, orchestrates the induction of a tailored immune response capable of neutralizing or containing the inciting agent (2, 3). Far more than the antithesis of inflammation, resolution is marked by production of soluble mediators, most notably, proresolution lipid agonists derived from polyunsaturated fatty acid precursors including lipoxins, resolvins, maresins, and protectins, which provide both negative feedback limiting the propagation of inflammation, as well as active restructuring of the inflammatory environment (4). Paramount to proper resolution is the removal of infiltrating leukocytes (efferocytosis), largely granulocytes, either through their migration out of affected tissues via lymphatics or through apoptotic and/or necrotic cell death. As the archetypic phagocyte, macrophages are essential mediators of this process. During effective resolution, circulatory monocytes undergo nonphlogistic recruitment to inflammatory sites, differentiating into tissue macrophages capable of efferocytosing apoptotic cells. This phenomenon provides a clean, quiet, orderly cell death without subsequent inflammatory alarm. Unique macrophages such as those expressing the evolutionarily conserved guidance receptor neuropilin-2, and specifically the neuropilin-2b isoform, may be specialized for proefferocytic function (5, 6). If efferocytosis of dead/dying/stressed cells is subverted, secondary necrosis of infiltrating leukocytes may result in the release of damage-associated molecular pattern molecules (DAMPs), prolonging or enhancing inflammation. As such, macrophages serve as the principal conductors, orchestrating wound healing/resolution or further

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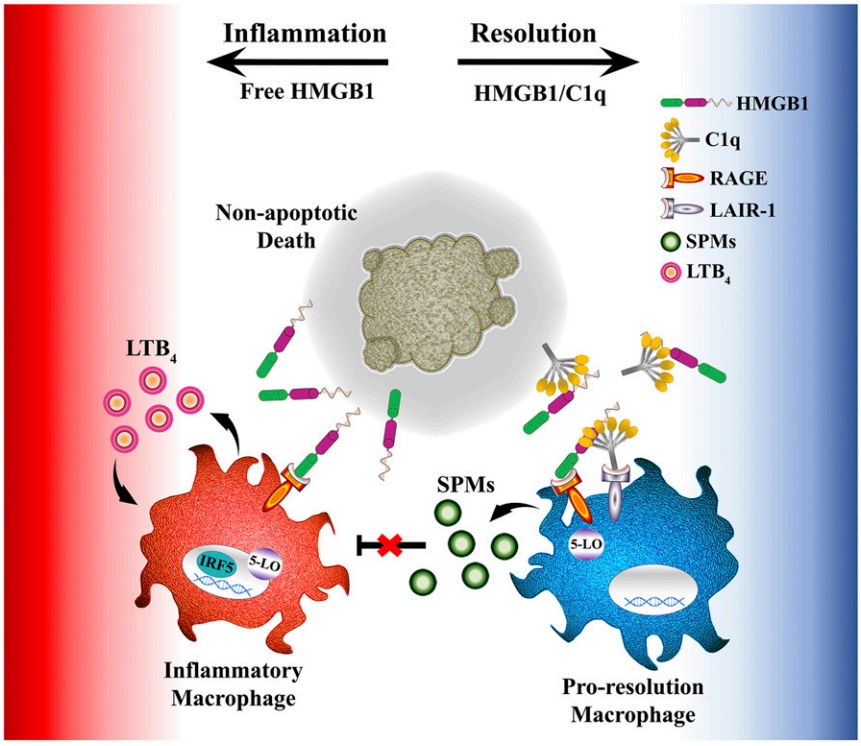

Fig. 1. The context of recognition of HMGB1 mediates proinflammatory or proresolution functionality in macrophages. Free HMGB1 released by nonapoptotic cell death signals through RAGE to induce 5-LO nuclear translocation and IRF5 expression, resulting in differentiation of proinflammatory macrophages and subsequent leukotriene B_4 production. By contrast, HMGB1 complexed with C1q forms a tetramolecular signaling complex with RAGE and LAIR-1 receptors, retaining 5-LO in the cytoplasm and inducing the production of specialized proresolving lipid mediators (SPMs), lack of IRF5 expression, and generation of proresolving macrophages. The context of HMGB1 recognition including location, kinetics, and duration may alternately affect the resolution or propagation of inflammation.

amplification of inflammation (7). Following clearance of effector cells and catabolism of proinflammatory signals, a postresolution state of adapted homeostasis establishes an immune experienced microenvironment defined by the local presence of epigenetically imprinted myeloid cells (trained immunity) and long-lived tissue-resident memory lymphocytes phenotypically poised to mediate the severity of subsequent immunologic challenges.

Here, Liu et al. (1) describe how exposure to the extracellular DAMP high-mobility group box 1 (HMGB1) molecule complexed with the complement factor C1q promotes resolution of inflammation via monocytes and macrophages. Together with their previous findings, the authors demonstrate in vitro and in vivo that HMGB1 signals via receptor for advanced glycation end product (RAGE) to induce leukotriene B4 production and IRF5 transcription, establishing a positive inflammatory feedback loop (Fig. 1). In the presence of C1q, HMBG1 forms a tetramolecular complex with RAGE and LAIR-1, retaining 5-lipoxygenase (5-LO) in the cytoplasm, resulting in production of specialized proresolving lipid mediators. These in turn prevent IRF5 transcription, subsequent leukotriene production, and, presumably in vivo, inflammation via inhibiting the differentiation of proinflammatory monocytes and macrophages. C1q, itself a pattern recognition receptor (PRR), facilitates the clearance of apoptotic cells by macrophages (8). Exposure to C1q alone or C1q-opsonized apoptotic cells dampens inflammatory responses, such as the lipopolysaccharide (LPS)-induced production of type I IFNs and TNFα, promoting an antiinflammatory phenotype and proefferocytic functionality (8). Monocyte-derived macrophages polarized in the presence of C1q fail to promote allogeneic and autologous T cell responses in mixed leukocyte reactions, implicating C1q in the cross talk between the innate and adaptive immune responses (8). Clinical observations support these findings, as individuals with homozygous deficiency of C1q experience early onset of high frequencies of systemic lupus erythematosus or lupus-like disease (~88%) and recurrent bacterial infections (~41%). In light of the current report, HMGB1/C1q regulation of monocyte/macrophage proresolution functionality may be crucial in subverting disease processes following chronic DAMP release.

This study's findings highlight how the contextual aspects of cell death, including timing, composition of cellular mediators, and location, are critically important to organized resolution of inflammation. However, macrophages themselves are prominent producers of C1q, and the role of C1q autocrine signaling or aspects of complement-specialized subsets of macrophages warrants further exploration. The production of C1q by tumor-associated macrophages is implicated in the establishment of an immunosuppressive tumor microenvironment and poor prognosis in clear-cell renal cell carcinoma (9). Additionally, single-cell RNA-seq analysis in melanoma models identified a unique, dominant antiinflammatory macrophage population defined by expression of C1q transcripts (10). Understanding the role of localized, macrophage-mediated C1q production will be essential to decipher how HMGB1/C1q complexes regulate tissue inflammation.

HMGB1 is a dynamic nuclear protein with pleiotropic roles as a regulator of transcriptional access to chromatin, central regulator of DNA damage repair and apoptosis inhibition, promoter of autophagy-mediated cellular responses to stress, and upon extracellular release, a DAMP and a cytokine-like molecule (11). The

most abundant nonhistone nuclear protein, HMGB1's function is dependent upon its location. Under homeostatic conditions, HMGB1 resides in the nucleus as a structural component of the nucleosome where, through its DNA-binding motifs and acidic tail, it facilitates interactions between DNA and other molecules such as transcription factors, DNA polymerases, V(D)J recombinases, and facilitates DNA repair. Upon cellular stress, HMGB1 translocates to the cytoplasm where it promotes autophagy, enhancing mitochondrial quality control and DNA repair, to preserve bioenergetic requirements requisite for cell survival. When present extracellularly, HMGB1's role shifts dramatically, serving as a distress signal, an indicator of stress and/or unscheduled cell death. Interestingly, HMGB1 may be secreted by activated monocytes, macrophages, and natural killer cells following LPS stimulation or engulfment of apoptotic corpses. Both proinflammatory and antiinflammatory roles have been attributed to exogenous HMGB1 (11). The findings by Liu et al. now ascribe a fourth role for HMGB1 functioning as a "rheostat" in the context of C1q to modulate the resolution mediated by the monocyte/macrophage compartment. Presumably at low levels of HMGB1, C1q limits inflammation but is unable to do so in tissues that are poorly perfused or in settings where large quantities of HMGB1 are released, perhaps suggesting why large amounts (microgram quantities) of HMGB1 are required to trigger receptor-mediated signaling in the presence of sera containing C1q. Further investigation is warranted to evaluate how levels of HMGB1/C1q complexes, and their constituents, facilitate the resolution of inflammation temporally (acute vs. chronic inflammation), in various tissues, and within the context of disease states (hypoxia vs. normoxia).

Defining the underlying cellular, molecular, and signaling mechanisms of chronic inflammation is of significant interest, as interventional strategies that facilitate resolution may inhibit the pathogenesis of autoimmune disease, diseases of aging, chronic infections, and cancer. Unresolved inflammation may lead to sustained local and/or systemic exposure to HMGB1/C1q complexes, the consequences of which are unknown. Recent studies have demonstrated that hematopoietic stem cells and hematopoietic progenitor cells (collectively, HSPCs) of the bone marrow are responsive to peripheral inflammation via pattern recognition receptors and cytokine signaling. Inflammatory signals can promote rapid myelopoiesis from HSPCs via differentiation and asymmetric division with distinct monocyte-committed progenitors giving rise to classical monocytes with unique transcriptomic profiles and function (12). Furthermore, epigenetic regulation, consisting of heritable histone modifications, DNA methylation, and the effects of noncoding RNAs, has been implicated in inflammatory gene expression, conferring a type of memory on these innate postmitotic cells. Exposure to environmental stimuli induces epigenetic reprogramming at the level of histone acetylation and methylation, which endows macrophages with proinflammatory and antiinflammatory properties, establishing a predilection toward anamnestic responses in the future (3). Heritable epigenetic modifications imprinted following HMGB1/C1q exposure that are maintained within self-renewing bone marrow progenitors, long-lived monocytes, or tissue-resident macrophages may establish a persistent state of maladaptive immunity by subverting effector functions necessary for either the induction or resolution of inflammatory responses. Furthermore, chronic HSPC exposure to HMGB1/C1q complexes may result in enhanced myelopoiesis leading to the overrepresentation of monocyte/macrophages, disrupting homeostasis, and promoting chronic inflammation. Understanding the mechanism of action of mediators of resolution and their context-dependent effects upon acute and chronic inflammatory disease will be instrumental in identifying clinical applications of these immunoregulatory agents not only in autoimmunity but also in chronic viral infections and cancer as well as acute events such as cerebrovascular accidents, myocardial infarction, and trauma.

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