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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Sugimoto et al, page 2896

# Reprogramming macrophages by plasmin

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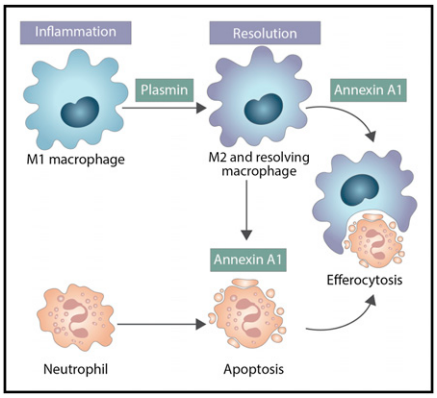
Resolution of inflammation is a key physiological process regulated at different levels, and failure to achieve resolution may result in chronic inflammation. In this issue of *Blood*, Sugimoto et al reveal the function of the plasmin system in resolving inflammation by inducing macrophage polarization toward M2 and proresolving phenotypes. Plasmin-derived M2 macrophages produce 2 key effector processes for the resolution of inflammation: annexin A1 and neutrophil efferocytosis.[1]

The correct management and treatment of chronic inflammation is a challenge in a population that includes increasingly elderly individuals affected by numerous degenerative diseases. Nonresolving inflammation is at the base of different chronic diseases, including cardiovascular disease, type 2 diabetes mellitus, or neurodegenerative diseases.[2] Resolution of inflammation is a tightly coordinated and active process necessary for restoring tissue integrity and avoiding excessive damage, fibrosis, and loss of tissue function. Although it is known that resolution of inflammation starts in the first few hours after an inflammatory response begins, we are starting to understand the different mechanisms orchestrating this process.[3]




M1 macrophages repolarize by plasmin to M2 and pro-resolving macrophages to produce annexin A1 and increase neutrophil apoptosis and efferocytosis, enhancing resolution of inflammation. Professional illustration by Somersault18:24.

Macrophages are central innate immune cells that control the inflammatory process. After sensing a noxious stimulus, macrophages polarize to a proinflammatory phenotype that initiates and maintains inflammation by producing different factors, including proinflammatory cytokines, chemokines, and lipids. These proinflammatory macrophages are termed classical or M1.[4] During the resolving phase of inflammation, macrophages polarize to anti-inflammatory phenotypes characterized by the production of anti-inflammatory cytokines and lipids, increased phagocytosis, and their ability to remodel the damaged tissue; these macrophages are termed alternative or M2.[4] Because of the high plasticity of macrophages, a plethora of intermediate polarization phenotypes that occur between these 2 extreme phenotypes has been described.[5,6] The modulation of macrophage plasticity toward M2 phenotypes is leading the way discovering novel anti-inflammatory targets by enhancing inflammation resolution.[6] Although macrophages found in inflammatory-resolving processes are phenotypically distinct to M2,[7] they resemble and derive from M2-like macrophages.[8]

The plasmin system has been widely recognized for its fibrinolytic activity; however, in chronic inflammatory diseases, this system is upregulated, suggesting that plasmin could play a role in maintaining chronic inflammation.[9] In fact, monocytes treated with plasmin elicit activation of the proinflammatory transcription factor NF-κB. However, plasmin also induces signaling in macrophages via STAT3, a strong M2-related transcription factor.[9] Sugimoto et al provide evidence indicating that plasmin is able to reprogram macrophages toward M2 and proresolving phenotypes in vivo. For this, the authors injected plasmin into the pleural cavity and found an increase of M2 macrophages; these resolving macrophages could be characterized by a low expression of CD11b. Plasmin also induces an increase of M2-related molecules, including arginase-1, transforming growth factor β, and interleukin-10, with a parallel decrease in the expression of proinflammatory genes. Furthermore, based on a model for resolving inflammation, there is an increase of plasminogen expression and plasmin activity during the resolution phase when compared with the inflammatory phase.

Extending their results to the mechanism of plasmin promoting an anti-inflammatory macrophage phenotype, Sugimoto et al found that the serine protease activity of plasmin is also necessary for increasing neutrophil apoptosis (see figure). Neutrophil apoptosis is a hallmark of inflammation resolution because phagocytosis of apoptotic bodies by macrophages is a potent M2-polarizing signal

to generate resolving macrophages.[3] Plasmin was also able to increase apoptotic-neutrophil efferocytosis by macrophages, indicating a potentiation of the resolution of the inflammation. Annexin A1 mediated the actions on plasmin-inducing neutrophil apoptosis and efferocytosis (see figure), and in vivo administration of plasmin is able to increase cell surface expression and secretion of annexin A1 by macrophages.[1] Annexin A1 is a potent anti-inflammatory effector molecule of the resolution of inflammation, being one of the main mediators of glucocorticoid anti-inflammatory actions, partially by mediating apoptosis and clearance of apoptotic neutrophils.[10] The work of Sugimoto et al suggests that resolving inflammation through modulation of the plasmin system could represent an advantageous therapy with fewer side effects than the use of glucocorticoids.

This study proposes the plasmin system as an important effector in establishing an efficient resolution of the inflammatory process, paving the way for further studies in the years ahead to test the pharmacological modulation of the plasmin system to achieve an efficient resolution of inflammation. This will ultimately affect the development of novel therapies for a wide range of chronic degenerative diseases with an inflammatory base that affect an increasing elderly population in developed countries.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Zaimoku et al, page 2908

# Immune insights into AA

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In this issue of *Blood*, Zaimoku et al demonstrate that the functional loss of the HLA-B4002 allele is common in aplastic anemia (AA) patients, suggesting that this allele plays a major role in the immune attack underlying the pathophysiology of this disease.[1]

The immune-mediated pathophysiology of AA is substantiated by the excellent clinical response to immunosuppressive treatment, and supported by a plethora of experimental studies.[2] Most studies suggest that AA is attributable to a T-cell–mediated immune attack targeting hematopoietic stem/progenitor cells (HSPCs). Indeed, different groups have documented in AA the presence of an oligoclonal T-cell response in vivo, with cytotoxic activity of these T-cell clones on autologous HSPCs in vitro.[3,4] However, although T-cell repertoire oligoclonality suggests the presence of an antigen-driven T-cell response, the identification of putative autoantigen(s) triggering such immune response remains elusive. Different HLA alleles were found associated with AA, including *DRB1*1501*, *DRB1*1502*, *B*5201*, and *B*4002*.[5] Furthermore, neutral copy-number loss of heterozygosity of the short arm of the chromosome 6 (6pLOH) emerged as a relatively common phenomenon in AA,[5] suggesting the hypothesis that it may represent a mechanism of immune escape for HSPCs.

In this work, Zaimoku et al confirm that *HLA-B*4002* is among the HLA alleles most frequently carried by AA patients, and that 6pLOH is particularly common in these *HLA-B*4002* AA patients.[5] Using an ultrasensitive flow cytometry assay exploiting a new anti-HLA-B4002 monoclonal antibody, Zaimoku et al demonstrate that HLA-B4002$^-$ granulocytes can be found not only in all *HLA-B*4002* patients with a 6pLOH, but also in the majority of patients without 6pLOH. Indeed, deep sequencing of *HLA-B*4002* in sorted HLA-B4002$^-$ granulocytes isolated from these AA patients without 6pLOH documents that the loss of HLA-B4002 was because of somatic mutations in the *HLA-B*4002* gene, leading to the specific phenotype of HLA-B4002$^-$A$^+$ granulocytes, which cannot be defined as 6pLOH. These HLA-B4002$^-$A$^+$ granulocytes were detected also in AA patients with 6pLOH, leading to the conclusion that HLA-B4002$^-$ granulocytes in these patients are a mosaic of cells truly carrying 6pLOH (HLA-B4002$^-$A$^-$) and cells lacking HLA-B4002 because of other structural gene mutations. Indeed, the authors were able to identify different *HLA-B*4002* somatic mutations leading to a loss-of-function phenotype. In addition, in the same patients a few missense mutations were found in phenotypically normal (HLA-B4002$^+$) granulocytes. All these observations suggest that these HLA-B4002$^-$ cells tend to expand as a result of continuous immune pressure from which they are spared.

The concept of possible immune escape in the context of AA is not a novel concept in bone marrow failure, since it was first introduced by Rotoli and Luzzatto to explain the pathophysiology of clonal expansion of glycosylphosphatidylinositol (GPI)–deficient cells in paroxysmal nocturnal hemoglobinuria (PNH).[6] Autoreactive T cells would target normal HSPCs via some GPI-linked protein or via the GPI anchor itself, eventually sparing