excluded from or included only in limited numbers in recent phase 3 studies aimed at establishing new standard-of-care regimens incorporating lenalidomide, bortezomib,[7] or carfilzomib[8] in early RRMM patients after a median of 1 to 2 prior lines of therapy. More recently, several studies have been designed to specifically address this patient population. Preliminary results from some of these studies in RRMM are summarized in the table.[5,6,9,10]

Taken together, these data suggest that Pd or carfilzomib-dexamethasone (Kd) represent the backbone for developing safe and effective triplets for the treatment of lenalidomide- or double- refractory MM through the addition of a third agent, such as bortezomib or mAbs targeting CD38 or SLAMF7 antigens. Promising results from these pilot studies have provided a compelling rationale for further investigation of these 3-drug regimens in the ongoing ICARIA-MM, APOLLO (daratumumab-Pd vs Pd), CANDOR (daratumumab-Kd vs Kd), and IKEMA (isatuximab-Kd vs Kd) large phase 3 clinical trials.

Note added in proof: An updated analysis of patients treated with daratumumab-carfilzomib-dexamethasone in the MMY1001 study was recently published ahead of print (Chari A, Martinez-Lopez J, Mateos M-V, et al. *Blood*. Published online ahead of print 21 May 2019). The authors reported a 1-year 74% estimate of PFS for all treated patients and a median PFS of 26 months for lenalidomide-refractory patients, results that differ from those that are reported in reference 10 and included in the table.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Freise et al, page 134

Novel pathways of self- and cross-tolerance in monocytes

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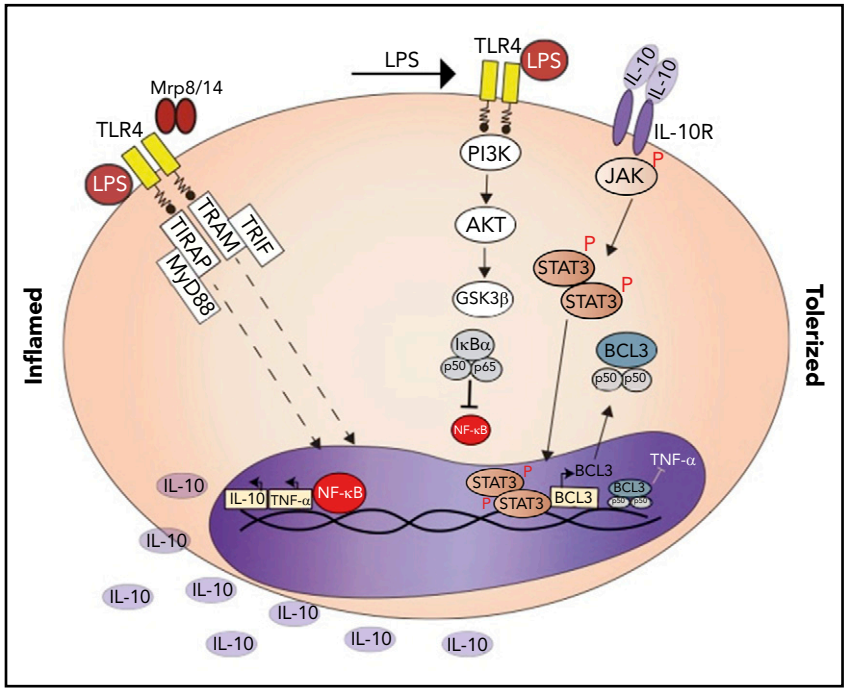
In this issue of *Blood*, Freise et al[1] describe novel pathways responsible for self- and cross-tolerance of monocytes evoked by endotoxin stimulation or by endogenous Toll-like receptor 4 (TLR4) ligands (see figure). This study helps unravel the pathways of innate immune memory of monocyte hyporesponsiveness (see figure), which occur in systemic inflammatory response syndrome (SIRS) and contribute to development of secondary infections and severe sepsis.

SIRS is characterized by an intense activation of the innate immune system, either from activation of pathogen-associated molecular patterns or damage-associated molecular patterns. The latter's activation explains the immune stimulation in absence of pathogens, caused by the release of endogenous nuclear, mitochondrial, or cytosolic molecules, collectively named alarmins. Myeloid-related proteins-8 (Mrp-8) and Mrp-14 alarmins are released by necrotic cells or secreted by phagocytes when tissue damage occurs, form heterodimers (Mrp-8/14), and activate TLR4.

The profound activation of the innate system triggered by endotoxins or alarmins during SIRS is followed by the compensatory anti-inflammatory response syndrome, which helps to avoid extensive tissue damage from inflammation. Nevertheless, compensatory anti-inflammatory response syndrome contributes to the development of opportunistic secondary infection and sepsis, and occurs from inactivation of monocytes, rather than paralysis of other phagocytes. Hyporesponsive monocytes express HLA-DR^{low}, produce lower amounts of inflammatory mediators, higher amounts of anti- inflammatory mediators, and display impaired phagocytic activity. Different mechanistic pathways have been described for monocyte hyporesponsiveness, such as increased expression of negative pro-inflammatory TLR signaling pathways leading to impaired NF-κB activation and expression of anti-inflammatory heat-shock proteins.[2]



LPS or Mrp-8 activates TLR-4 and downstream NF-κB translocation into the nucleus; in the nucleus, NF-κB induces the synthesis of TNF-α and IL-10. Secondary stimulation with LPS activates PI3K/AKT and phosphorylates GSK3β; inactive phosphorylated GSK3β does not degrade IκBα and impairs NF-κB activation. IL-10, via IL-10R, phosphorylates STAT3 that induces BCL-3 synthesis. BCL-3 promotes binding of inactive NF-κB to the TNF promoter. R, receptor

The incomplete blockade of self- or cross-monocyte tolerance by blocking known mechanisms suggests there are multiple and independent intracellular pathways involved with inactivation.

In the Freise et al study, elegant and robust in vitro and ex vivo experimental approaches in mice and human monocytes were carried out to define the intracellular pathways activated by lipopolysaccharide (LPS) or alarmin Mpr-8 in monocyte tolerance to LPS. Pharmacological and genetic manipulations in the used models confirmed the pathways. Translational data from humans with sterile SIRS evoked by cardiopulmonary bypass surgery also corroborate the findings.

LPS or Mrp-8 preactivation induced early and long-lasting inactivation of glycogen synthase kinase (GSK-3β), a process dependent on persistent activation of phosphatidylinositol 3-kinase (PI3K)/AKT pathway. Indeed, in vitro and ex vivo monocyte hyporesponsiveness was induced by pharmacological blockade of GSK-3β, and inhibiting AKT evoked complete blockade of the hyporesponsive state. Interestingly, MAPK p38 was activated in hyporesponsive monocytes, pointing out the specificity of the pathway PI3K/AKT/GSK-3β. Furthermore, increased intracellular levels of IκBα, a GSK-3β substrate, confirmed the inactivation of GSK-3β. Because IκBα downregulated NF-κB, the PI3K/AKT/GSK-3β/NFκB pathway was proposed as a potential mediator in monocyte hyporesponsiveness. The specificity of AKT/GSK-3β in the anti-inflammatory axis has been demonstrated in diverse models of phagocytes activation. Conversely, recent data showed self- and cross-hyporesponsiveness evoked by Mrp-8 prestimulation in human and both murine peritoneal or bone marrow derived macrophages is primarily through downregulation of phosphorylated p38 MAPK rather than the inactivation of NF-κB.[3] Given the similarity of the experimental aims in both studies, the results suggest that different concentrations and schedules of stimulation and restimulation may activate distinct intracellular pathways.

It is noteworthy that in the Freise et al study, LPS or Mrp-8 tolerized monocytes expressed high levels of B-cell lymphoma-3 (BCL-3), also a member of the IκB family of NF-κB regulatory protein. Furthermore, clear involvement of BCL-3 in hyporesponsiveness was shown by normal response of BCL-3$^{-/-}$ Hoxb8 cells to a second stimulation of LPS. A possible connection between GSK-3β and BCL-3 was prevented because pharmacological blockade of GSK-3β did not cause overexpression of BCL-3. Meanwhile, gene expression of BCL- 3 was induced for 4 and 24 hours after monocyte stimulation by endotoxin or alarmin, and an additional transcriptional mechanism was then proposed for monocyte hyporesponsiveness. A connection between IL-10 and BCL-3 had already been suggested in models of immune tolerance, and Chuang et al[4] demonstrated BCL-3 is a key protein in IL-10 induced immunosuppressed macrophages. Moreover, IL-10 secretion is enhanced by TLR-4 stimulation and higher systemic levels of the cytokine are found in SIRS models.[5] Data from the Fraise study showed IL-10, via IL-10 receptor, caused downstream STAT-3 phosphorylation, which triggered BCL-3 expression in LPS or Mrp-8 tolerized monocytes. Early and long-lasting STAT-3 phosphorylation was detected in in vitro and in vivo tolerized monocytes. Pharmacological blockade of STAT-3 reduced messenger RNA BCL-3 levels and reversed LPS or Mrp-8 induced human monocytes hyporesponsiveness. Likewise, monocytes of patients with dominant-negative mutations in the STAT-3 gene were not self-tolerant to LPS. The translational confirmation of IL-10/STAT3/BCL-3 in monocyte hyporesponsiveness was demonstrated in a model of sterile SIRS induced by cardiopulmonary bypass surgery. Long-lasting, elevated serum levels of Mrp8/14 were detected after bypass completion in association with higher levels of IL-10. In the meantime, monocytes from patients had high levels of phosphorylated STAT-3 and were hyporesponsive to ex vivo stimulation with LPS. Although Fraise and coauthors present convincing data for the proposed pathway, a recent study did not corroborate STAT3 phosphorylation by IL-10 in ex vivo stimulated monocytes collected from SIRS and septic patients.[6] It is important to take into account the diversity of alarmins released in the different damaged tissues, which are released by different mechanisms, and induce distinct pathways of cell activation. The proposed mechanisms in the Freise et al study occur in in vitro and in vivo TLR4 activation by LPS or Mrp-8 alarmins, and further investigation must be carried out to elucidate the participation of the pathways in monocyte tolerance evoked by other alarmins. Data will be pivotal to

corroborate the proposal of PI3K/AKT/GSK-3β/NF-κB and IL-10/STAT3/BCL3 pathways as targets for reversal of monocyte tolerance.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Albeituni et al, page 147

Calming the storm in HLH

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In this issue of *Blood*, Albeituni et al demonstrate the pleiotropic immunomodulatory effects by which the Janus kinase (JAK) inhibitor ruxolitinib ameliorates hypercytokinemia and organ injury in 2 distinct murine models of hemophagocytic lymphohistiocytosis (HLH).[1] By demonstrating both interferon-γ (IFN-γ)–dependent and IFN-γ–independent effects, the authors provide compelling preclinical data supporting the potential superiority of ruxolitinib when compared with IFN-γ inhibition alone. In doing so, the authors also identify a previously underrecognized role of neutrophil cytotoxicity in the pathobiology of HLH-induced multiorgan dysfunction.

HLH is a hyperinflammatory disorder characterized by massive immune cell activation leading to severe multiorgan injury that culminates in mortality in up to 50% of affected children and adults.[2,3] Immune cell activation can be triggered by infection, malignancy, or autoimmunity and is propagated by primary or acquired defects in T-cell and natural killer (NK)–cell cytotoxicity that preclude their ability to terminate the immune response. Thus, hypercytokinemia is both a result and driver of immune cell activation. The cytokine storm of HLH involves elevations in IFN-γ, tumor necrosis factor-α (TNF-α), and interleukin-1 (IL-1), IL-2, IL-6, and IL-18. While $CD8^+$ T lymphocytes and macrophages are known to propagate HLH-related inflammation, the role of other cellular lineages that may be activated in the wake of this cytokine storm and that contribute to end-organ damage remains unclear. Most patients are treated based on the HLH 94 and 2004 protocols with dexamethasone and etoposide. While efficacious in a subset of patients, systemic toxicities are signficant.[4,5]

The hypercytokinemia of HLH has become an attractive alternative therapeutic target. Compelling preclinical and clinical data led to the recent US Food and Drug Administration approval of the IFN-γ–blocking antibody emapalumab (Gamifant) for patients with relapsed, refractory, or progressive primary HLH (NCT01818492). However, a potential limitation of targeting a single cytokine is that it does not impact the source of cytokine production or other cytokines that may propagate immune activation and end-organ damage. Several case reports and preclinical models have highlighted the JAK inhibitor ruxolitinib as an alternative approach. JAK inhibition represents an appealing target for the treatment of hypercytokinemia, as JAKs are expressed on numerous immune cell lineages, including myeloid, lymphoid, dendritic, and $CD56^+$ NK cells, and mediate signaling of multiple cytokines, including IFN-α, IFN-β, IFN-γ, IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-12, IL-10, IL-15, IL-21, granulocyte macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor.[6] However, while inhibition of multiple cytokines could be theoretically more beneficial, increased toxicity is also a possibility. Studies comparing these strategies are lacking.

Albeituni et al address this issue by evaluating whether JAK1/2 inhibition to block signaling of multiple cytokines is superior to single-cytokine blockade of IFN-γ. In a model of primary HLH (lymphocytic choriomeningitis virus [LCMV] infection in perforin-deficient mice), JAK1/2 inhibition led to less thrombocytopenia, less hepatosplenomegaly with less $CD8^+$ T-cell/monocyte/neutrophil infiltration and activation, and lower cytokine levels (IL-6, TNF-α, GM-CSF, MCP-1, and MIP-1α) relative to IFN-γ inhibition alone. Further, treatment with ruxolitinib on days 4 to 9 after disease induction was associated with significantly greater 35-day survival than IFN-γ inhibition alone (14/14 vs 5/13 mice). In a model of secondary HLH (TLR9 stimulation with IL-10 receptor blockade), JAK1/2 inhibition was also superior to IFN-γ inhibition.

This work highlights 2 important findings that merit discussion. First, the use of JAK1/2 inhibition was clearly superior to IFN-γ inhibition in reducing hypercytokinemia, immune activation, organomegaly, and death in these murine models of HLH. Teleologically, this is perhaps not surprising in that an anti-IFN-γ antibody can serve as a sponge to decrease the amount of deleterious cytokine present while JAK1/2 inhibition is akin to turning off the faucet and blocking the effects of multiple cytokines (see figure). Second, Albeituni et al identified a critical role of neutrophil-mediated tissue injury in the pathobiology of HLH. Specifically, the authors noted that splenic neutrophils from LCMV-infected perforin-deficient mice upregulated triggering receptor expressed on myeloid cells-1 (TREM-1) and increased production of