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The opposite impact of Janus kinase inhibitor Ruxolitinib on the function of bone marrow mesenchymal stem cells and immune cells in acute GVHD recipients

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Allogeneic hematopoietic cell transplantation (Allo-HCT) is a curative therapy for hematologic malignancies owing to graft-versus-leukemia/lymphoma (GVL) activity mediated by alloreactive donor T cells, but graft-versus-host disease (GVHD) mediated by the donor T cells remains a major obstacle for a wide-spread clinical application of Allo-HCT.[1] The GVHD target tissues are variable in different patients and can involve gastrointestinal (GI) track, liver, lung, skin, spleen, lymph nodes, thymus, and bone marrow (BM).[2] Although the principal organs of acute GVHD are GI track, liver, and lung, BM GVHD can delay reconstitution of lymphohematopoietic cells, resulting in subsequent bleeding and infection that account for ~30% of deaths after Allo-HCT.[3] The pathogenesis of BM GVHD was proposed to result from damage of BM stroma niches by donor $CD4^+$ T cells in a Fas/FasL-dependent manner, and depletion of donor $CD4^+$ T cells effectively prevent BM GVHD.[3,4] Besides direct suppression of primitive donor hematopoietic stem cells (HSCs) and blocking megakaryocyte differentiation,[5] the cytokines (ie, GM-CSF, IFN-γ, and TNF-α) from donor T cells also inhibited HSC differentiation into regulatory plasmacytoid dendritic cells (DCs) that is important for immune tolerance.[6] However, how BM GVHD impact on host-type stroma niches and their interactions with HSCs remains unclear, and a recent publication in JCI by Lin et al from joint groups of Xiaoxia Hu, Hui Cheng, Tao Cheng, and Weiying Gu has provided novel insights.[7]

Acute GVHD is predominantly mediated by Th/Tc1 cells that produce proinflammatory cytokines such as IFN-γ. The cytokines play an important role in persisting the disease, and IFN-γR-deficiency in donor T cells markedly reduced acute GVHD while preserving GVL activity.[8] IFN-γR signaling activates the Janus kinase (JAK) and signal transducers and activators of transcription signaling pathways, and pharmacological blockade of JAK1 and JAK2 by Ruxolitinib prevents GVHD while preserving GVL activities.[9] JAK family members can be differentially activated by various cytokines and growth factors and play a central role in myeloid cell differentiation and proliferation.[10] Prevention of GVHD by Ruxolitinib inhibition of JAK1 and JAK2 was also found to reduce activation of DCs and neutrophils.[11] Recent clinical trial showed that administration of Ruxolitinib effectively ameliorated steroid resistant acute GVHD, and it is the first trial with clinical effectiveness.[12] The effect of amelioration of acute GVHD may be more related to inhibition of JAK2 by Ruxolitinib because more selective inhibition of JAK1 by Itacitinib did not lead to amelioration of acute GVHD.[13]

The impact of Ruxolitinib treatment on host-type BM stroma niches and donor-type hematopoiesis during acute GVHD was not previously studied, and Lin et al described their systemic studies and novel observations in the recent JCI publication[7] (**Fig. 1A**). First, Lin et al observed that acute GVHD resulted in defective BM stroma niches that consist of BM mesenchymal stem cells (BMSCs) and their progenies including fibroblast stroma cells, osteoblasts, adipocytes, and chondrocyte.[14] BMSCs produce key niche factors, such as C-X-C motif chemokine ligand 12 (CXCL12) and stem cell factor to promote homing and maintenance of HSCs.[15] In a haplo-matched murine Allo-HCT model of $CD45.1^+$ B6.SJL donor to $CD45.2^+$ CB6F1 recipient, acute GVHD was associated with marked reduction in BMSCs and osteolineage cells (OLCs)[7] (**Fig. 1A**). The BMSCs from acute GVHD BM showed impairment in their stemness and differentiation potential, as indicated by their downregulation of the stemness-related gene Grem1, differentiation-related genes Adipoq and Bmp4, and differentiation pathway genes, as well as with upregulation of the calcification-inhibiting gene Mgp. The BMSCs also showed reduced survival and function, with upregulated expression of apoptosis-related pathway genes, reduced expression of niche factors (Cxcl12 and Scf), reduced osteogenesis, and reduced adipogenesis, as well as disrupted mitochondria metabolism and mitochondria transfer–related pathways [phosphoinositide 3-kinase (PI3k/Akt) signaling and gap junction][7] (**Fig. 1A**). Thus, acute GVHD damages the fitness of BM stroma niches via damaging the BMSCs.

Second, consistent with upregulation of JAK/STAT pathway in donor-type lymphohematopoietic cells during acute GVHD,[11] Lin et al observed that acute GVHD also led to upregulation of JAK/STAT pathway in the BMSCs, and inhibition of JAK2/STAT1 pathway by Ruxolitinib treatment restored the fitness of BM stroma niches[7] (**Fig. 1A**). Ruxolitinib directly reduced phosphorylation of JAK2 and STAT1 in the BMSCs, resulting in sustainable improvement

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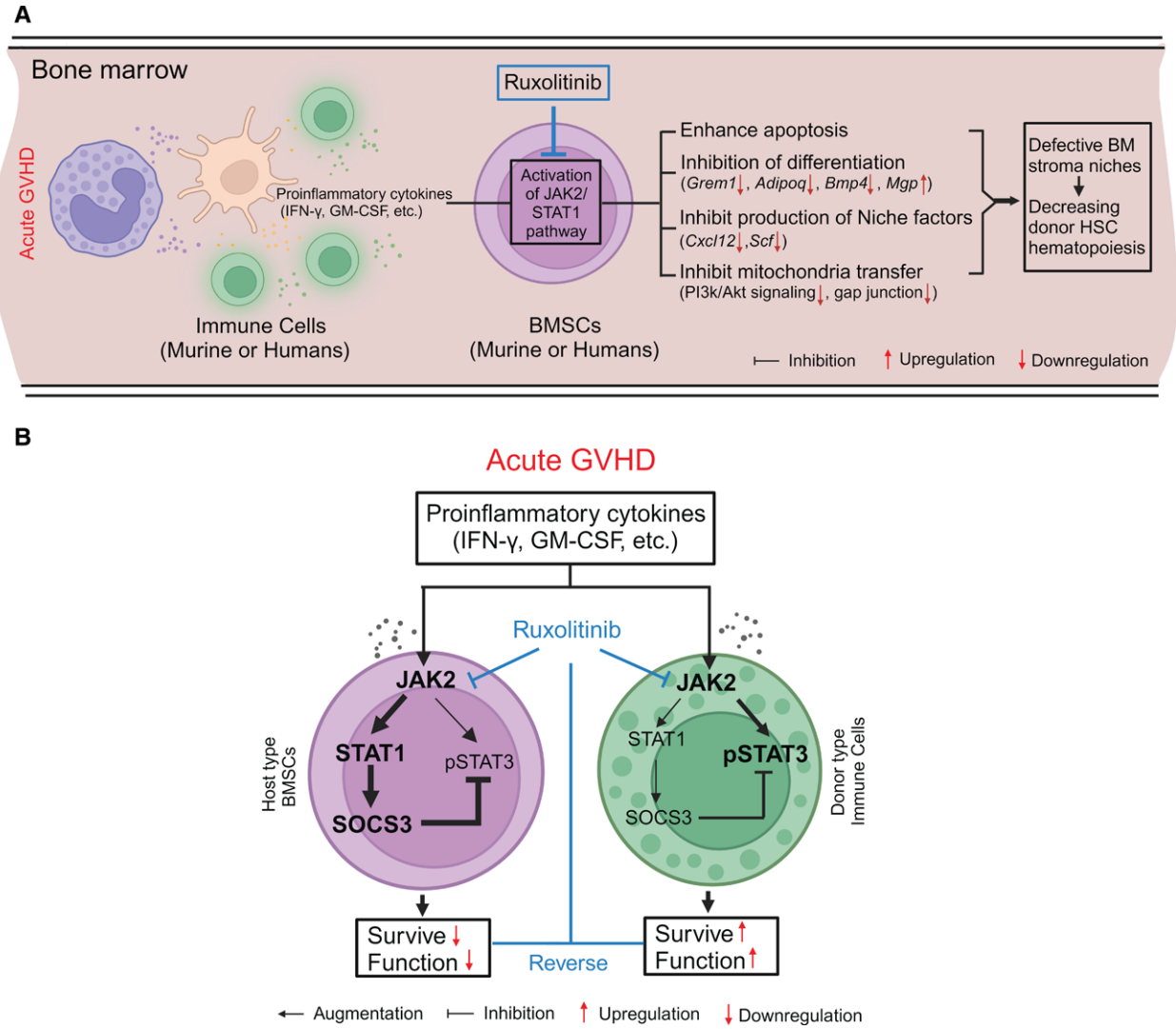

Figure 1. Inhibition of JAK2/STAT1 pathway by Ruxolitinib treatment restores the function of BMSC niches that augments hematopoiesis of donor HSCs in murine and human acute GVHD recipients while inhibiting immune cell function. (A) Acute GVHD activates JAK2/STAT1 pathway in the BMSCs, leading to the disruption of functional BM stroma niches, with increased apoptosis, reduced stemness and differentiation potential, downregulation of differentiation-related genes, reduced production of niche factors, and reduced mitochondria transfer from recipient BMSCs to donor-type HSCs. Direct inhibition of JAK2/STAT1 activation in the host-type BMSCs by Ruxolitinib reverses the negative impact of acute GVHD and restores the fitness of BMSCs, leading to augmenting hematopoiesis of donor-type HSCs. (B) Stronger JAK2-STAT1 activation in the BMSCs may lead to stronger activation of inhibitory SOSC3 that reduces activation of STAT3, leading to their dysfunction and increase of apoptosis. In contrast, stronger JAK2-STAT3 activation in the immune cells may lead to their improved function and increased survival. Direct inhibition of JAK2/STAT1 activation by Ruxolitinib may differentially reverse the activation of SOSC3 and STAT3 in the host-type BMSCs and donor-type immune cells, leading to restoring or destroying the function of the BMSCs or immune cells, respectively. BMSC = BM mesenchymal stem cell, HSC = hematopoietic stem cell, GVHD = graft-versus-host disease, JAK = Janus kinase, STAT = signal transducers and activators of transcription.

in BMSC functions, such as increased survival, production of niche factor CXCL12, osteogenesis, and chondrogenesis, as well as improved mitochondria function and enhanced PI3/AKT pathway that augments mitochondria transfer into donor HSCs to augment hematopoiesis in the HCT recipients[7] (**Fig. 1A**). Thus, amelioration of acute GVHD by Ruxolitinib is associated with restoring BM stroma niches that augment donor-type hematopoiesis.

Third, the observations with murine models also occurred in allo-HCT patients. BMSCs from acute GVHD patients also showed reduced capacity in colony-forming, osteogenesis, and adipogenesis. Ruxolitinib also directly inhibited the phosphorylation of JAK2 and STAT1 in BMSCs of acute GVHD patients[7] (**Fig. 1A**). The Ruxolitinib treatment restored the fitness of patient BMSCs by improving mitochondria function and augmenting osteogenesis and adipogenesis, leading to augmenting donor-type hematopoiesis, as indicated by increase in Hb content and platelet count in the blood of the patients[7] (**Fig. 1A**).

In summary, Lin et al demonstrated that in murine models and patients acute GVHD damages the BM stoma niches by augmenting BMSC apoptosis and dysfunction, and amelioration of acute GVHD by administration of Ruxolitinib is associated with direct inhibition of JAK2/STAT1 in the BMSCs and restoring the function of BMSCs, which augments the hematopoiesis of donor-type HSCs[7] (**Fig. 1A**). However, there are important remaining questions, as depicted in Figure 1B. First, the proinflammatory cytokines and factors that activate JAK2/STAT1 pathways in the host-type BMSCs of acute GVHD recipients remains unclear, although activation of JAK2/STAT1 pathway by proinflammatory cytokines (ie, IFN-γ,GM-CSF, and IL-6) in donor-type lymphohematopoietic cells

is established.[11] Second, it also remains unclear why activation of JAK2/STAT1 pathways in the BMSCs increases their apoptosis and dysfunction, but activation of JAK2/STAT1 pathways in the donor-type lymphohematopoietic cells augments survival and enhances their function. We theorize that the opposite outcome of activation of JAK2/STAT1 pathway in host-type BMSCs and donor-type lymphohematopoietic cells may result from differential expression of cytokine receptors (ie, IFN-γR vs IL-6R) and differential activation of JAK2-STAT1-SOCS3 and JAK2-STAT3 axis. As reported,[16] stronger JAK2-STAT1 activation in the BMSCs may lead to stronger activation of inhibitory SOSC3 that can reduce activation of STAT3 and reduce cell function and survival (**Fig. 1B**). STAT3 plays a critical role in survival and expansion of immune cells and mesenchymal cells.[17] Inhibition of JAK2/STAT1 activation by Ruxolitinib may differentially reverse the activation of SOSC3 and STAT3 in the BMSCs and immune cells, leading to restoring or destroying the function of the BMSCs or immune cells, respectively (**Fig. 1B**).

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