Sterile α motif domain–containing 9 (SAMD9) and SAMD9-like (SAMD9L) syndromes are inherited bone marrow failure syndromes known for their frequent development of myelodysplastic syndrome with monosomy 7. In this issue of the JCI, Abdelhamed, Thomas, et al. report a mouse model with a hematopoietic cell–specific heterozygous Samd9l mutation knockin. This mouse model resembles human disease in many ways, including bone marrow failure and the nonrandom loss of the mutant allele. Samd9l-mutant hematopoietic stem progenitor cells showed reduced fitness at baseline, which was further exacerbated by inflammation. TGF-β hyperactivation was found to underlie reduced fitness, which was partially rescued by a TGF-β inhibitor. These findings illustrate the potential role of TGF-β inhibitors in the treatment of SAMD9/SAMD9L syndromes.
Inflammation fuels bone marrow exhaustion caused by Samd9l mutation

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SAMD9/SAMD9L syndromes

Sterile α motif domain–containing 9 (SAMD9) and SAMD9–like (SAMD9L) syndromes are inherited bone marrow failure (BM) failure syndromes due to mutations in SAMD9 or SAMD9L. The genes are paralogs located on chromosome 7q21 and their function remains elusive but has a growth inhibitory effect. Patients with SAMD9/SAMD9L syndromes have diverse clinical manifestations, including cytopenias, myelodysplastic syndrome (MDS), growth restriction, and immune dysregulation. Previously, SAMD9 and SAMD9L mutations were thought to cause MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) syndrome and ataxia pancytopenia syndrome, respectively (1, 2). But, more recent studies suggest those syndromes can be observed in patients with either gene mutations (3).

SAMD9/SAMD9L syndromes are notorious for their frequent association with monosomy 7, which carries one of the worst prognoses in myeloid malignancies as a single cytogenetic abnormality (4). Because the gain-of-function mutants are severely growth inhibitory, cells that lost the mutant allele (i.e., monosomy 7, uniparental isodisomy 7q, or loss-of-function second-site mutation) have a growth advantage leading to an expansion of clones that lost SAMD9/SAMD9L–mutation-containing chromosome 7 (5). In fact, patients carrying germline SAMD9/SAMD9L mutations comprise 8% to 17% of childhood MDS with monosomy 7 (3, 6) and it has been described as familial monosomy 7 syndrome (7). Selection pressure for monosomy 7 underlies a predisposition to MDS and acute myeloid leukemia.

In this issue of the JCI, Abdelhamed, Thomas, and colleagues established a mouse model of SAMD9/SAMD9L syndromes. The mice (referred to as Samd9l–Mut) possessed a heterozygous Samd9l p.W1171R mutation, which is equivalent to the human SAMD9L p.W1180R mutation noted in patients with impaired hematopoietic cell function (8).

Lessons learned from Samd9l mouse models

The mouse genome lacks Samd9 and only has Samd9l on chromosome 6, which opens the opportunity to study the function of Samd9l without redundancy of Samd9.

We have learned from prior Samd9l mouse models that (a) Samd9l haploinsufficiency (heterozygous knockout) predisposes mice to myeloid malignancies spontaneously, which is even more accelerated by viral infections, and it increases the repopulating capacity of hematopoietic stem progenitor cells (HSPCs) (2); (b) Samd9l plays an important role in the degradation of cytokine receptors by endocytosis and endosome fusion with lysosomes, thereby its deficiency increases the availability of cytokine receptors on the cell surface and promotes growth (2, 9); and (c) a gain-of-function Samd9l homozygous mutant mouse model develops BM failure, growth retardation, and both homozygous and heterozygous mutant mice show reduced repopulating capacity (9).

In their study, Abdelhamed, Thomas, et al. generated conditional knockin mice that had a skewed myeloid commitment at the expense of decreased lymphoid commitment (8). B cell lymphopenia was the most notable finding in the peripheral blood. Single-cell RNA sequencing (RNA-Seq) further showed a differentiation block in the B cell lineage. Samd9l–Mut HSPCs also showed decreased colony formation upon replating and were outcompeted in competitive transplantation assays, suggesting decreased overall fitness of Samd9l–Mut HSPCs, similar to a prior study (2). Interestingly, 4 out of 26 Samd9l–Mut mice had a partial loss of chromosome 6 on which mouse Samd9l is located, phenocopying nonrandom loss of a mutant allele on chromosome 7 in patients with SAMD9/SAMD9L syndromes. This finding suggests that loss of the Samd9l–mutant allele confers a growth advantage in mice, as in humans (8).
Inflammation and BM failure

Inflammation caused by infection or autoimmune conditions triggers HSCs to transition rapidly from quiescence to active cell cycling with the eventual loss of self-renewal capacity (10–13). Proinflammatory cytokines, including IFN-α and IFN-β, in response to viral infections can cause BM suppression or even aplastic anemia (14–16). This pathology may be due to a direct effect on HSCs or an indirect effect via the niche.

**SAMD9** and **SAMD9L** are IFN-responsive genes and they play an important role in innate immunity against viral infections (17–19). The authors show that one way to directly affect HSCs in the setting of inflammation is through the type I IFN signaling–induced expression of **SAMD9L** in HSCs. Increased **SAMD9L** expression favors the elimination of inflamed (or infected) cells by inhibiting their growth. In vitro IFN-α or in vivo polynosinic/polyribidylic acid (pI:pC) injections, which induce type I IFNs (IFN-α and IFN-β) in vivo, increased the expression of both WT and mutant **SAMD9L** proteins. However, the treatment substantially increased apoptosis and decreased colony-forming capacity only in **SAMD9L-Mut** BM cells.

Interestingly, inflammation induced by pI:pC reduced the engraftment potential of both **SAMD9L-WT** and **SAMD9L-Mut**, but it is noteworthy that inflammation further decreased the already reduced engraftment potential and increased apoptosis of **SAMD9L-Mut** BM cells. Similarly, both **SAMD9L-WT** and **SAMD9L-Mut** mice decreased their lymphocyte counts upon pI:pC challenge, but a greater degree of reduction along with myeloid hyperplasia was observed in **SAMD9L-Mut** mice. Subsequent RNA-Seq analysis of lineage-negative cKit-positive HSCs showed that the TGF-β pathway was upregulated in pI:pC-treated **SAMD9L-WT** HSCs, which was confirmed by flow cytometry as increased p-SMAD2/3 signaling (Figure 1). This upregulation of p-SMAD2/3 was most notable in B cells, which may explain B cell lymphopenia in **SAMD9L-Mut** mice. The TGF-β small molecule inhibitor, SD-208, partially rescued **SAMD9L-Mut** mice, albeit not to the WT levels (8). These findings suggest that TGF-β inhibitors may boost the colony-forming capacity of the **SAMD9L-Mut** mouse and patient BM; however, whether the inhibitors can actually reverse BM failure in mice and humans requires future studies.

Interestingly, TGF-β hyperactivation is not specific to **SAMD9/SAMD9L** syndromes but is also observed in other inherited BM failure syndromes, such as Fanconi anemia (20), Diamond-Blackfan anemia (21, 22), and Shwachman-Diamond syndrome (23). These observations suggest that TGF-β hyperactivation may be a common pathway leading to HSC exhaustion due to various underlying mechanisms.

**Summary and future directions**

Abdelhamed, Thomas, et al. generated a **SAMD9L** mouse model with a heterozygous human **SAMD9L** mutation equivalent at its endogenous locus. This mouse model phenocopies human disease in that it develops BM failure and nonrandom loss of the mutant allele. The authors further investigated the effects of inflammation using in vitro and in vivo models and showed that inflammation exacerbates apoptosis of lymphocytes, and reduces colony-forming capacity and engraftment potential, particularly in **SAMD9L-Mut** HSCs. They discovered that TGF-β hyperactivation was one of the mechanisms underlying reduced HSC fitness in the setting of inflammation, and a TGF-β inhibitor rescued the colony-forming capacity of mouse and human mutant HSCs at least partially. It will be critically important to confirm whether TGF-β hyperactivation is present also in patient HSPCs and whether it is only induced with inflammation or also present at a steady state, before moving TGF-β inhibitors to clinical trials. It would also be interesting if TGF-β inhibitors could reverse growth restriction and prolong survival in their mouse model. The work by Abdelhamed, Thomas, et al. opens the possibility of TGF-β inhibitors as therapeutics in this rare disease with no current treatment options other than BM transplant (8).

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