Rapamycin targets several pathophysiological features of immune-mediated bone marrow failure in murine models

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In this issue of *Haematologica*, Feng *et al.*[1] compare the efficacy of treatment with cyclosporine A (CsA) and rapamycin to ameliorate pancytopenia, improve bone marrow (BM) cellularity, and extend survival in murine models of immune-mediated aplastic anemia (AA). Interestingly, while the efficacy of CsA and rapamycin to attenuate immune-mediated bone marrow failure (BMF) in murine AA models is similar, CsA and rapamycin achieve their effects through different mechanisms.[1]

Immune-mediated aplastic anemia (AA) is an acquired form of BMF and is characterized by an abnormally low number of BM cells (hypoplasia) and severe reduction in blood cells (pancytopenia), which in the severe form of AA (SAA) can be life-threatening. The immune and hematologic pathophysiology of AA is quite complex and includes: a) development and oligoclonal expansion of autoreactive T cells, including $CD8^+$ cytotoxic T cells, $CD4^+$ Th1 cells, and Th17 cells; b) effector T-cell-mediated apoptosis and depletion of hematopoietic stem and progenitor cells (HSPCs) and mature blood cells, leading to BM hypoplasia and pancytopenia; c) production of proinflammatory cytokines (e.g. TNFα and IFNγ); d) severe reduction and functional impairment of immunosuppressive regulatory T cells (Tregs); and e) karyotype abnormalities, genomic instability, and somatic mutations in different myeloid cancer-associated genes that positively and negatively correlate with response to immunosuppresive therapy (IST) and risk of development of myelodysplasia and acute myeloid leukemia (AML).[2-6]

Current standard treatments for AA include IST with horse anti-thymocyte globulin (ATG) and cyclosporine A (CsA), or allogeneic HLA-matched sibling or well-matched unrelated donor BM transplant. While IST is effective in 60-70% of AA patients, a significant proportion of patients who responded to IST undergo relapse after CsA withdrawal or are refractory to IST. Moreover, IST is not effective in treating refractory and relapsed AA.[7-12]

Recently, combined application of eltrombopag, a thrombopoietin mimetic, and standard IST has proven to be very effective in treating patients with refractory and severe AA. However, relapse and clonal evolution remain important post-therapy concerns.[13,14]

Different murine models were developed to study the etiology and pathophysiology of AA, and the MHC partially mismatched lymphocyte infusion models, based on alloantigen recognition, are the best characterized and most relevant pre-clinical AA models. The induced AA in these models exhibits many of the clinical and pathological features of acquired AA in patients, and can be modulated using IST and Treg cell therapies. These models provided important insights into the cellular and molecular immune effectors implicated in AA, and are a powerful and relevant *in vivo* system for testing new drugs and therapeutic approaches for treating SAA.[15,16]

The AA in lymphocyte infusion models is induced by infusing parental lymph node cells (LNCs) from $H2^b$ C57BL/6 mice into MHC partially mismatched non-irradiated or sublethally irradiated F1 hybrid $H2^{b/d}$ B6D2F1 (C57BL6 x DBA/2J) or CByB6F1 (C57BL6 x BALB/c) recipients. Among mismatched minor-H antigens, H60 contributes the most to AA development in the C.B10 mouse AA model, which is generated by infusion of LNCs from BL6 mice into C.B10 mice which are pre-conditioned with 5 Gy of sublethal total body irradiation (TBI).[15,16]

Feng *et al.* have shown that treatment of AA mice with rapamycin for 12 days and treatment with CsA for nine days resulted in similar and statistically significant improvements in BM cellularity, number of white blood cells (WBCs) and platelets (PLTs), and 100-day survival in comparison to untreated AA mice and control mice that received 5 Gy TBI. Temporal studies of recovery of complete blood counts (CBCs) and BM cellularity in rapamycin-treated mice and TBI control mice revealed a similar degree of recovery at days (d)28, 42 and 100, except for a delayed WBC recovery in rapamycin-treated mice.[1]

Importantly, delayed treatment with rapamycin was also effective in decreasing pancytopenia and BMF in mice with ongoing AA, with a better response from treatment initiated at d5 *versus* d7 after LN cell infusion. Moreover, the therapeutic effects of a 5-day delay of rapamycin treatment lasted for ten weeks in this experiment, albeit with a significantly slower recovery of WBCs.[1]

Subsequent experiments have demonstrated that both CsA and rapamycin rescued mice from BM failure by suppressing $CD8^+$ and $CD4^+$ T-cell infiltration in the BM. However, treatment of AA mice with rapamycin led to an increase in functional regulatory T cells in the BM, lymph node and spleen in comparison to mice treated with CsA and untreated AA mice. Furthermore, rapamycin more efficiently eradicates $CD8^+$ effector T cells in a CByB6F1 AA model and antigen-specific $CD8^+$ effector T cells in a minor histocompatibility antigen H60 mismatched AA model. Additional *in vitro* and *in vivo* experiments have revealed that rapamycin treatment is more efficient in reducing memory-like and effector T cells than CsA treatment.[1]

Transcriptome analyses of BM $CD4^+$ and $CD8^+$ T cells from BMF mice with or without rapamycin or CsA treatment have discovered important differences in the transcription profile of effector molecules important for immune activity of cells, indicating that rapamycin and CsA exert their immuno-modulating effects through different molecular pathways. Furthermore, the analysis of cytokine secretory profiles of T cells from rapamycin and CsA-treated mice

revealed significant differences in the effects of rapamycin and CsA on cytokines related to Th1 and Th2 immune responses.[1] These important mechanistic findings warrant further molecular and functional studies to uncover the full spectra of molecular and physiological mechanisms of immunosuppression through which rapamycin and CsA ameliorate BMF.

Through significant depletion of effector T cells and increase in Treg cells, treatment with rapamycin resulted in improved BM cellularity, significantly lower pancytopenia, and significantly increased numbers of HSPCs in the BM and long-term survival of mice with AA. Thus, similar to other experimental anti-inflammatory and immunosuppressive approaches,[16,17] treatment with rapamycin simultaneously and efficiently targets several pathophysiological features of AA in murine models.[1]

The analysis of HSPC populations in the BM of control, TBI, untreated, CsA-treated and rapamycin-treated mice on d13, has revealed that rapamycin treatment resulted in a 2-3-fold increase in the frequency and numbers of c-Kit$^+$Sca1$^+$Lin$^-$ (KSL), c-Kit$^-$Sca1$^+$Lin$^-$ and KSLCD150$^+$ BM cells, which greatly surpass the numbers observed in control mice. Interestingly, in contrast to control, TBI, untreated and CsA-treated mice, c-Kit$^+$Sca1$^+$Lin$^-$ (KSL) and c-Kit-Sca1$^+$Lin$^-$ cells from rapamycin-treated mice exhibited significantly increased numbers of cells with high expression of Sca-1 marker. It is well established that inflammatory conditions (radiation, chemotherapy, infections) and inflammatory cytokines such as IFNs and TNFα increase Sca-1 expression on HSPCs.[18,19]

Thus, it is unclear at this point what the cause of significant Sca-1 upregulation is, since the cytokine profiling of plasma from rapamycin-treated mice on d13 has shown that rapamycin significantly down-regulated both IFNγ and TNFα. It would be very interesting to analyze c-Kit$^+$Sca1$^+$Lin$^-$ (KSL), c-Kit$^-$Sca1$^+$Lin$^-$ and KSLCD150$^+$ BM cells in rapamycin-treated mice at later time points during their long-term survival.

Analysis of effects of CsA and rapamycin on mTOR and NFAT signaling pathways suggests that CsA suppresses immune activity by interfering with the NFAT1 signaling pathway, whereas rapamycin promotes differentiation of Th2 effector lineages and suppresses pro-inflammatory Th1 and Th17 T cell lineages by modulating mTOR activity.

In conclusion, the study by Feng *et al.* demonstrates that, similar to treatment with standard dose of CsA, rapamycin effectively and reproducibly attenuated immune-mediated BM failure in mouse models of AA.[1]

Although treatment of AA patients with standard IST and rapamycin in a recent clinical trial was not more efficient than standard IST,[20] due to its immunosuppressive activity and tolerogenic role in organ transplantation, rapamycin has clinically relevant potential and will be tested in an upcoming phase II clinical trial as a prophylactic treatment of AA patients at high risk of relapse after withdrawal of CsA treatment.

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