

inhibits production of immunoregulatory interleukin-10 by activated CLL cells in vitro (Samantha Drennan and F.F., unpublished data, 2014). In addition, continuous treatment by ibrutinib was reported to associate with recovery of humoral immunity, with an increase in serum immunoglobulin levels, predominantly of the IgA isotype,<sup>2</sup> but there is no mention of the serum immunoglobulin levels in this work with extended follow-up.<sup>1</sup> The data leave the question on how ibrutinib favors immune recovery and set the basis for further investigation in larger cohorts of patients requiring treatment for the first time.

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

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## ● ● ● IMMUNOBIOLOGY

Comment on Nguyen et al, page 2519

# TIF-IA and Ebp1 regulate RNA synthesis in T cells

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In this issue of *Blood*, Nguyen et al show that mycophenolic acid (MPA) induces GTP depletion, which inhibits the function of transcription initiation factor I (TIF-IA) and impacts the interaction of TIF-IA with ErbB3-binding protein 1 (Ebp1), a key in regulating proliferating cell nuclear antigen (PCNA) expression and ribosomal RNA (rRNA) synthesis in T cells during activation.<sup>1</sup>

The figure summarizes the main findings of the report and shows how MPA can regulate T-cell proliferation and activation at different levels by targeting the interaction between TIF-IA and Ebp1, thus inhibiting rRNA synthesis and regulating the expression of key factors involved in cell proliferation, such as PCNA and p53. Moreover, this study suggests that the use of MPA together with sotrastaurin suppresses T-cell activation even more potently than each drug alone, by also inhibiting the TIF-IA-Ebp1 interaction.

Mycophenolate mofetil (MMF) is an immunosuppressive drug that has been used more frequently over the past 20 years.

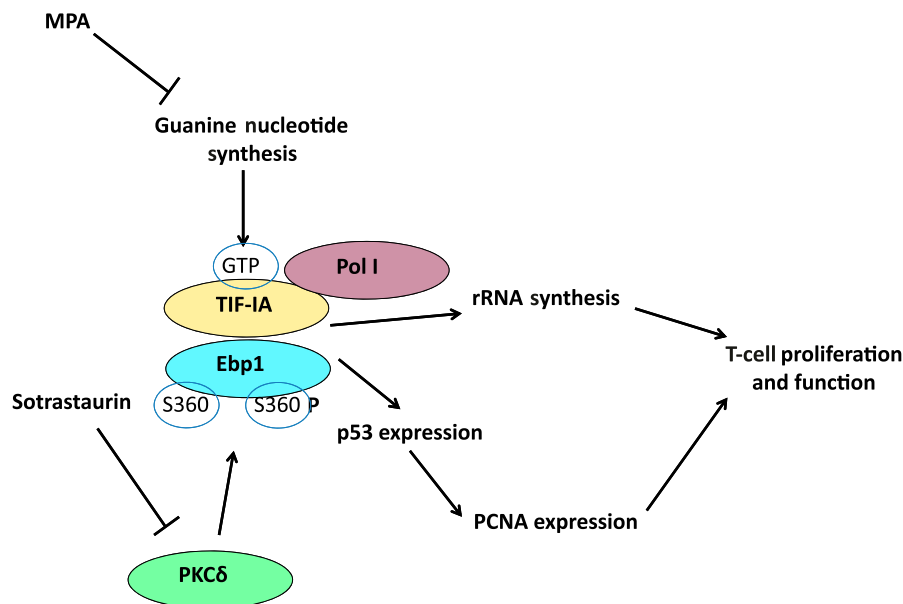
Currently, MMF is used in combination with other drugs to treat certain autoimmune diseases, is part of the immunosuppressive regimen to modulate graft-versus-host disease following hematopoietic stem cell transplantation, and is used to prevent graft rejection post-organ transplantation. MMF is a prodrug of MPA, which is a purine analog. MPA inhibits specifically T-cell and B-cell activation and function by inhibiting the type II isoform of inosine 5'-monophosphate dehydrogenase, a rate-limiting enzyme in the de novo synthesis of guanosine, thus depleting guanine nucleotides.<sup>2</sup> Notably, previous studies by Huang et al, Dayton et al, and others

have shown that MPA could also inhibit the synthesis of rRNA<sup>3,4</sup>; however, so far, the MPA mechanisms that could mediate this effect remain to be understood.

TIF-IA is involved in the regulation of rRNA synthesis and is expressed by all mammalian cells.<sup>5,6</sup> In their recent work, Nguyen et al tested whether TIF-IA requires GTP binding in order to regulate RNA synthesis in T cells while interacting with other proteins. A systematic approach was used by the authors to assess the effects of MPA on cell lines, primary T cells, or cells from patients when possible, using chromatin immunoprecipitation assays and RNA analysis, and by knocking down or overexpressing the factor of interest.

Interestingly, they found that TIF-IA is a GTP-binding protein and that MPA treatment induced GTP depletion that then modified TIF-IA function and localization to the periphery of nucleolus into T cells, while blocking RNA polymerase I (Pol I) binding to the rDNA promoter. In addition, MPA treatment also led to increased p53 expression while decreasing PCNA expression, explaining how MPA can inhibit rRNA synthesis as well as proliferation in T cells. The investigators then went on to assess the role of Ebp1, which negatively regulates p53,<sup>7</sup> and found that Ebp1 is upregulated in proliferating T cells and binds to TIF-IA. They showed that the interaction between TIF-IA and Ebp1 plays a key role in the regulation of cell proliferation and PCNA expression. In addition, they found that MPA reduced the interaction between TIF-IA and Ebp1 while upregulating p53 but decreasing PCNA expression, which suggests that the interaction between TIF-IA and Ebp1 might depend on TIF-IA binding to GTP.

It has been reported that Ebp1 can interfere with rRNA processing; however, whether Ebp1 regulates RNA synthesis was unknown. In the present study, the authors demonstrate for the first time that Ebp1 is key for TIF-IA retention where RNA synthesis occurs, and that both TIF-IA and Ebp1 play key roles in the regulation of rRNA. Moreover, phosphorylation at S360 of Ebp1 by protein kinase C  $\delta$  (PKC $\delta$ ) was necessary for TIF-IA-mediated regulation of RNA synthesis. Interestingly, inhibiting phosphorylation of Ebp1 by using sotrastaurin, a specific inhibitor of PKC $\delta$ , together with MPA led to a very



MPA inhibits guanine nucleotide synthesis and depletes GTP. This impacts the function of TIF-IA and in particular the capacity of TIF-IA to interact with Ebp1, an interaction that regulates rRNA synthesis via the interaction of TIF-IA with Pol I and transcription of PCNA, thus impacting T-cell proliferation and function. In addition, sotrastaurin inhibits PKC $\delta$ , which phosphorylates Ebp1 at position S360, a step required for PCNA expression. MPA and sotrastaurin synergize and inhibit T-cell activation and proliferation very potently by inhibiting the functional interaction between TIF-IA and Ebp1.

potent inhibition of RNA synthesis, PCNA expression, T-cell proliferation, and reduced interleukin-2 production by T cells.

Sotrastaurin is a drug that is currently being used to inhibit graft rejection in renal transplantation and to treat certain inflammatory diseases such as psoriasis.<sup>8,9</sup> Therefore, these results are of great interest because they suggest that the combination of MPA and sotrastaurin could be used in the future as a highly potent regimen to modulate lymphocyte activation (see figure) in the context of transplantation or to treat certain autoimmune diseases. However, sotrastaurin is a paninhibitor and more studies are warranted to assess whether other immune cells will be affected. In addition, sotrastaurin may affect more than just rRNA synthesis or growth within 1 cell and this needs to be addressed as well to truly evaluate the possibility of using this drug combination safely in patients. These studies will be highly relevant because therapies such as transplantation rely heavily on successful and effective immunosuppression.

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

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## ● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Hijazi et al, page 2558

# The traumatic side of fibrinolysis

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In this issue of *Blood*, Hijazi et al challenge the view that consumptive coagulopathy that accompanies traumatic brain injury (TBI) results in a sequence of events that lead to intracranial hemorrhage (ICH).<sup>1</sup>

**W**hy is it that an antifibrinolytic drug that is given to reduce bleeding in severe trauma patients can sometimes cause the very thing it is intending to stop? The paper highlighted in this commentary provides an interesting insight that goes a considerable way toward explaining this paradox and also how the fibrinolytic system can be inadvertently turned on when it should be turned off.

One of the greatest causes of mortality associated with TBI is ICH. Consumptive coagulopathy that accompanies TBI is widely assumed to underlie the sequence of events leading to ICH expansion. Not only do Hijazi et al challenge this view, but they imply

that coagulopathy is not a cause of ICH at all but rather occurs as a consequence of the fibrinolytic system being activated within the brain.

To explore the role of the fibrinolytic system in the promotion of ICH following TBI, wild-type (WT) mice and mice deficient in tissue-type plasminogen activator (tPA) or urokinase plasminogen activator (uPA) were subjected to a closed head injury model of TBI, and the degrees of ICH and coagulopathy were evaluated. WT mice had an increase in plasma D-dimer levels and a drop in platelet count within 2 hours, but there was no change in the international normalization ratio (INR),