

blood

2008 112: 2171-2172
doi:10.1182/blood-2008-06-160523

TPO signaling: when the tyrosines go marching in(side)

Roberto Pozner and Mirta Schattner

Updated information and services can be found at:
<http://bloodjournal.hematologylibrary.org/cgi/content/full/112/6/2171>

Information about reproducing this article in parts or in its entirety may be found online at:
http://bloodjournal.hematologylibrary.org/misc/rights.dtl#repub_requests

Information about ordering reprints may be found online at:
<http://bloodjournal.hematologylibrary.org/misc/rights.dtl#reprints>

Information about subscriptions and ASH membership may be found online at:
<http://bloodjournal.hematologylibrary.org/subscriptions/index.dtl>



inside blood

15 SEPTEMBER 2008 | VOLUME 112, NUMBER 6

● ● ● CHEMOKINES

Comment on Hitchcock et al, page 2222

TPO signaling: when the tyrosines go marching in(side)

Roberto Pozner and Mirta Schattner HEMATOLOGICAL RESEARCH INSTITUTE

In this issue of *Blood*, Hitchcock and colleagues provide novel insights into the molecular pathways regulating TPO-mediated c-Mpl trafficking, highlighting the essential role of AP2 in receptor internalization and lysosomal degradation as new pieces in the TPO/c-Mpl signaling puzzle.

Thrombopoietin (TPO) and its receptor c-Mpl are the primary regulators of megakaryocytopoiesis and play a critical role in hematopoietic stem cell biology. Upon TPO binding, c-Mpl facilitates tyrosine (Y) phosphorylation of cytoplasmic signaling proteins and

activation of several pathways that control cellular proliferation, megakaryocyte development, and survival. Turning off the TPO signal is critical to prevent uncontrolled proliferation; however, the molecular processes governing this process are still not fully elucidated. Suppressors

of cytokine signaling (SOCS), phosphatases, and other proteins, such as focal adhesion kinase and members of the Src family kinases (SFKs), have recently emerged as negative modulators of TPO signaling.^{1,2}

Although it is still a matter of debate, soon after the discovery of TPO it was suggested that internalization of TPO/c-Mpl complexes by platelets and megakaryocytes is a primary means of regulating plasma TPO levels.

A phosphotyrosine (Y₁₁₂) and 2 dileucine motifs (L₅₄L₅₅ and I₅₇L₅₈) within the cytoplasmic domain of the receptor have been identified to promote TPO-mediated c-Mpl internalization and proliferative signaling. Furthermore, c-Mpl recycling

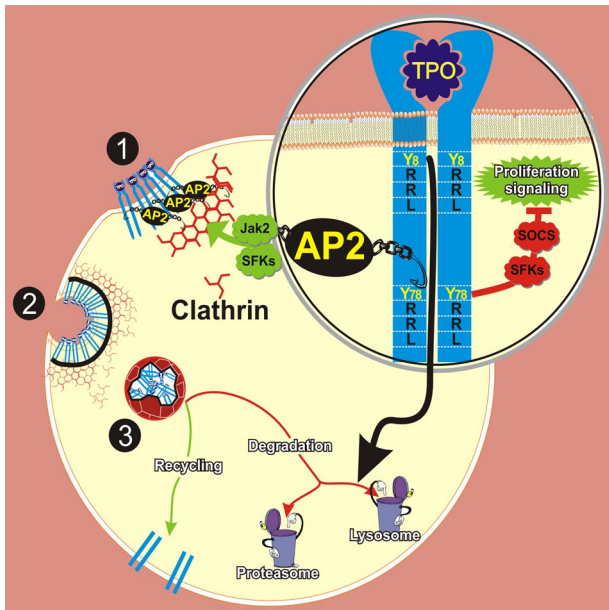
from intracellular pools has also been demonstrated.³

Clathrin-mediated endocytosis constitutes the major route for selective receptor internalization in higher eukaryotes. Clathrin molecules are recruited to the cell surface where they interact with transmembrane proteins via adaptor proteins (AP) such as AP2 to form clathrin-coated pits. YXX ϕ and [DE]XXXL[IL] motifs in the cytoplasmic tails of transmembrane proteins have been identified as the binding sites for AP2.⁴

Hitchcock et al, in a series of elegant experiments, have demonstrated that AP2 has a key role in TPO-stimulated clathrin-mediated c-Mpl internalization in both Jak2 and SFKs activation-dependent pathways. Bearing in mind that c-Mpl contains 2 YXX ϕ motifs in its cytoplasmic domain at Tyr8 (Y₈RRL) and Tyr78 (Y₇₈RRL) and using BaF3-Mpl-expressing cells with point mutations at Y₈ or Y₇₈, the authors have identified Y₇₈RRL as the binding motif for AP2 in the c-Mpl receptor. Interestingly, they also observed that cells expressing the Y₇₈F mutation exhibited increased proliferation and prolonged activation of Jak2, STAT5, AKT, and ERK1/2 in response to TPO. The observation that knocking down AP2 did not have the same effects on signaling as the mutation led the authors to suggest that Y₇₈ may be responsible not only for the internalization but also for the activation of other signaling pathways that restrain the TPO signal.

Although the clearance of c-Mpl was recently attributed only to the proteasome,⁵ this study unveiled that lysosomal degradation is another control mechanism of c-Mpl expression and also identifies Y₈RRL as the lysosomal targeting motif. Accordingly, Y₈F c-Mpl cells showed an increase in c-Mpl recycling to the cell surface (see figure).

This paper is the first report that describes the YXX ϕ motif for receptor internalization and lysosome targeting in the hematopoietic growth factor receptor family and raises many new questions that await an answer. What



Regulation of TPO-mediated c-Mpl receptor internalization and degradation. After TPO binding, clathrin-coated pits are formed through the interaction between the AP2 and Y₇₈RRL motif in the cytoplasmic tail of c-Mpl. The internalization process is regulated by Jak2 as well as SFKs activation (1). Following invagination and budding, (2) clathrin-coated vesicles are routed to endocytic pathways that direct the c-Mpl receptor to recycling and/or degradation (3). While Y₇₈RRL negatively regulates proliferation, Y₈RRL targets c-Mpl lysosomal degradation.

mechanisms control the balance between c-Mpl degradation/recycling? What contribution does c-Mpl internalization make to the TPO proliferative signal? What would be the biological impact of the c-Mpl Y₇₈ and Y₈ mutations in primary cells? To what extent are Y mutations linked to the pathogenesis of myeloproliferative diseases? Investigating these questions should help us to understand the interplay between the actors controlling the TPO/c-Mpl scenario.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

● ● ● CLINICAL OBSERVATIONS

Comment on Till et al, page 2261

Test-driving CARs

Laurence J. N. Cooper UNIVERSITY OF TEXAS M.D. ANDERSON CANCER CENTER

Redirecting T-cell specificity through the introduction of a chimeric antigen receptor (CAR) is emerging as a clinically feasible approach for adoptive immunotherapy. In this issue of *Blood*, Till and colleagues now demonstrate that autologous T cells genetically modified to express a CD20-specific CAR can be safely infused in patients with B-lineage lymphomas.

Approximately 15 years after Zelig Eshhar demonstrated how to redirect the specificity of T cells,¹ investigators are reporting their early clinical experiences with infusing T cells genetically modified to express chimeric antigen receptors (CARs). By combining T-cell therapy with gene therapy in compliance with current good manufacturing practice (cGMP) for phase 1 and 2 trials, investigators have bypassed tolerance to enable clinical-grade T cells to recognize desired cell-surface antigens independent of MHC. T cells are rendered tumor-specific through introduction of a CAR, which is typically composed of the scFv from a monoclonal antibody (mAb) that forms part of the CAR ectodomain and which, upon binding antigen, activates T cells by phosphorylation of conserved immunoglobulin tyrosine activation motifs within a chimeric CD3- ζ or Fc ϵ RI endodomain. To limit potential deleterious off-target effects, the first human trials for hematopoietic malignancies have infused genetically modified CAR⁺ T cells that target lineage-restricted antigens, such as CD19 and CD20 expressed on malignant (and healthy) B cells,^{2,3} and generally have used first-generation CARs (activating T cells solely through CD3- ζ ⁴). Build-

REFERENCES

1. Hitchcock IS, Fox NE, Prevost N, et al. Roles of focal adhesion kinase (FAK) in megakaryopoiesis and platelet function: studies using a megakaryocyte lineage specific FAK knockout. *Blood*. 2008;111:596-604.
2. Kaushansky K. Lineage-specific hematopoietic growth factors. *N Engl J Med*. 2006;354:2034-2045.
3. Dahlen DD, Broudy VC, Drachman JG. Internalization of the thrombopoietin receptor is regulated by 2 cytoplasmic motifs. *Blood*. 2003;102:102-108.
4. Bonifacino JS, Lippincott-Schwartz J. Coat proteins: shaping membrane transport. *Nat Rev Mol Cell Biol*. 2003;4:409-414.
5. Royer Y, Staerk J, Costuleanu M, et al. Janus kinases affect thrombopoietin receptor cell surface localization and stability. *J Biol Chem*. 2005;280:27251-27261.

ing on clinical experiences of therapeutic mAbs targeting CD20, Till and colleagues demonstrate that an inpatient dose-escalation study infusing autologous clinical-grade T cells expressing a CD20-specific CAR with or without low-dose IL-2 can be undertaken in patients with non-Hodgkin lymphomas. In the current financial and regulatory climate, publishing the results of a gene-therapy trial is a singular accomplishment. However, like many initial human experiences, the results raise more questions than they answer.

Sustaining the survival of adoptively transferred CAR⁺ T cells is one of the major impediments to achieving significant therapeutic responses. One way to enhance persistence is to infuse a heterogeneous population of T cells so that subpopulations can participate in lymphopenia-induced proliferation. This has been championed by Rosenberg and colleagues⁵ at the National Institutes of Health who demonstrated that clinical responses can be achieved when melanoma-specific T cells are infused after lymphodepleting chemotherapy and when bulk populations of T cells are given rather than T-cell clones. Thus, most infusions of CD20-specific T cells were

given after chemotherapy and the trial was altered to infuse populations of genetically modified T cells rather than clones. Cumulatively, up to $4.4 \times 10^9/m^2$ cells were infused within 10 days, and while a limited number of infused T cells could be detected in the peripheral blood of some patients for up to 3 months, the question remains as to why the infused T cells did not persist longer and at increased levels leading to loss of normal CD20⁺ B cells. Perhaps it was due to the cells entering replicative senescence after nonviral gene transfer and ex vivo expansion to clinically meaningful numbers. Perhaps it was due to insufficient T-cell help, despite the use of low-dose IL-2. Perhaps it was due to the level of CAR expression or competency of CAR-dependent signaling, which might have been insufficient to sustain a proliferative T-cell signal. Perhaps it was due to a lack of CAR⁺ central-memory T cells in the inoculum that were capable of long-term in vivo persistence.⁶ Or perhaps it was due to incomplete lymphodepletion resulting from the choice of pre-infusion chemotherapies used.

The trial described by Till et al drives home the observation that multiple infusions of autologous CAR⁺ T cells targeting a B-lineage antigen are both safe and feasible. This clinical experience can now serve as a platform for future endeavors to answer questions concerning the improvement of persistence and the resolving of issues regarding homing to tumor deposits, thereby improving the therapeutic potential of their CAR⁺ T cells.

The clinical data in this issue of *Blood* are among the first reports on the potential of T cells manufactured under cGMP that have been genetically modified to redirect specificity.⁷⁻⁹ With this publication, the authors have advanced the promising technology of CARs, which combine the specificity of mAbs with the replicative and homing potentials of T cells.

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

REFERENCES

1. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci U S A*. 1993; 90:720-724.
2. Cooper LJ, Topp MS, Serrano LM, et al. T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. *Blood*. 2003;101:1637-1644.
3. Brentjens RJ, Latouche JB, Santos E, et al. Eradication