Comment on Fauriat et al, page 1166

**NK-cell education: KIR-S come into play**

**Thierry Walzer**  IMMUNITY, INFECTION AND VACCINATION, INSERM

In this issue of *Blood*, Fauriat and colleagues find that the expression of KIR2DS1 by human NK cells is associated with a decreased responsiveness to various stimuli in HLA C2/C2 but not in C1/C1 persons.

How natural killer (NK)–cell responsiveness is controlled is an unresolved and fascinating question. A major breakthrough in this field of investigations was made when NK cells expressing inhibitory KIR (human) or Ly49 (mouse) capable of interacting with endogenously expressed major histocompatibility complex (MHC) molecules were found to be hyperresponsive to stimulation through various receptors. This counterintuitive result led to the concept of “licensing” or “education,” a process by which a chronic interaction of inhibitory KIR or Ly49 with their MHC ligands would promote NK-cell responsiveness through unknown mechanisms. Recent data in mouse models suggested that activating receptors also participate in this educational process but in the opposite direction. Indeed, it was found that overexpression of ligands for an activating Ly49 led to a decreased responsiveness of NK cells. In this issue of *Blood*, Fauriat et al investigate the consequences of KIR2DS1 expression on NK-cell responsiveness. KIR2DS1 is an activating KIR known to interact with HLA-C molecules of the group 2 but not to those of the group 1. They took advantage of a combination of anti-KIR antibodies to track KIR2DS1 positive NK cells in human peripheral blood mononuclear cells. Next, they set up a 9-color flow cytometry procedure to track every combination of KIR and NKG2A/CD94 molecule expression on NK cells and assess their responsiveness to various stimuli. Of note, this flow cytometry procedure is certainly powerful but potentially limited by steric hindrance of epitopes, considering that 7 antibodies coupled to sometimes very big fluorochromes are used to stain the same cells. Despite this potential technical limitation, data by Fauriat et al clearly indicate that KIR2DS1 influenced NK-cell responsiveness in donors of the C2 group but not in donors of the C1 group. More precisely, they found that KIR2DS1 single positive NK cells are hyporesponsive to stimulation through various receptors in comparison with KIR-negative NK cells. Moreover, they found that expression of KIR2DS1 by NKG2A-positive NK cells decreases their responsiveness (see figure). These results provide the first evidence that the interaction of an activating KIR with its cognate ligand may modulate NK-cell responsiveness in a physiological setting.

As discussed by the authors, these results fit well with the “rheostat” model of NK-cell responsiveness. This model proposes that NK-cell responsiveness varies in a wide range, regulated by the level of expression of MHC molecules and the number of inhibitory receptors capable of interacting with these molecules. They propose that activating KIR would be another parameter of this equation, working against responsiveness. However, several issues remain to be addressed. In particular, when, where, and under which conditions does education occur? How do NK cells integrate the various educating stimuli? And perhaps most importantly, how can the same ligand/receptor pair (eg, KIR-L/MHC) increase NK-cell responsiveness in an educating context and inhibit NK-cell activation upon interaction with a target cell? Exploration of signaling complexes downstream activating and inhibitory receptors in educating or triggering conditions may provide clues to understand this complex phenomenon.

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

**REFERENCES**


---

**REFERENCES**


5. Barlogie B, Haessler J, Pineda-Roman M. Completion of premaintenance phases in total therapies 2 and 3 improves clinical outcomes in multiple myeloma: an important variable to be considered in clinical trial designs. *Cancer*. 2008 15(12):112270-2725.


---

**Fine tuning of NK-cell responsiveness by NK-cell receptors.** In this model, the interaction of inhibitory receptors with their ligands increases NK-cell responsiveness, whereas the interaction of activating receptors with their ligands has the opposite outcome.
Comment on Saur et al, page 1254

A means to an end: ubiquitination of Mpl

Wei Tong  CHILDREN’S HOSPITAL OF PHILADELPHIA

In this issue of Blood, Saur and colleagues report that ubiquitin-mediated degradation of the Mpl receptor constrains Tpo-mediated cell proliferation, highlighting the importance of the E3 ubiquitin ligase c-Cbl in rapid down-regulation of Tpo/Mpl signaling.

Thrombopoietin (Tpo), via interaction with its cognate receptor Mpl, is the primary cytokine regulating megakaryocyte development and platelet production in addition to its role in hematopoietic stem/progenitor cell (HSPC) homeostasis. Mpl belongs to the type I cytokine receptor family, which includes the erythropoietin receptor (EpoR) and prolactin receptor (PrlR). Ligand binding activates the JAK2 tyrosine kinase, which in turn triggers a cascade of positive and negative signaling events to ensure proper cellular responses. Failure to terminate receptor signaling can lead to oncogenic transformation. Indeed, mutations that constitutively activate Mpl and JAK2 have been found in patients with myeloproliferative neoplasms. Therefore, it is of fundamental importance to understand the mechanisms responsible for restricting cytokine receptor signaling.

One such mechanism that offers swift termination of cytokine signaling involves ubiquitination and internalization of the receptor itself. The mature form of Mpl is subject to rapid internalization, degradation, and clearance from the cell membrane upon Tpo stimulation through both lysosome-dependent and proteasome-dependent mechanisms. In this issue of Blood, Saur et al provide new insights into the turnover of Mpl. Specifically, the authors show for the first time that Mpl is ubiquitinated upon Tpo stimulation to regulate Mpl stability, surface expression, and Tpo-mediated cell proliferation in BaF3 cells. The authors identify 2 cytoplasmic lysine residues (K553 and K573) as the targets for ubiquitination. Arginine substitutions of K553 and K577 not only impaired Mpl ubiquitination and degradation, but also conferred hyperproliferation of cells in response to Tpo. Moreover, ubiquitination is shown to be mediated at least in part by the RING domain-containing E3 ligase c-Cbl. Thus, siRNA-mediated knockdown of c-Cbl, or overexpression of an inactive form of c-Cbl (G379A) mutant, significantly reduced Mpl ubiquitination and turnover. The authors extended their studies to primary cells and found that megakaryocytes and platelets predominantly express the mature form of Mpl, and the Mpl proteins are more stable than those in cell lines with forced Mpl expression. Challenging but rewarding future studies will address to what extent these results apply to Mpl signaling in HSPCs to further elucidate.

c-Cbl docks directly or indirectly through adaptor proteins to phosphorylated receptors upon ligand-induced activation. The fact that JAK2 kinase activity appears dispensable for Mpl ubiquitination raises the question as to how c-Cbl is recruited to the Mpl receptor. Furthermore, when compared with the effects of the Mpl K553 + S727R mutant that fails to be ubiquitinated showed decreased cell-surface turnover in the absence of Tpo. Whether ubiquitination of Mpl also affects its internalization upon Tpo stimulation requires further elucidation.

The present report found that inhibition of JAK2 fails to impede Mpl ubiquitination or degradation. This contrasts with the authors’ previous findings that JAK2 kinase activity is important for Mpl internalization through tyrosine phosphorylation of the Mpl cytoplasmic domain. It will therefore be of great interest to investigate the JAK2-independent signaling pathway that ubiquitinates Mpl. When expressed in BaF3 cells, the Mpl K553 + S727R mutant that fails to be ubiquitinated showed decreased cell-surface turnover in the absence of Tpo. Whether ubiquitination of Mpl also affects its internalization upon Tpo stimulation requires further elucidation.

Nonetheless, accumulating evidence supports a role for c-Cbl in constraining signal transduction by multiple receptors. c-Cbl-deficient mice show a marked expansion in HSC numbers with enhanced repopulating activities. Moreover, c-Cbl-deficient HSCs exhibit accelerated proliferation and are hypersensitive to Tpo in activating Stat5. The work from the present report supports the notion that these observations might reflect elevated and/or sustained Mpl surface expression in Cbl-deficient HSCs. In agreement, c-Cbl mutations found in human myelodysplasias might exert their oncogenic functions by that the ubiquitinated receptor (often mono-ubiquitinated) is recognized by ubiquitin-binding domain-containing adaptor proteins, which enable receptor internalization, post-internalization sorting, and subsequent degradation. Another mechanism exemplified by interferon receptor 1 (IFNAR1) suggests that ubiquitination at specific lysines increases receptor endocytosis by triggering exposure of an “endocytic motif.” In all of the above cases, ligand-induced activation of JAK2 and subsequent posttranslational modification of the receptor is required to dock E3 ligases to the receptor to initiate receptor ubiquitination and the down-regulation process.

Surprisingly, the present report found that inhibition of JAK2 fails to impede Mpl ubiquitination or degradation. This contrasts with the authors’ previous findings that JAK2 kinase activity is important for Mpl internalization through tyrosine phosphorylation of the Mpl cytoplasmic domain. It will therefore be of great interest to investigate the JAK2-independent signaling pathway that ubiquitinates Mpl. When expressed in BaF3 cells, the Mpl K553 + S727R mutant that fails to be ubiquitinated showed decreased cell-surface turnover in the absence of Tpo. Whether ubiquitination of Mpl also affects its internalization upon Tpo stimulation requires further elucidation.

The present report found that inhibition of JAK2 fails to impede Mpl ubiquitination or degradation. This contrasts with the authors’ previous findings that JAK2 kinase activity is important for Mpl internalization through tyrosine phosphorylation of the Mpl cytoplasmic domain. It will therefore be of great interest to investigate the JAK2-independent signaling pathway that ubiquitinates Mpl. When expressed in BaF3 cells, the Mpl K553 + S727R mutant that fails to be ubiquitinated showed decreased cell-surface turnover in the absence of Tpo. Whether ubiquitination of Mpl also affects its internalization upon Tpo stimulation requires further elucidation.

Nonetheless, accumulating evidence supports a role for c-Cbl in constraining signal transduction by multiple receptors. c-Cbl-deficient mice show a marked expansion in HSC numbers with enhanced repopulating activities. Moreover, c-Cbl-deficient HSCs exhibit accelerated proliferation and are hypersensitive to Tpo in activating Stat5. The work from the present report supports the notion that these observations might reflect elevated and/or sustained Mpl surface expression in Cbl-deficient HSCs. In agreement, c-Cbl mutations found in human myelodysplasias might exert their oncogenic functions by that the ubiquitinated receptor (often mono-ubiquitinated) is recognized by ubiquitin-binding domain-containing adaptor proteins, which enable receptor internalization, post-internalization sorting, and subsequent degradation. Another mechanism exemplified by interferon receptor 1 (IFNAR1) suggests that ubiquitination at specific lysines increases receptor endocytosis by triggering exposure of an “endocytic motif.” In all of the above cases, ligand-induced activation of JAK2 and subsequent posttranslational modification of the receptor is required to dock E3 ligases to the receptor to initiate receptor ubiquitination and the down-regulation process.

Nonetheless, accumulating evidence supports a role for c-Cbl in constraining signal transduction by multiple receptors. c-Cbl-deficient mice show a marked expansion in HSC numbers with enhanced repopulating activities. Moreover, c-Cbl-deficient HSCs exhibit accelerated proliferation and are hypersensitive to Tpo in activating Stat5. The work from the present report supports the notion that these observations might reflect elevated and/or sustained Mpl surface expression in Cbl-deficient HSCs. In agreement, c-Cbl mutations found in human myelodysplasias might exert their oncogenic functions by that the ubiquitinated receptor (often mono-ubiquitinated) is recognized by ubiquitin-binding domain-containing adaptor proteins, which enable receptor internalization, post-internalization sorting, and subsequent degradation. Another mechanism exemplified by interferon receptor 1 (IFNAR1) suggests that ubiquitination at specific lysines increases receptor endocytosis by triggering exposure of an “endocytic motif.” In all of the above cases, ligand-induced activation of JAK2 and subsequent posttranslational modification of the receptor is required to dock E3 ligases to the receptor to initiate receptor ubiquitination and the down-regulation process.

Nonetheless, accumulating evidence supports a role for c-Cbl in constraining signal transduction by multiple receptors. c-Cbl-deficient mice show a marked expansion in HSC numbers with enhanced repopulating activities. Moreover, c-Cbl-deficient HSCs exhibit accelerated proliferation and are hypersensitive to Tpo in activating Stat5. The work from the present report supports the notion that these observations might reflect elevated and/or sustained Mpl surface expression in Cbl-deficient HSCs. In agreement, c-Cbl mutations found in human myelodysplasias might exert their oncogenic functions by that the ubiquitinated receptor (often mono-ubiquitinated) is recognized by ubiquitin-binding domain-containing adaptor proteins, which enable receptor internalization, post-internalization sorting, and subsequent degradation. Another mechanism exemplified by interferon receptor 1 (IFNAR1) suggests that ubiquitination at specific lysines increases receptor endocytosis by triggering exposure of an “endocytic motif.” In all of the above cases, ligand-induced activation of JAK2 and subsequent posttranslational modification of the receptor is required to dock E3 ligases to the receptor to initiate receptor ubiquitination and the down-regulation process.
NK-cell education: KIR-S come into play

Thierry Walzer