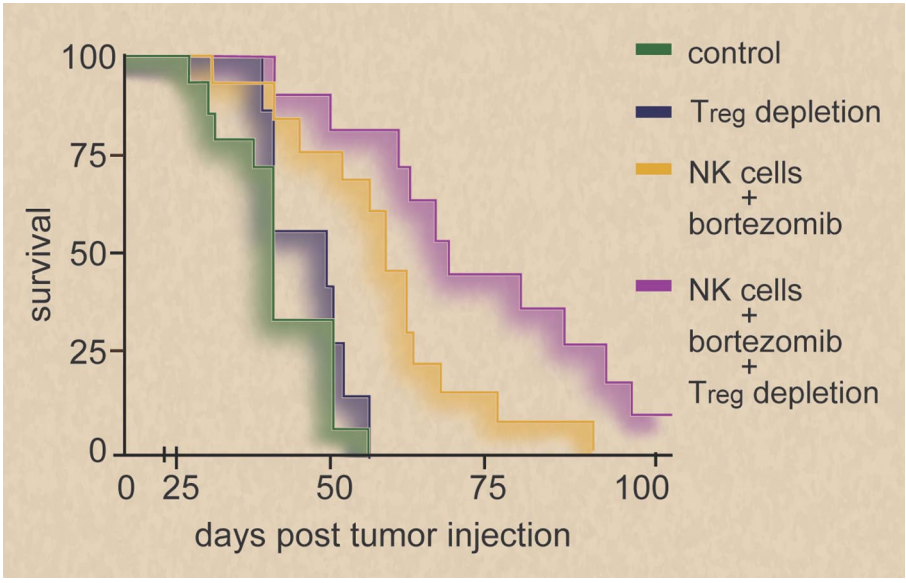


**Survival of BALB/c mice inoculated with RENCA tumor cells is enhanced when mice are treated with bortezomib before adoptive infusion of syngeneic NK cells. Survival is further improved by depletion of regulatory T cells (Tregs) before bortezomib treatment and NK-cell infusion. Professional illustration by Debra T. Dartez.**

NK-cell cytotoxicity.[6,7] NKG2D is an activating immunoreceptor on cytotoxic lymphocytes known to engage MHC I–related surface molecules induced by various forms of cell stress, including genotoxic stress, and it contributes significantly to tumor immunosurveillance.[2] Of note, up-regulation of mouse NKG2DLs H60 and Mult-1 on bortezomib-treated cells was also observed in the present study. However, circumstantial evidence suggests that bortezomib may also impair cellular antitumor immunity: high doses of bortezomib have been shown to inhibit NK-cell reactivity.[6] In the present study, NK-cell transfer was performed 1 day after bortezomib treatment, and thus bortezomib may not have directly affected NK-cell functionality.

Considering the variety of mechanisms by which bortezomib affects both immune and tumor cells, it will be challenging to elucidate in greater detail how bortezomib impacts tumor cell-NK cell interaction in vivo. Nevertheless, independently of the underlying mechanism(s), Lundqvist and coworkers convincingly demonstrate that in vivo application of bortezomib can serve to enhance antitumor reactivity of adoptively infused NK cells. Additional depletion of Tregs further enhanced NK-cell antitumor reactivity. This is in line with previous studies, which demonstrated that Tregs suppress NK cell–mediated antitumor activity, particularly against NKG2DL-positive tumors.[8] It remains to be determined whether a combination of these treatment modalities can also boost NK reactivity in other tumor models and ultimately may be beneficial in a human setting. The present work constitutes one step forward in the attempt to exploit NK cells in the treatment of cancer by combining strategies that relieve NK-cell suppression and enhance tumor cell susceptibility to NK cytolysis.

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

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● ● ● IMMUNOBIOLOGY

Comment on Kang et al, page 6128

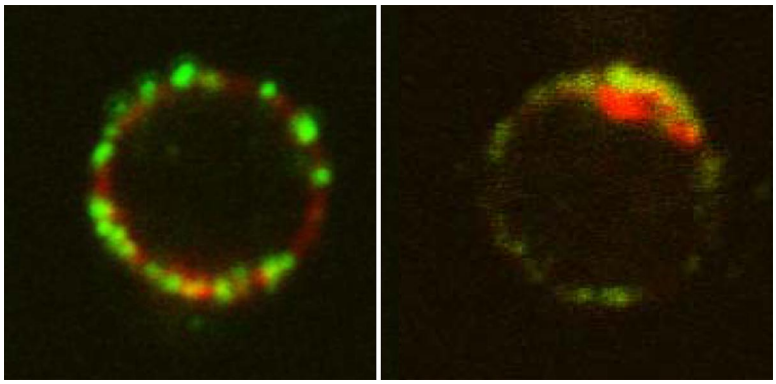
# 4.1R: a FERM player at the immunologic synapse

**Judy L. Cannon** UNIVERSITY OF CHICAGO

In this issue of *Blood*, Kang and colleagues show that 4.1R localizes to the immunologic synapse to negatively regulate T-cell activation. These findings contrast with other FERM family members and show that FERM proteins affect T-cell function through multiple mechanisms.

T-cell activation requires the coordination of signaling events downstream of T-cell receptor (TCR) ligation with intracellular cytoskeletal reorganization, leading to T-cell effector function. Regulators of the actin cytoskeleton have been shown to be critical mediators of the formation of the immunologic synapse and productive T-cell activation.[1] 4.1 proteins belong to a larger FERM (FERM: F-4.1, E-ezrin, R-radixin, M-moesin) family of cytoskeletal regulators, which also include the ERM (ezrin-radixin-moesin) proteins. In T cells, ERM proteins were initially identified as canonical markers of the pole opposite to the immunologic synapse (distal pole complex, DPC),[2,3] and perturbing ERM function leads to defects in T-cell activation.[4] 4.1R is a key player in determining erythrocyte shape and function, but until now, its function in T cells has been unknown. This new study by Kang et al provides the first description of the role of 4.1R in T cells.[5]

Kang and colleagues find that, compared with wild-type T cells, 4.1R-deficient T cells are hyperproliferative. 4.1R deficiency also



**Localization of 4.1R in T cells. See the complete figure in the article beginning on page 6128.**

leads to increased cytokine production by T cells and hyperphosphorylation of 2 key signaling intermediates downstream of T-cell activation, LAT and ERK. 4.1R colocalizes with LAT at the site of TCR activation (see figure) and mediates its effects on T-cell activation through direct association with LAT. 4.1R binding to LAT inhibits LAT phosphorylation by ZAP-70. These results show that 4.1R is a negative regulator of T-cell function through effects on LAT phosphorylation.

Interestingly, while 4.1R and ERM proteins share significant sequence homology, this report shows that 4.1R and ERM family members play fundamentally opposing roles in their regulation of T-cell activation. ERM proteins move negative regulators to the DPC to positively regulate T-cell activation, while 4.1R localizes to the immunologic synapse to negatively regulate T-cell signaling. 4.1R negatively regulates T-cell activation via binding to LAT to inhibit LAT phosphorylation. Like ERM proteins, 4.1R binds both the actin cytoskeleton and signaling molecules downstream of TCR ligation. However, unlike ERM proteins that localize binding partners away from the immunologic synapse via its interaction with the actin cytoskeleton, 4.1R does not appear to move its binding partner LAT in order to regulate its phosphorylation. Instead, 4.1R colocalizes with LAT and ZAP-70 at the synapse. These results show that 4.1R and ERM proteins differ in the mechanism by which each links the actin cytoskeleton and signaling molecules downstream of the TCR. While the precise mechanism by which 4.1R mediates the inhibition of LAT phosphorylation by ZAP-70 remains to be determined, Kang et al contribute to our broader understanding of how actin cytoskeletal regulators can affect T-cell signaling and function.

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

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● ● ● LYMPHOID NEOPLASIA

Comment on Cullion et al, page 6172

# Notch targeting 2.0

**Jon C. Aster** BRIGHAM AND WOMEN'S HOSPITAL

In this issue of *Blood*, Cullion and colleagues add an encouraging chapter to the saga of Notch1 as a therapeutic target in T-ALL.

Notch1 is a member of a family of highly conserved receptors that normally signal by way of a series of ligand-induced proteolytic cleavage events. These permit the intracellular portion of Notch1 (ICN1) to gain access to the nucleus, where it forms a short-lived transcriptional activation complex. The final cleavage that liberates ICN1 is carried out by γ-secretase, a multiprotein complex that also implicated the generation of amyloidogenic peptides from β-amyloid precursor protein in the brains of patients with Alzheimer disease. Interest in Notch1 in T-cell acute lymphoblastic leukemia (T-ALL) has been sparked by the recognition that acquired Notch1 mutations leading to elevated levels of ICN1 are found in the majority of human T-ALLs as well as many murine T-ALL models.[1] Subsequent studies have shown that ICN1 drives the growth of T-ALL cells, in large part due to its ability to up-regulate c-Myc expression and enhance signaling through the PI3-kinase/AKT/mTOR pathway.

The increases in ICN1 levels caused by Notch1 mutations are counteracted by drugs that inhibit γ-secretase, a large number of which are in preclinical development due to the link between γ-secretase and Alzheimer disease. This fortuitous circumstance made Notch1 a very attractive rational therapeutic target, but the first attempt to treat patients with refractory/relapsed T-ALL with an oral γ-secretase inhibitor (GSI) was plagued by both treatment failures and "on-target" gut toxicity.[2] The latter probably resulted from goblet cell metaplasia, as in the absence of Notch signaling the differentiation of epithelial cells lining the small bowel and colon is skewed toward goblet cell fate and away from enterocyte fate. Although these were the early days, the disappointing results of this trial raised serious questions about the future of Notch-directed therapeutics.

The tide may have turned, however, based on 2 recent reports. Earlier this year, Ferrando's group reported that GSI and dexamethasone, long known to be highly active against ALL, have strongly synergistic anti–T-ALL effects in vitro and in murine xenografts.[3] This, in and of itself, is not completely surprising, as Notch1 signaling had been shown through retroviral mutagenesis screens conducted more than a decade ago to protect against dexamethasone-mediated killing of murine T-cell lines.[4] What was entirely unexpected was that dexamethasone also protected mice against GSI-induced gut toxicity by blocking goblet cell development and shifting differentiation back toward enterocyte fate. One critical uncertainty hangs over this remarkable observation, however. The dose of