

Identification of a severe bleeding disorder in humans caused by a mutation in CalDAG-GEFI

In this issue, Canault et al. report for the first time a point mutation in the *RAS guanyl-releasing protein 2* (*RASGRP2*) gene that results in a severe bleeding defect in humans.

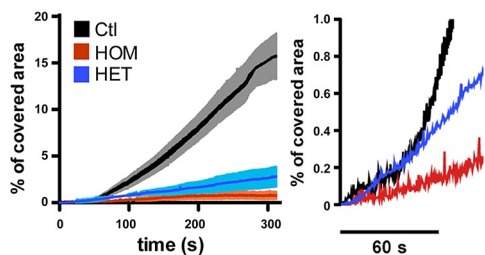
The study of inherited platelet disorders has shed light on the molecular mechanisms of physiologic thrombosis and hemostasis and led to the development of several therapies to prevent pathologic clotting. The *RASGRP2* gene encodes the guanine nucleotide exchange factor (GEF) calcium- and DAG-regulated GEF 1 (CalDAG-GEFI), which is crucial for activation of Rap1, a small GTPase that regulates integrin-mediated activation and granule secretion in platelets and other cells. The function of CalDAG-GEFI has been studied in vitro and in mice—mice lacking CalDAG-GEFI have impaired thrombi formation by platelets as well as a defect in neutrophil function—but to date, no pathological *RASGRP2* mutations have been identified in humans.



Insight from
William Muller

Now, Canault et al. have investigated the cause of an inherited platelet disorder in three siblings from a consanguineous marriage that are all affected by a severe bleeding disorder. Whole genome sequencing was used to identify a mutation (cG742T) in the *RASGRP2* gene. This mutation reduces Rac1 GTP binding (secondary to decreased Rap1 activation), impairing the ability of platelets to aggregate in response to a variety of stimuli, to form thrombi under flow, and to undergo normal spreading. Heterozygotes also have platelets that fail to adhere normally under flow and have a spreading defect (see figure) but they do not suffer from bleeding because their platelets aggregate normally. The functional deficiency induced by the mutation was confined to platelets and megakaryocytes with no obvious alteration in leukocytes. This is probably because other GEFs in leukocytes are able to activate Rap1 or, as the authors speculate, that CalDAG-GEFI with this point mutation is still able to function in leukocytes. In fact, even in platelets, the aggregation defects can be overcome by high doses of agonists in vitro, so while CalDAG-GEFI may be the preferred way for platelets to activate Rap1, it is clearly not the only way.

Adhesion of calcein-AM-labeled platelets to fibrillar collagen under flow (750 s^{-1}) from a homozygous (HOM), heterozygous (HET), and healthy (Ctl) subject. Percentage of covered area was assessed over 300 seconds (left). The initial 60 seconds are magnified (right).



Mutations in CalDAG-GEFI had previously been reported to be responsible for leukocyte adhesion deficiency type III, which was later found to be caused by the absence of kindlin-3. To demonstrate that the mutation in CalDAG-GEFI is truly responsible for the phenotype, the authors showed that cells transfected with wild-type *RASGRP2* can activate Rap1, whereas those transfected with the mutation found in the affected siblings cannot.

Looking at these data from a different perspective, the presence of a single normal allele is sufficient to prevent bleeding, but not platelet adhesion to collagen. This suggests that partial inhibition of CalDAG-GEFI might be a novel and potentially safe therapeutic target to prevent thrombosis without causing bleeding—a holy grail of vascular medicine and surgery.

Canault, M., et al. 2014. *J. Exp. Med.* <http://dx.doi.org/10.1084/jem.20130477>.

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A gallon of sweat to make a pint of blood



Insight from
Margaret Goodell

From multipotent stem cells, the hematopoietic system undergoes continuous differentiation to generate a remarkable array of mature cells throughout life. These hematopoietic stem cells (HSCs) hold promise for cell therapy through bone marrow transplantation, as well as serving as a paradigm for stem cell biology, so they are the focus of intense investigation. However, studying these cells is impeded by their scarcity—they are present at around 1 per 50,000 bone marrow cells and there is still no reliable method to expand them in vitro.

Gazit et al. analyzed data from hundreds of microarrays to identify sets of genes uniquely expressed in individual hematopoietic cell types. They then mined the HSC-specific gene list to identify candidate markers for HSCs that might be useful to study these rare cells. They found that when mCherry was knocked-in to the locus of one of these genes, *Fgd5*, red fluorescence could be used to purify virtually all of the HSCs, as rigorously tested by both phenotypic and functional criteria. Moreover, they inserted a tamoxifen-inducible Cre recombinase into the locus, offering the possibility of using this mouse line for HSC-specific deletion or lineage tracing.

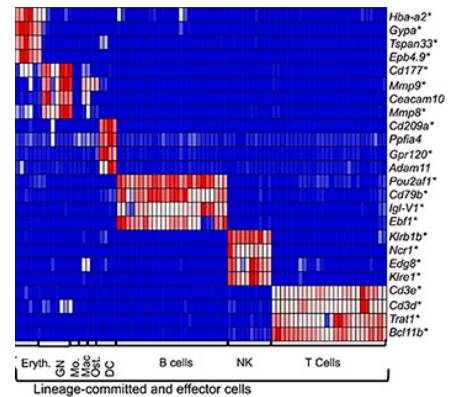
There are a number of genes that are highly expressed in HSCs, but most of these are shared with their immediate downstream progeny. Thus, establishing these *Fdg5*-reporter mouse lines and demonstrating the high and specific expression pattern that can be achieved with insertions into this locus is a significant contribution to the field. The power of the study lies in the utility of the mouse lines, but it also provides a benchmark for such reporters in the future.

This strategy could also be exploited to generate other lineage-specific reporter or deleter mouse lines. The bioinformatics approach generates sets of genes that can serve as what my lab previously termed “cellular fingerprints” that can be used as cell-type identifiers, and some may confer unique cellular properties that merit functional investigation.

Whereas General George S. Patton said, “A pint of sweat saves a gallon of blood,” here we have a valiant effort, undoubtedly representing gallons of sweat, that will be useful for making a pint or more of blood.

Gazit, R., et al. 2014. *J. Exp. Med.* <http://dx.doi.org/10.1084/jem.20130428>.

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“Fingerprints” of different hematopoietic lineages with clustering showing high expression (red) of small numbers of genes that are not detected (blue) in most other cell types.

LUBAC: A new function in immunity

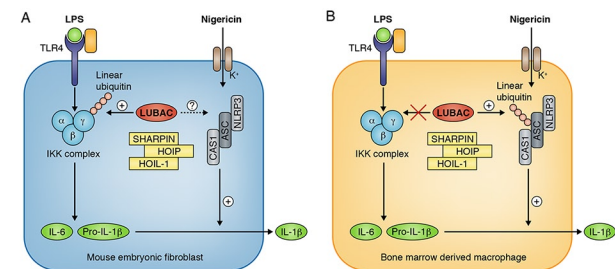
In this issue, Rodgers et al. report a new substrate of the linear ubiquitin assembly complex (LUBAC) and a new function of this complex in mice. Linear ubiquitination has been implicated as a key regulator of the NF- κ B pathway. LUBAC consists of three components: HOIL-1, HOIP, and Sharpin. It adds linear ubiquitin to NEMO, driving the formation of a TNF-R–IL-1R signaling complex and leading to the nuclear translocation of NF- κ B. Linear ubiquitination thus plays an important role in the regulation of immunity via the transduction of NF- κ B-dependent signals, such as those mediated by the TLR/IL-1 and TNF receptor families.

Rodgers et al. now show that LUBAC is essential for NF- κ B activation in mouse embryonic fibroblasts (MEFs) stimulated with LPS, but dispensable in bone marrow–derived macrophages (BMDMs). They also demonstrate that the activation of caspase 1 by the complex formed by NLRP3 (NLR family, pyrin domain containing 3, encoded by *CIAS1*) and ASC (apoptosis-associated speck-like protein containing CARD) is dependent on LUBAC in LPS-primed, nigericin-stimulated macrophages. Using different approaches, they show that the formation of the NLRP3–ASC complex in response to nigericin stimulation involves the linear ubiquitination of ASC. Linear ubiquitination is required for the transformation of pro-IL-1 β into secreted and bioactive IL-1 β by caspase 1. HOIL1-deficient mice are protected against LPS toxicity in vivo.

This work reveals new aspects of linear ubiquitination in mice. First, it reveals a cell specificity of LUBAC in the activation of NF- κ B. It shows, for the first time, that LUBAC is dispensable for NF- κ B activation in certain cell types, although the mechanism involved remains elusive. Second, it shows that LUBAC plays an essential role in the activation of caspase-1 upon stimulation of the NLRP3–ASC inflammasome. This finding extends the cellular function of LUBAC and linear ubiquitination to processes independent of NEMO and the NF- κ B pathway.

If extended to human cells, these findings would provide a novel angle from which to tackle the pathogenesis of the autoinflammation, immunodeficiency, and amylopectinosis observed in patients with inherited HOIL-1 deficiency. Surprisingly, these patients also display a cell-specific pattern of response to IL-1 β , with hyper-responsive monocytes and hyporesponsive fibroblasts. LUBAC-dependent NLRP3 activation would also pave the way for the targeting of linear ubiquitination in patients with autoinflammatory diseases due to mutations of *NLRP3*, such as cryopyrin-associated periodic syndrome (CAPS) and Muckle-Wells syndrome.

Rodgers, M.A., et al. 2014. *J. Exp. Med.* <http://dx.doi.org/10.1084/jem.20132486>.



Cell specificity of linear ubiquitin dependent activation of IKK complex and inflammasome in mice. In mouse embryonic fibroblasts (A), expression of IL-6 and pro-IL-1 β upon LPS is dependent of LUBAC activity. In BMDM (B), expression of IL-6 and pro-IL-1 β upon LPS is independent of LUBAC activity but IL-1 β secretion is dependent of linear ubiquitination of ASC.



Insight from Bertrand Boisson (left) and Jean-Laurent Casanova (right)

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