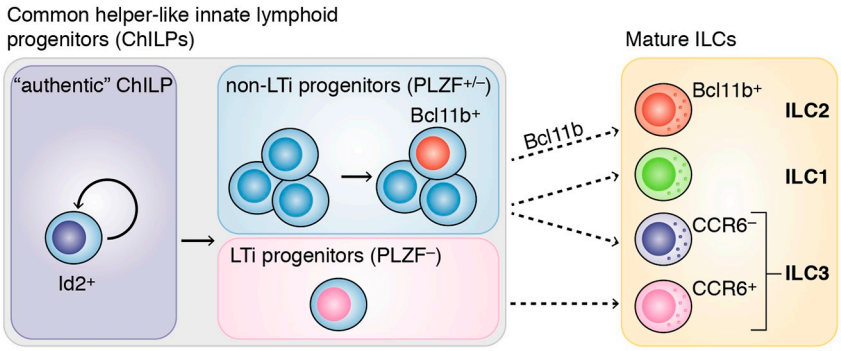
# Bcl11b drives the birth of ILC2 innate lymphocytes

Insight from Chao Zhong (left) and Jinfang Zhu (right)

Effective immune responses to pathogens require the activation and differentiation of both innate and adaptive lymphocytes, such as innate lymphoid cells (ILCs) and T cells, respectively. ILCs consist of distinct helper-like subsets representing innate versions of $CD4^+$ T helper subsets, whereas natural killer cells represent the innate counterpart of $CD8^+$ cytotoxic T cells. By producing effector cytokines, T helper subsets and helper-like ILCs are also involved in the pathogenesis of many inflammatory diseases. Strikingly, the development of ILCs and T cells seems to depend on a shared transcriptional network. For example, GATA3 and Tcf7, which play essential roles during T cell development, are also indispensable for the development of multiple ILC subsets. Bcl11b is another critical component of a T cell lineage fate-specifying transcriptional network. In this issue, Yu et al. and Walker et al. independently describe the role of Bcl11b in specifying ILC2 development.

One would have expected that, similar to GATA3 and Tcf7, Bcl11b would be critical for the development of multiple ILC lineages. However, the two papers in this issue report that Bcl11b appears to be essential for ILC2 development only. Consequently, Bcl11b deficiency leads to impaired immune responses to either papain treatment, influenza virus infection (Yu et al.), or *Nippostrongylus brasiliensis* helminth infection, without affecting the ability of mice to clear bacterial *Citrobacter rodentium* infection (Walker et al.). The authors show that Bcl11b is expressed in mature ILC2s as well as in a subset of $Id2^+$ common helper-like innate lymphoid progenitors (ChILPs); Walker et al. but not Yu et al. also show that a proportion of other mature ILC subsets, such as $CCR6^-$ ILC3s, express Bcl11b. The discrepancy in Bcl11b expression by mature ILC3s between these two studies has not been resolved. Nevertheless, the Bcl11b-expressing ChILPs only give rise to ILC2s but not other ILCs, suggesting that the previously identified ChILPs contain a mixture of distinct committed ILC progenitors. This is consistent with another report in *Nature* suggesting that within the ChILP compartment, the $PLZF^+$ progenitors are largely committed to specific ILC subsets and fail to generate lymphoid tissue inducers, which represent another subset of ChILP-derived ILCs.

Both reports also show that Bcl11b-expressing ILC2 progenitors express high levels of GATA3. However, other studies have reported that $GATA3^{hi}$ progenitors also give rise to other ILCs. In addition, GATA3 is constantly required for the maintenance of ILC2s, whereas inducible deletion of Bcl11b in mature ILC2s does not result in loss of functional ILC2s. Thus, Bcl11b either functions independently of GATA3 or collaborates with GATA3 during ILC2 development, but the detailed mechanism requires further investigation. Moreover, it is not known whether Bcl11b is critical for ILC2 development in humans.




**Scheme of helper-like innate lymphoid cell (ILC) development. LTi: hematopoietic lymphoid tissue inducer lineage.**

It is intriguing that Bcl11b, which is critical for the development of all T cell subsets, appears to be dispensable for the development of ILC1s and ILC3s. Does this mean that T cells have preferentially adopted the ILC2 transcriptional program during their early development in the thymus? If so, one would predict that T cell progenitors, particularly $CD4^-CD8^-$ thymocytes, might display certain features of ILC2s. This idea is consistent with the notion that T cells carry an endogenous Th2-prone program that makes Th2 lymphocyte differentiation a default cellular fate.

The studies of Yu et al. and Walker et al. not only identify Bcl11b as an ILC2 lineage-specifying factor, they also provide important general insights into the mechanisms of ILC development. Furthermore, this work raises the possibility that dysregulation of Bcl11b in ILC progenitors may contribute to human diseases involving ILC2 function.

**Acknowledgments:** C. Zhong and J. Zhu are supported by the Division of Intramural Research (DIR), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), USA.

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Walker, J.A., et al. 2015. *J. Exp. Med*. http://dx.doi.org/10.1084/jem.20142224
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The Journal of Experimental Medicine

# Pentraxin 3 innately preps damaged tissue for wound healing

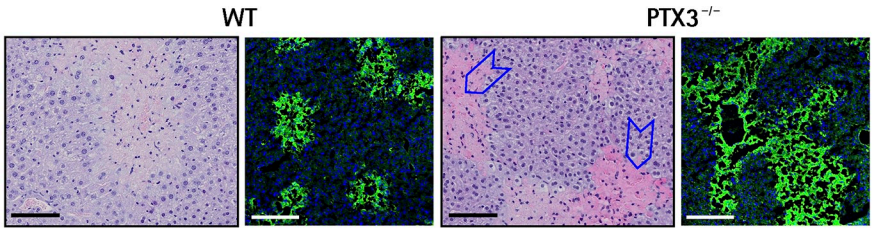
Insight from Rejane Rua (left) and Dorian McGavern (right)

The mechanisms regulating wound injury and tissue repair involve complex enzymatic cascades and inflammatory processes, and new components are still being discovered. In this issue, Doni et al. report that the innate immune pattern recognition molecule (PRM), pentraxin 3 (PTX3), has a novel and nonredundant role in the orchestration of tissue repair and remodeling during wound healing.

The innate immune system is equipped with an array of PRMs that detect conserved microbial motifs and mobilize early defense against foreign invaders. This pattern recognition system is multifunctional and can also detect endogenous alarmins that are released from damaged tissue. Detection of alarmins, such as extracellular matrix components and nucleic acids, is known to facilitate wound-healing reactions, in part through the induction of inflammation. PTX3 is a secreted, fluid-phase PRM that is rapidly produced by stromal and myelomonocytic cells. Its functions include opsonization, regulation of the complement cascade, and antimicrobial resistance, among others.

Using four different models of tissue damage (including skin damage and liver and lung sterile tissue injury), the authors showed that PTX3 deficiency caused marked alterations in wound healing, including excessive fibrin accumulation, enhanced collagen deposition, epithelial hyperplasia, and defective formation of mature tissue. Interestingly, PTX3 expression was associated with wound-healing cells such as macrophages within the lesion, suggesting a role for this glycoprotein in tissue remodeling. Consistent with this theory, *Ptx3*$^{-/-}$ macrophages were less efficient at degrading fibrin matrix in vitro. Fibrin is a provisional extracellular matrix protein that is transiently deposited after tissue injury, but must be degraded for proper tissue remodeling and scar formation to occur.

To understand the link between PTX3 and fibrin matrix degradation, the authors conducted an elegant series of experiments involving tissue acidification showing that PTX3 serves as a molecular bridge between fibrin and plasminogen and promotes fibrinolysis. So, PTX3 appears to have an important role in fibrin breakdown, and tissue acidification serves as the switch that promotes this wound-healing activity following injury.




Hematoxylin–eosin and confocal images (green = fibrin; blue = nuclei) of fibrin in liver sections of wild-type (left) and Ptx3$^{-/-}$ (right) mice. Blue arrowheads indicate deposits of fibrin in necroinflammatory areas in the lobular spaces 48 hours after chronic injury. Bars, 100 µm.

These data demonstrate that PRMs such as PTX3 can have different functions that depend on the tissue milieu and the manner by which they are activated. In fact, another recently published study from this same group in *Cell* showed that PTX3 also has anti-oncogenic properties linked to its ability to quench complement activation and reduce tumor infiltration by macrophages. We now know that induction of this protein has the potential to accelerate wound healing, ward off microbes, and suppress tumor growth. Given its ability to coordinate such diverse immune activities, PTX3 is a relevant and interesting target for therapeutic manipulation in damaged tissue as well as in a variety of diseases. Future avenues of research may uncover other unexpected roles for this fascinating molecule.

Bonavita, E., et al. 2015. *Cell.* 160:700–714.

Doni, A., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem. 20141268

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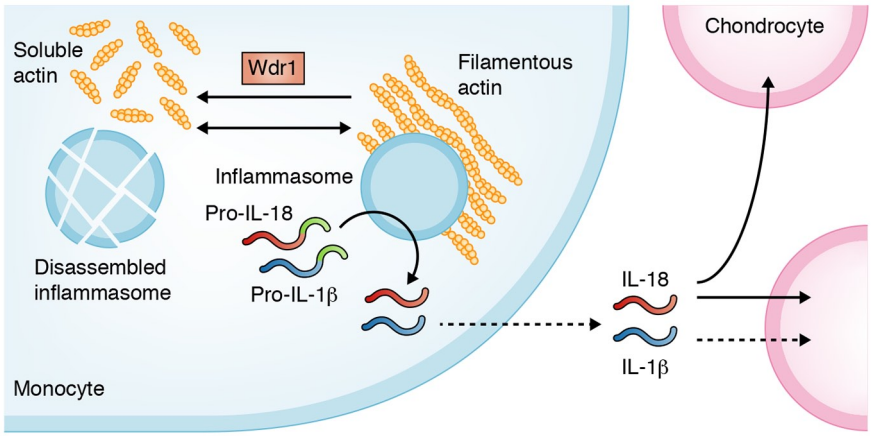
# Inflammation on the move

Insight from Niels Borregaard

Inflammation is a major cause of morbidity, as illustrated by debilitating conditions such as rheumatoid arthritis and inflammatory bowel diseases. IL-1β and TNF are key mediators of inflammation, and agents that neutralize their effects have provided relief for millions of patients, but we may have yet more to discover about inflammation.

In this issue, Kim et al. show that mice with a hypomorphic mutation of *Wdr1* (i.e., a mutation that results in underproduction of an otherwise normal product) develop inflammation caused by IL-18.

This is interesting for several reasons. To the cell biologist, Wdr1 is a protein that interacts with filamentous actin. It is the murine homologue of human actin-interacting protein 1 (AIP1). The dynamics of cellular actin is crucial in cell biology and underlies fundamental cellular functions such as cell division, movement, and phagocytosis. Actin oscillates between filamentous actin and soluble actin. Cofilin binds




**Inflammasomes assemble on filamentous actin and generate IL-1β and IL-18. Wdr1 accelerates dissolution of filamentous actin and of the inflammasome. Mice hypomorphic for Wdr1 have more filamentous actin and their monocytes generate IL-1β and IL-18. The mice develop inflammation of cartillage tissue driven by IL-18, not by IL-1β.**

filamentous actin and severs the filaments, causing these to dissemble, yielding soluble actin. Wdr1 in mice and AIP1 in humans bind the cofilin/filamentous actin complex and energize cofilin's ability to sever the filaments. Null mutations of *Wdr1* are embryonic lethal; mice with hypomorphic mutations of *Wdr1* are viable but have thrombocytopenia and their neutrophils have crawling defects. These mice also develop autoinflammation.

The inflammasome is to immunologists what actin is to cell biologists. Intracellular sensors, such as the NOD-like receptors (NLRs) and the absent in melanoma 2 (AIM2)–like receptors (ALRs), sense damage or danger signals and recruit ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain). In this complex, ASC generates caspase-1 by proteolytic cleavage of procaspase-1. Caspase-1 in turn cleaves pro–IL-1β and pro–IL-18, generating the potent proinflammatory cytokines IL-1β and IL-18 that are secreted from the cell.

Kim et al. report on a significant amount of work involving a comprehensive analysis of this novel mouse model. They show that myeloid cells from hypomorphic *Wdr1* mutants have more filamentous actin than wild-type mice with spontaneous activation of ASC and caspase-1; the cells generate huge amounts of IL-1β and IL-18 as expected. This points to a critical role for actin dynamics in controlling the inflammasome. Surprisingly, the mice suffer inflammation of cartilage tissue rather than a generalized inflammation, and the inflammation is caused not by the elevated IL-1β but by IL-18. Furthermore, monocytes and not macrophages, neutrophils, or dendritic cells are responsible for the production of IL-18. The authors observed massive infiltration of neutrophils, but it was monocytes that migrated to the tissue, produced IL-18 locally, and initiated the inflammation.

This study provides a cellular framework for understanding the role of IL-18 in inflammation. This is a novel and intriguing paper that implicates the actin-depolymerizing cofactor Wdr1 in the regulation of the pyrin inflammasome and IL-18 production by monocytes. These results add to the growing literature on the role of actin polymerization in inflammation and may have translational implications for the therapy of patients with autoinflammatory conditions.

Kim, M.L., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20142384

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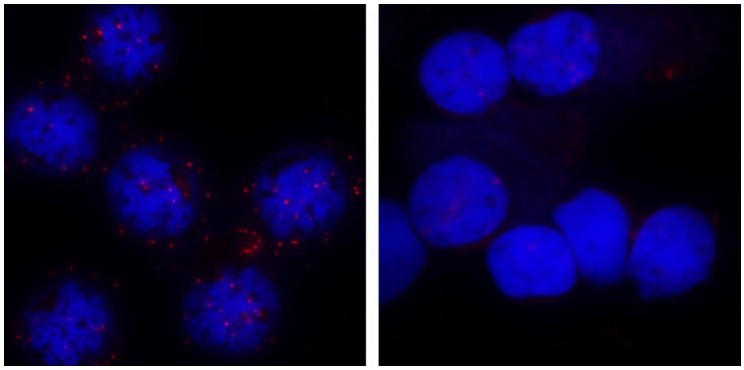
## Augmenting NF-κB in poor-risk CLL: A general paradigm for other cancers?

**Insight from David Tuveson (left) and Kanti Rai (right)**

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder of B lymphocytes. It has an extremely variable clinical course. Some patients have a rather indolent course, whereas others are known to have a rapidly progressive disease. Most patients die from causes related to CLL that can be due to bone marrow failure, infection, or transformation to a high-grade lymphoma. Clinical stratification of CLL has revealed that a subset of patients with poor prognosis harbor cytogenetic alterations and lack mutations at the immunoglobulin locus. Therefore, the development of additional molecular biomarkers for patients at high risk for early lethality from CLL could help direct their care toward enrollment in clinical trials of promising experimental approaches such as inhibitors of BCL2 or BCR signaling or CD19 chimeric antigen receptor T cells (which have been shown to eradicate CLL in patients who have failed other approaches). In this issue, Mansouri et al. report that somatic mutations in the *NFKBIE* gene occur in 7% of poor prognosis patients, and this may be a common mechanism contributing to disease progression by sustaining the survival of malignant CLL cells.

The NF-κB pathway is ubiquitously activated in CLL cells by cell surface receptor signaling cascades through the B cell receptor and Toll-like receptors, so this identification of additional mutational events that reinforce NF-κB signaling appeared biochemically redundant. *NFKBIE* encodes the IκBε protein, a potent and seemingly stoichiometric inhibitor of the IκB kinase (IKK) in CLL cells as demonstrated by Mansouri et al. Surprisingly, even partial depletion of *NFKBIE* with RNA interference in cell culture was sufficient to activate IKK and promote NF-κB transcriptional activation, consistent with the findings that the *NFKBIE* mutations are heterozygous in CLL samples and that these samples had modest increases in NF-κB signaling. Whether the most recurrent *NFKBIE* mutation identified in this work (a 4-bp deletion in exon 1 that resulted in a frame shift and potentially expressed truncated protein) has neomorphic oncogenic functions besides the inability to inhibit IKK remains unknown.



**Heterozygous gene dosage for *NFKBIE* is sufficient to disrupt NF-κB regulation. Fluorescent microscope images of the interaction between IκBε and the transcription factor p65 in cells from CLL patients that are either wild type for *NFKBIE* (left) or heterozygous for *NFKBIE* deleted (right). Blue color indicates cell nuclei; each red dot represents a single interaction.**

More broadly, the finding of heterozygous *NFKBIE* mutations as a marker of poor prognosis raises the possibility that similar mutations or heterozygous loss of *NFKBIE* caused by genetic deletion or epigenetic silencing may also further sculpt NF-κB signaling and thereby promote the progression of more common malignancies. Unfortunately, most of the publicly available tumor genomes include a significant amount of contaminating normal cells, and therefore haploid loss of *NFKBIE* may not be appreciated. Single nuclei sequencing of neoplastic cells isolated freshly from tumors will address this and other issues of potential haploidy in cancer by unambiguously determining the cancer genome and concurrent genetic events. For CLL patients, the finding of the *NFKBIE* mutations in high-risk patients refocuses our attention on developing NF-κB inhibitors to use in tandem with other approaches and for considering experimental clinical trials for such patients.

Mansouri, L., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20142009

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