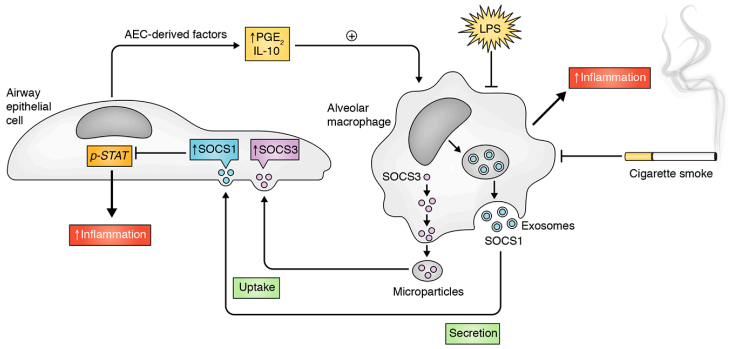
Special delivery: How macrophages get the message across

Insight from Clare Lloyd

Communication between cells is vital for maintenance of immune homeostasis and is also required for rapid and effective immune responses. Nowhere is this balance of activity more important than at mucosal surfaces such as the respiratory tract, where resident cells must ignore potentially antigenic inhaled material while responding swiftly to clear viruses and bacteria. In this issue, Bourdonnay et al. describe a novel form of intercellular communication that helps to explain how alveolar macrophages and epithelial cells maintain pulmonary homeostasis.

The function of SOCS proteins is to constrain JAK-STAT signaling and repress inflammatory reactions. We do not usually expect to find these intracellular signaling molecules in extracellular compartments, but Bourdonnay et al. showed that lung-resident alveolar macrophages (AMs) were able to secrete SOCS1 within exosomes and SOCS3 within extracellular microparticles. Uptake of these extracellular vesicles by alveolar epithelial cells (AECs) allowed SOCS1 and -3 to suppress STAT signaling following activation by cytokines. The authors demonstrated that this novel extracellular transfer system was promoted by IL-10 and PGE_2 and inhibited by LPS. The phenomenon was demonstrated in a number of model systems including both rats and mice and also, importantly, in human AMs. Moreover, secretion was not simply dependent on expression of SOCS3, because fibroblasts expressed high levels of SOCS3 but did not secrete it.



SOCS proteins are secreted in cross-talk between AMs and AECs for control of inflammation in the lungs.

Any in vitro finding needs to be robustly tested in vivo to demonstrate a physiologic effect. The authors showed that SOCS3 was abundant in bronchiolar lavage (BAL) even in naive mice; SOCS3 levels increased after administration of PGE_2 to the lungs and decreased after pulmonary delivery of LPS. Moreover, SOCS3 was retrieved from BAL from healthy human volunteers. Interestingly, exposure to cigarette smoke reduced levels of both SOCS1 and -3 in both mice and humans; so, smoking could lead to unchecked JAK-STAT activation, thereby contributing to inflammatory responses.

Although it remains to be seen whether this particular form of epithelial–macrophage communication operates during respiratory infection, these data are important because they shed some light on the complex interactions that maintain homeostasis within the lung. It may be possible to exploit this system in order to down-regulate inflammation following infection and ultimately facilitate the restoration of immune homeostasis.

Bourdonnay, E., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141675.

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DUSP4 phosphatase puts the brakes on DLBCL

Insight from Robert Rickert

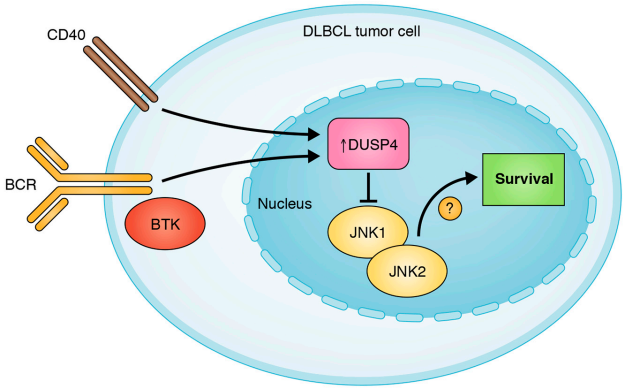
Diffuse large B cell lymphoma (DLBCL) is a heterogeneous B cell malignancy that can be stratified into three molecular subtypes using gene expression profiling: activated B cell–like DLBCL (ABC-DLBCL), germinal center B cell–like (GCB-DLBCL), or mediastinal B cell–like. In this issue, Schmid et al. report that aberrant methylation results in loss of DUSP4 expression in most (75%) DLBCL cases, suggesting that DUSP4 functions as a novel tumor suppressor.

The dual-specificity phosphatases (DUSPs) are a family of 25 phosphatases that can dephosphorylate both tyrosine and serine/threonine residues. A primary substrate of the DUSPs are the MAP kinases, which are inhibited by dephosphorylation of a critical motif in the kinase domain.

In this study, the authors first screened for potential DLBCL tumor suppressors that could be epigenetically silenced by DNA methylation. They used genome-wide DNA methylation analyses coupled with gene expression profiling after pharmacological DNA demethylation and identified *DUSP4* as one of eight genes impairing cell survival when ectopically expressed in DLBCL lines. Of the MAP kinase targets of DUSP4, they focused on JNK1/2, because these kinases, unlike ERK1/2 and p38, were dephosphorylated in both ABC-DLBL and GCB-DLBCL. Further experiments showed that DUSP4 acts on nuclear JNK and that a small molecule JNK inhibitor targets the survival of selected DLBCL lines. This finding was validated in xenograft experiments, showing tumor reduction in animals receiving the JNK inhibitor. Interestingly, an additive effect was observed

in ABC-DLBCL lines after coinhibition with a JNK inhibitor and ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, suggesting that JNK and BTK are synthetic lethal targets downstream of the BCR. Lastly, the absence of DUSP4 negatively correlated with patient survival in all subtypes of DLBCL.

This study illustrates the power of systematic epigenetic approaches in understanding dysregulated gene expression. DUSP4 is a relatively understudied target in cancer, whereas its substrates, the MAP kinases, are well-known regulators of cell fate. Additional investigations will be required to determine the relevance of the DUSP4/JNK1/2 axis in the stratification and treatment of other lymphoma types that also lack DUSP4. Moreover, the physiologic targets of JNK that promote lymphoma cell viability remain to be determined. The combinatorial effects of BTK and JNK inhibitors are intriguing and worthy of further study. In particular, it will be important to attribute the relative efficacy and synergy of these inhibitors to particular receptors/pathways versus prevalent off-target effects.



B cell activation (e.g., via BCR and CD40 engagement) induces the expression of DUSP4, which negatively regulates JNK1/2 by dephosphorylation, resulting in apoptosis. Inhibition of BTK and JNK1/2 act synergistically to promote apoptosis of lymphoma cells.

Schmid, C.A., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141957.

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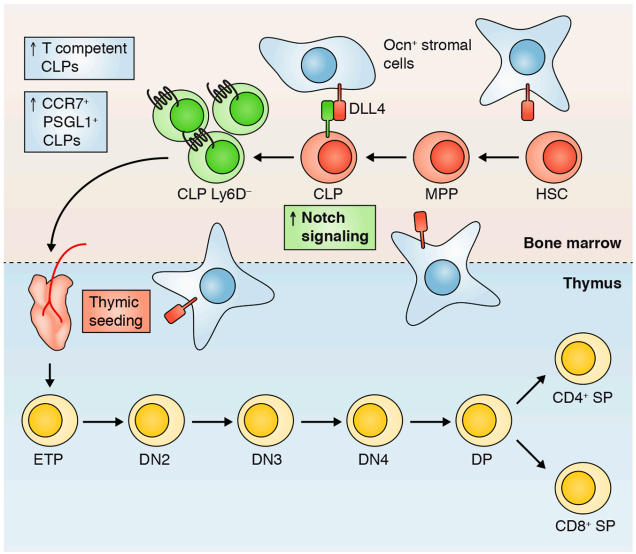
T cell development runs marrow deep

Insight from Juan Carlos Zúñiga-Pflücker

Notch signaling induces and supports T cell lineage differentiation in the intrathymic microenvironment. Although the antigen-independent specification of the B cell lineage is known to occur in the bone marrow, it is not clear whether engagement of Notch1 receptors on hematopoietic progenitors is required to initiate T cell lineage development.

In this issue, Yu et al. assessed the influence of bone marrow stromal cells on the generation of thymic-seeding progenitors (TSPs). The authors took advantage of several in vivo mouse models to tackle this question and showed that osteoblasts appear to influence the generation of TSPs through expression of DLL4, thus providing an initial Notch signal within the bone marrow and initiating T cell lineage specification. When osteoblasts were destroyed, or their ability to express DLL4 was compromised, lower numbers of putative TSPs (defined as Lineage$^-$Sca$^+$cKit$^+$IL7R$^+$ Thy1.2$^-$Ly6D$^-$ cells) were detected in the bone marrow. This also led to a reduced number of early thymocyte progenitors (ETPs, typically defined as CD4$^-$CD8$^-$CD44$^+$CD117$^+$(ckit$^+$) CD24$^-$CD25$^-$ cells) and other subsets of developing thymocytes, including reduced numbers of mature CD4$^+$ and CD8$^+$ T cells.



Model of osteocalcin-positive cell control of T lymphopoeisis. Osteocalcin-positive osetoblasts and osteocytes (and, potentially, stromal cells in the thymus) express the Notch ligand DLL4, which binds to Notch receptors on CLPs. The presence of osteocalcin-positive mature osteolineage cells is necessary for adequate production of T-cell component CLPs and their expression of CCR7 and PSGL1.

These results strongly point to a prethymic defect in the generation of several lymphocyte progenitor subsets. However it is curious, and somewhat unexpected, to note a decreased number of intrathymic precursor cells, such as later CD4$^-$ CD8$^-$ subsets; due to their strong proliferative capacity, these cells typically compensate for defects associated with decreased progenitor cell numbers. A cursory analysis of genes expressed by immature thymic epithelial cells (TECs), or subsets of TECs, points to the possibility that the osteocalcin gene promoter (used to drive the loss of osteoblasts or loss of DLL4 expression) may also be expressed by these cells. Additionally, it is well established that reduced Notch signaling during T cell development can lead to severe blocks in differentiation and cell survival of early thymocyte subsets. So, although the authors conclude that the "data demonstrate the role of Notch ligands

expressed by osteocalcin-positive cells as the basis for the decrease in T lymphopoiesis," this may not entirely reside with the bone marrow and could potentially be due to osteocalcin-positive stromal cells within the thymus.

Nevertheless, it is clear that Notch signaling is important for T-lineage specification prior to thymic entry, and that this is mediated by bone marrow stromal cells. This knowledge will likely influence how we view the branching points of B and T lymphopoiesis, as well as the development of therapies to alleviate deficiencies in immune cell reconstitution.

Yu,V.W.C., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141843.

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Going with the flow: How shear stress signals the emergence of adult hematopoiesis

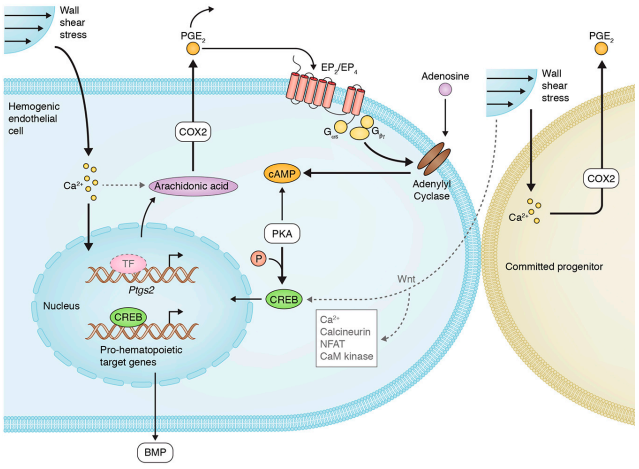
Insight from David Traver

It has long been noted that the initiation of definitive hematopoiesis occurs just after blood circulation begins during embryonic development. In this issue, three companion papers demonstrate that this is not just a coincidence and describe a mechanism by which the shear stress of blood flow triggers a cascade of molecular events leading to the birth of hematopoietic stem cells (HSCs). In all vertebrate animals studied to date, HSCs arise from the transdifferentiation of hemogenic endothelial cells comprising the floor of the dorsal aorta. Although the complete set of signals regulating this unusual event remain to be described, an emerging theme is that flow shear stress acts as a key inducer of the endothelial-to-hematopoietic transition (EHT). The molecular mechanisms underlying shear stress sensing, however, have remained poorly defined.

The three papers published in this issue describe how blood flow sensing leads to the production of several factors that synergize towards HSC emergence from arterial endothelium. Interestingly, each factor serves to increase cyclic adenosine monophosphate (cAMP) production to up-regulate the activity of the cAMP response element–binding protein (CREB) via protein kinase A (PKA) intermediates. Each paper shows that activation of CREB, through the functions of different G protein–coupled cell surface receptors, leads to the transcription of a variety of genes that each play key roles in HSC emergence.

Previous studies have demonstrated that shear stress across endothelial surfaces leads to the production of extracellular ATP, which is rapidly converted to adenosine. Jing et al. show that binding of adenosine to the A_{2b} adenylyl cyclase-stimulatory receptor on vascular endothelium leads to up-regulation of the cAMP–PKA–CREB pathway to activate the CXCL8 cytokine gene, which is in turn required for HSC development. Importantly, the authors show that this novel requirement for adenosine is conserved across vertebrate evolution through comparative experiments in the zebrafish and mouse embryo. Kim et al. show that flow-induced cAMP–PKA–CREB signaling also leads to secretion of bone morphogenetic proteins (BMPs) to promote HSC emergence. These authors suggest that CREB function leads to production and secretion of BMP2 and BMP4, which bind to type I BMP receptors on hemogenic endothelium to promote EHT. Finally, Diaz et al. demonstrate that blood flow promotes the synthesis and release of prostaglandin E_2 (PGE_2) by vascular endothelium. This also leads to stimulation of the cAMP–PKA–CREB signaling axis via the EP_2/EP_4 receptors to activate several master regulators of the hematopoietic program. Importantly, these authors show that provision of flow conditions to cultured E9.5 murine tissues that normally lack HSC activity confers long-term, multilineage engraftment potential.

That ectopic provision of flow conditions or PGE_2 analogues can confer precocious HSC potential to cultured precursors suggests that this strategy could help in vitro efforts to instruct HSC fate from human pluripotent cells. Despite decades of efforts, this feat has not yet been achieved. Collectively, these studies suggest that exposing cultured cells to flow, or experimentally modulating the cAMP–PKA–CREB signaling pathway, may be a key step toward achieving this important milestone of regenerative medicine.



Model of flow-induced signal transduction events. Shear force induces signaling events that are orchestrated by the cAMP–PKA–CREB pathway to produce PGE_2, adenosine, and BMP, which drive HSC production.

Diaz, M.F., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20142235.
Jing, L., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141528.
Kim, P.G., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141514.

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