the intermediate stages of DC subset development from hematopoietic stem cells? What is the nature of the LC precursors in human peripheral blood potentially giving rise to LCs under inflammatory conditions? What are the inflammatory signals that may convert such a human blood–derived precursor into an LC?

Human peripheral blood contains monocyte subsets as well as minute populations of circulating DCs, among them myeloid cell–related BDCA-1^+ ($CD1c^+$) and BDCA-3^+ DCs as well as plasmacytoid DCs (reviewed in Ziegler-Heitbrock et al[6]). Martínez-Cingolani et al[1] demonstrated that BDCA-1^+ peripheral blood DCs undergo rapid differentiation into LC-like DCs in response to the inflammatory factor TSLP in synergism with the previously identified LC differentiation stimulus transforming growth factor-β1 (TGF-β1).[2] Other candidate DC precursor cell populations (including phenotypically similar cells isolated from tonsils, blood monocytes, BDCA-3^+ blood DCs) lacked similar LC differentiation potential in response to TSLP plus TGF-β1 despite expressing functional TSLP receptor complexes. Moreover, higher percentages of LCs were present in cultures of BDCA-1^+ DCs as compared with cultures of monocytes, even under conditions previously optimized for obtaining LC-like cells from monocytes (ie, granulocyte macrophage–colony-stimulating factor plus interleukin-4 and TGF-β1).[7] TSLP was previously identified as a factor induced in lesional skin from atopic dermatitis/eczema patients.[8] The critical novel point made by the current study was that TSLP synergizes with TGF-β1 for driving LC differentiation and that such a TSLP/TGF-β1–dependent LC precursor is included within the (potentially heterogenous) population of BDCA-1^+ blood DCs (see figure). The authors elegantly identified TSLP as a potential LC differentiation factor when they initially performed microarray gene profiling of blood DCs stimulated with TSLP, leading to the identification of an LC signature. Based on these observations, they then added TSLP alone, or together with TGF-β1, to cultures of candidate LC precursors. The authors subsequently identified BDCA-1^+ blood DCs as the main precursors capable of differentiating into LCs under these conditions. Indeed, the authors confirmed that these LCs are characterized by hallmark markers for LCs such as high levels of CD1a, Langerin (CD207), and CCR6, as well as the ultrastructural presence of numerous intracellular Birbeck granules and by the lack of other markers such as CD11b and CD209 (DC-Sign) affiliated with dermal/interstitial DCs and inflammatory dendritic epidermal cells (IDECs).

LCs represent a unique DC subset that performs long-lasting interactions with epithelial cells in the steady-state epidermis. Interestingly, TSLP-induced LCs lacked the expression of E-cadherin, typically expressed at high levels by LCs. During skin allergy or infection, TSLP is expressed at high levels by apical keratinocytes within the inflamed epidermis and is released by activated mast cells. TSLP might facilitate LC differentiation in inflamed skin and the lack of epithelial adhesion molecules might allow these cells to rapidly egress from the epidermis to skin-draining lymph nodes. The lineage relationship of these TSLP-induced LCs to other inflammatory DCs such as IDECs (reviewed in Segura and Amigorena[9]) remains to be analyzed. Moreover, alternative inflammatory signals might also lead to the generation of LCs. For example, the TGF-β family member Activin-A, induced by keratinocytes during inflammation and wound healing, promotes LC differentiation from human monocytes.[10] The absence/low expression of these inflammatory signals in the steady-state epidermis may indicate that the factors inducing LC differentiation during inflammation or in the steady-state epidermis differ. LCs were functionally implicated in tolerance induction, skin inflammation, and cancer; therefore, deciphering these LC-inducing signals is of substantial clinical interest. Apart from providing in vitro models for the study of LCs, these findings might lead to the identification of novel molecular targets for pharmacologic interference with skin diseases. Moreover, knowledge of these LC-instructive factors and the nature of blood precursors of LCs might allow for the improved generation of LCs for cell therapy applications and for gaining novel insights into the pathogenesis of monocyte/DC/LC-related neoplasms.

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REFERENCES

1. Martínez-Cingolani C, Grandclaudon M, Jeanmougin M, Jouve M, Zollinger R, Soumelis V. Human blood BDCA-1 dendritic cells differentiate into Langerhans-like cells with thymic stromal lymphopoietin and TGF-β. *Blood.* 2014;124(15):2411-2420.

2. Malissen B, Tamoutounour S, Henri S. The origins and functions of dendritic cells and macrophages in the skin. *Nat Rev Immunol.* 2014;14(6):417-428.

3. Kanitakis J, Morelon E, Petruzzo P, Badet L, Dubernard J-M. Self-renewal capacity of human epidermal Langerhans cells: observations made on a composite tissue allograft. *Exp Dermatol.* 2011;20(2):145-146.

4. Schulz C, Gomez Perdiguero E, Chorro L, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science.* 2012;336(6077):86-90.

5. Ginhoux F, Tacke F, Angeli V, et al. Langerhans cells arise from monocytes in vivo. *Nat Immunol.* 2006;7(3):265-273.

6. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood.* 2010;116(16):e74-e80.

7. Geissmann F, Prost C, Monnet JP, Dy M, Brousse N, Hermine O. Transforming growth factor beta1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin 4, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells. *J Exp Med.* 1998;187(6):961-966.

8. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol.* 2002;3(7):673-680.

9. Segura E, Amigorena S. Inflammatory dendritic cells in mice and humans. *Trends Immunol.* 2013;34(9):440-445.

10. Musso T, Scutera S, Vermi W, et al. Activin A induces Langerhans cell differentiation in vitro and in human skin explants. *PLoS ONE.* 2008;3(9):e3271.

● ● ● PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS

Comment on Iqbal et al, page e33

Macrophages shine bright

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In this issue of *Blood*, Iqbal et al created a novel mouse model with a strong expression of green fluorescence protein (GFP) in monocytes, tissue resident

macrophages, and inflammatory macrophages, and may provide an important tool for future studies focusing on macrophage biology.[1] Several transgenic mice with expression of fluorescent proteins in myeloid cells exist, among them the CCR2-RFP and the CX_3CR1^{GFP} mouse. However, both of these mice have several limitations: they are knock-in constructs under control of chemokine receptors with potential effects on monocyte mobilization from the bone marrow, recruitment to sites of inflammation, or survival.[4,5] Alteration of chemokine receptor expression during macrophage differentiation may affect expression of fluorescent proteins and thus render macrophages nonfluorescent.

Here, the authors report a mouse expressing GFP under the control of the human CD68 promoter (see figure). The authors convincingly show that resident macrophages in the spleen, liver, brain, and lungs express high levels of GFP (see figure). Interestingly, they also identify 3 populations of resident peritoneal macrophages carrying different GFP levels. This raises the question that if these represent functional subpopulations, this may require further attention. In contrast to existing monocyte-reporter mice, the design of the transgenic hCD68-GFP mouse supposedly does not affect monocyte trafficking or survival. In addition, the authors provide experimental evidence that emigrated monocytes in hCD68-GFP mice retain high levels of GFP expression even after differentiation into macrophages; this was not the case in CX_3CR1^{GFP} mice. In conclusion, the hCD68-GFP mouse may be superior in studying several aspects of monocyte trafficking and macrophage behavior in vivo.

Recruitment and activation of macrophages are key aspects in many inflammatory diseases. In particular, circumstances of chronic inflammation visualization of macrophage origin, behavior, and activation might harbor the key to development of novel therapeutic approaches.[6] During atherosclerosis—a chronic inflammation of the arterial vessel wall—blood monocytes are recruited to the inflamed vessel wall,[7] where they give rise to inflammatory macrophages and foam cells. However, recent reports suggest that lesional macrophages might also arise from differentiated fetal macrophages that persist from early embryonic development and self-renewal throughout adulthood.[8] Given recently established protocols for stable intravital visualization of inflammatory myeloid cell behavior in high resolution,[9] the hCD68-GFP mouse might offer a powerful tool to further dissect aspects of macrophage origin and activation in atherosclerosis and similar chronic inflammatory processes. In addition, these mice might also afford direct visualization of macrophage egress from established atherosclerotic lesions.

Although it is not described in the study, the results suggest that the human CD68 promoter may be a good driver of Cre-recombinase expression to specifically delete floxed alleles in macrophages. The widely used Lysm-cre mouse is clearly not ideal for macrophage-specific deletion, and there are many reports of leaky or inefficient deletion. Thus the human CD68 promoter may be a better driver of macrophage-specific Cre expression than other currently available Cre lines. However, the problem with deriving myeloid-specific Cre-expressing mice using this expression cassette seems to be a transient expression of the hCD68 promoter in hematopoietic stem cells and progenitor cells that leads to deletion of floxed transgenes in nearly all hematopoietic cell lineages.[10] To circumvent this issue, one would need to employ an inducible construct. However, given the shortage of macrophage-specific deletion strategies, this might be a worthwhile investment for future development.

Although the study by Iqbal et al does not present any particularly novel information on myeloid cell biology, it provides a fresh tool that clearly adds to the existing arsenal of transgenic mice and may hence be interesting to many investigators interested in myeloid cell origin, trafficking, differentiation, and fate. Widespread distribution and testing of this mouse line in studies on fate mapping, steady-state trafficking, and inflammation will shed light on the suitability and superiority of this strain over existing ones and likely help deliver exciting data in emerging questions of macrophage biology.

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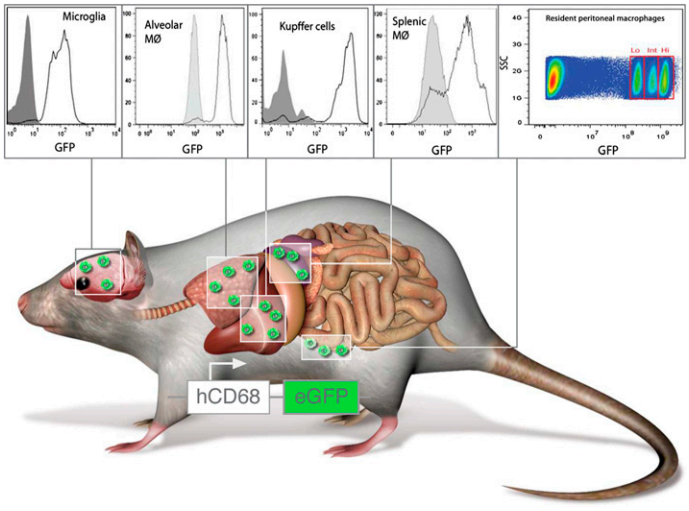

hCD68-GFP labels resident and inflammatory macrophages. Mice expressing GFP under control of the human CD68 promoter carry resident macrophages with high levels of GFP in the spleen, liver, lungs, brain, and peritoneum. In addition, circulating monocytes are GFP^+ and retain high levels of GFP even after transmigration and differentiation toward macrophages. Panels from Figure 2 and Figure 5 in the article by Iqbal et al beginning on page e33. Professional illustration by Luk Cox, Somersault1824.

REFERENCES

1. Iqbal AJ, McNeill E, Kapellos TS, et al. Human CD68 promoter GFP transgenic mice allow analysis of monocyte to macrophage differentiation in vivo. *Blood.* 2014;124(15):e33-e44.

2. Saederup N, Cardona AE, Croft K, et al. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS ONE.* 2010;5(10):e13693.

3. Jung S, Aliberti J, Graemmel P, et al. Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol.* 2000;20(11):4106-4114.

4. Landsman L, Bar-On L, Zernecke A, et al. CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. *Blood.* 2009;113(4):963-972.

5. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol.* 2006;7(3):311-317.

6. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol.* 2014;14(6):392-404.

7. Soehnlein O, Drechsler M, Döring Y, et al. Distinct functions of chemokine receptor axes in the atherogenic mobilization and recruitment of classical monocytes. *EMBO Mol Med.* 2013;5(3):471-481.

8. Robbins CS, Hilgendorf I, Weber GF, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat Med.* 2013;19(9):1166-1172.

9. Chèvre R, González-Granado JM, Megens RT, et al. High-resolution imaging of intravascular atherogenic inflammation in live mice. *Circ Res.* 2014;114(5):770-779.

10. Franke K, Kalucka J, Mamlouk S, et al. HIF-1α is a protective factor in conditional PHD2-deficient mice suffering from severe HIF-2α-induced excessive erythropoiesis. *Blood.* 2013;121(8):1436-1445.

● ● ● PLATELETS & THROMBOPOIESIS

Comment on Fan et al, page 2421

Self-control of platelets: a new ITIM story

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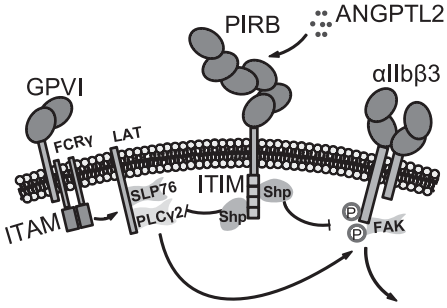
In this issue of *Blood*, Fan et al discover the presence of a new receptor-ligand pair that inhibits platelet activation.[1]

Platelets play important roles in maintaining the integrity of blood vessels and preventing bleeding. However, the activities of platelets are tightly regulated to avoid thrombosis and allow vascular permeability. The classical mechanism for the negative regulation of platelets includes prostacyclin binding to its receptor, which inhibits platelet activation by activating Gs protein, elevation of cyclic adenosine monophosphate (cAMP), and activation of cAMP-dependent protein kinase. P_2Y_{12} adenosine 5′-diphosphate (ADP), receptor antagonists such as clopidogrel block ADP-induced activation of Gi protein (which inhibits cAMP elevation), thus indirectly elevating cAMP levels and contributing to their antiplatelet effect. The effect of another often-mentioned inhibitor, nitric oxide (NO) is debatable. NO plays biphasic roles of stimulating and negatively regulating platelet activation mainly by activating cyclic guanosine monophosphate (cGMP)- and cAMP-dependent protein kinases.[2,3] Possibly due to its biphasic roles, no NO- or cGMP-based antiplatelet agents have been successfully developed.



Outside-in signaling of integrins requires additional molecules not depicted in the schematic.[3] See Figure 7E in the article by Fan et al that begins on page 2421.

Recent studies in platelets indicate the importance of a family of receptors containing an immunoreceptor tyrosine-based activation motif (ITAM), which includes the collagen receptor GPVI/Fc receptor γ chain complex and podoplanin receptor Clec-2. They are important not only in thrombosis but also in regulating vascular permeability during inflammation and maintaining hemostasis at the junction of blood and lymphatic vessels.[4,5] The signaling of ITAM-containing receptors requires phosphorylation of ITAM by Src family kinases and subsequent Syk binding to ITAM and activation. Syk induces formation of the complex of linkers for activated T cells (LATs), the Src homology 2 domain-containing leukocyte phosphoprotein of 76-kDa (SLP76) and phospholipase Cγ2, which induces calcium elevation and activation of protein kinase C.[6] The ITAM signaling pathway is important not only in transducing GPVI and Clec-2 signals but also in amplifying signals induced by adhesion receptors, the glycoprotein Ib-IX complex, and integrins.[7,8] Similar to that found in other immune cells, the counterbalance for the ITAM receptors are the receptors containing an immunoreceptor tyrosine-based inhibition motif (ITIM), which inhibits ITAM signaling by recruiting phosphatases. The currently known platelet ITIM receptor includes platelet endothelial cell adhesion molecule-1 (PECAM-1), which is an adhesion receptor that binds to PECAM-1 receptors in other cells and plays an inhibitory role in collagen- and von Willebrand factor-induced platelet activation.[9] G6B, another ITIM receptor expressed in platelets, appears to be important in normal platelet production by megakaryocytes.[10]

Fan et al demonstrate that platelets express the leukocyte immunoglobulin-like receptor subfamily B (LILRB), and its murine homolog, the paired immunoglobulin-like receptors B (PIRB). LILRB/PIRB are members of the immunoglobulin superfamily containing the ITIM motif. As expected for a functional ITIM receptor, platelet PIRB is important in inducing activation of protein tyrosine phosphatase Shp1/2 and inhibition of ITAM signaling. The authors further report that a ligand for the LILRB/PIRB, angiopoietin-like-protein 2 (ANGPTL2), inhibits platelet activation induced by GPVI agonists and integrin outside-in signaling. Importantly, mouse platelets expressing a functionally deficient mutant of PIRB showed enhanced platelet activation. These results suggest a new ligand-ITIM receptor pair that inhibits platelet activation. Different from previously identified ITIM receptor ligands, ANGPTL2 is a soluble protein that is stored in platelet granules and released during platelet activation, which raises interesting questions: whether ANGPTL2 serves as a self-control mechanism that negatively regulates ITAM signaling during platelet activation and inflammation and whether ANGPTL2 or its analogs can be developed as a new type of platelet inhibitor.

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REFERENCES

1. Fan X, Shi P, Dai J, et al. Paired immunoglobin-like receptor B regulates platelet activation. *Blood.* 2014;124(15):2421-2430.

2. Li Z, Xi X, Gu M, et al. A stimulatory role for cGMP-dependent protein kinase in platelet activation. *Cell.* 2003;112(1):77-86.

3. Li Z, Delaney MK, O'Brien KA, Du X. Signaling during platelet adhesion and activation. *Arterioscler Thromb Vasc Biol.* 2010;30(12):2341-2349.

4. Herzog BH, Fu J, Wilson SJ, et al. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature.* 2013;502(7469):105-109.

5. Boulaftali Y, Hess PR, Getz TM, et al. Platelet ITAM signaling is critical for vascular integrity in inflammation. *J Clin Invest.* 2013;123(2):908-916.