however, in view of the possible mechanisms of kindlin activity discussed above, that αLβ2 and αMβ2 clustering appeared to be unaffected in kindlin-3–deficient granulocytes.

The authors also compared the amounts of platelet and granulocyte talin-1, kindlin-3, and integrin in the various kindlin-3-deficient mice by using a novel mass spectrometry–based method. They found that decreasing the amount of kindlin-3 in platelets did not affect the amount of talin-1 or β3 integrin and that kindlin-3 and talin-1 were present in essentially stoichiometric amounts in WT cells. They also found approximately twice as much kindlin-3 and talin-1 as integrin in platelets, whereas the opposite was the case for granulocytes, a finding that may contribute to differences in integrin activation kinetics in these cells.

The results reported by Klapproth et al[1] demonstrate that despite the presence of talin-1 concentrations sufficient to saturate its β-integrin binding sites, integrin function in platelets and granulocytes can be titrated by varying the amount of kindlin-3. This is consistent with the idea that kindlin-3 plays a permissive role in enabling talin to activate integrins. They also found that relatively little active integrin is required to support basal platelet and granulocyte function, perhaps explaining the surprisingly mild bleeding diathesis (in the absence of stress) of many adult patients with Glanzmann thrombasthenia[7] and the relatively mild infectious complications experienced by some patients with leukocyte adhesion deficiency III.[8] Despite the insights into integrin activation provided by these studies, important questions remain about the dual roles for talin and kindlin in regulating integrin function. In resting platelets, the integrin-binding site in the talin-1 FERM domain is masked by sequences located in the talin-1 rod domain.[9] But there is no clear explanation of how platelet stimulation relieves this apparent talin-1 autoinhibition. Kindlins are structurally similar to talin FERM domains but have a lipid-binding Pleckstrin homology (PH) domain inserted into their F2 subdomain and, in contrast to talins, they are not autoinhibited.[10] However, as with talin-1, it is unclear how agonist stimulation causes kindlin binding to β-integrin tails. Kindlins bind to lipids as well as integrins, and the PH domain of kindlin-2, a kindlin family member more widely expressed than kindlin-3 whose expression is limited to hematopoietic cells, binds to membrane phosphoinositides.[11] Thus, it is possible that in platelets and granulocytes, agonist-stimulated phosphorylation of membrane phosphoinositides drives kindlin to the plasma membrane where it encounters a high density of integrin cytoplasmic tails.

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● ● ● PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Hofer et al, page 2601

A slan-based nomenclature for monocytes?

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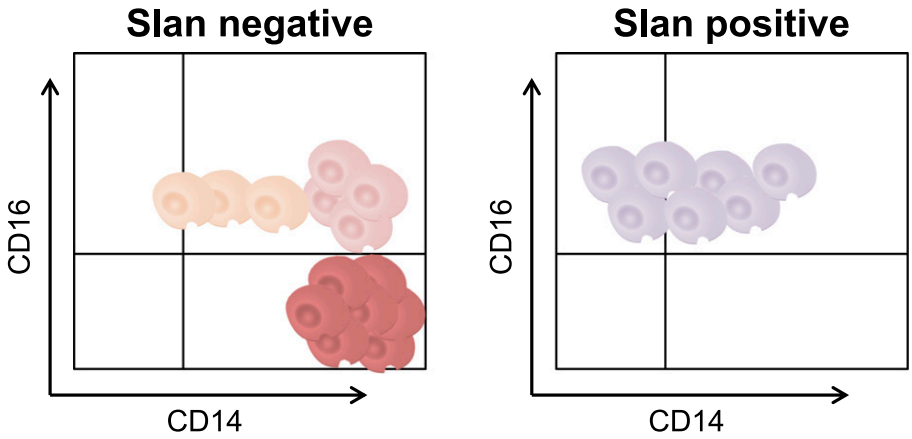
In this issue of *Blood*, Hofer et al report 6-sulfo LacNAc (slan) as a marker for transcriptionally distinct subsets of CD16-positive ($CD16^+$) monocytes that are expanded in male subjects with sarcoidosis (slan-negative $CD16^+$) or depleted during hereditary diffuse leukodystrophy with axonal spheroids (HDLS) (slan-positive $CD16^+$).[1]

Monocytes originate from bone marrow precursors, circulate through the blood for a few days, and further differentiate into macrophages or dendritic cells upon recruitment into tissues under homeostatic or inflammatory conditions. Similar to other leukocyte types, peripheral blood monocytes are highly heterogeneous in morphology, phenotype, and immune functions. Monocyte differentiation may occur within the blood stream as a consequence of their abortive interactions with endothelial beds, a process known as marginalization[2] or patrolling.[3] In addition, certain studies in mice provide evidence that monocytes are recruited into nonlymphoid tissues, where they can take up antigens and then recirculate into lymph nodes with no major changes in their phenotype.[4] Therefore, the pool of peripheral blood monocytes includes cells freshly derived from the bone marrow, as well as cells more advanced in their developmental program as a consequence of their marginalization/patrolling and likely recirculation.

In 1989, the development of flow cytometry and monoclonal antibodies facilitated the discovery of a subset of small monocytes expressing Fcγ receptor III/CD16.[5] This discovery opened a new chapter in our understanding of monocyte heterogeneity



A new slan-based nomenclature for circulating CD16$^+$ monocytes. slan identifies subsets of peripheral blood CD16$^+$ monocytes that are differentially altered in frequency during sarcoidosis (increased frequency of slan-negative CD16$^+$ monocytes in males) vs HDLS (reduced frequency of slan-positive CD16$^+$ monocytes). This new slan-based classification offers a better understanding of monocyte development during homeostasis and disease pathogenesis.

during homeostasis and disease pathogenesis. In 2010, a panel of experts proposed a novel nomenclature for monocytes based on the differential expression of CD14 and CD16: classical (CD14^{++}CD16$^-$), intermediate (CD14^{++}CD16$^+$), and nonclassical (CD14$^+$CD16^{++}) monocytes.[6] Indeed, these 3 monocyte subsets represent distinct stages of monocyte differentiation with distinct functional features.[3] They are known to be recruited into different tissues via chemokine C-C motif receptor 2 for classical and intermediate monocytes, or via chemokine CX3C motif receptor 1 for nonclassical monocytes.[6]

slan is a carbohydrate modification of P-selectin glycoprotein ligand 1 (PSGL-1) recognized by the monoclonal antibody M-DC8.[7] Although initially considered to be dendritic cells, the current consensus is that slan-positive CD16$^+$ cells represent a fraction of nonclassical monocytes.[1,3] In their article, Hofer et al revisit the CD14/CD16–based nomenclature[6] and propose that a slan-based definition of CD16$^+$ monocytes allows a better identification of monocyte subsets with unique transcriptional signatures and frequency variation during disease pathogenesis.[1] Briefly, the authors identified and isolated monocyte subsets using 2 flow-cytometry gating approaches, CD14/CD16 and CD14/slan/CD16. In healthy subjects, the number of nonclassical CD14$^+$CD16^{++} monocytes (48.1 $\pm$ 27.5 cells per microliter) was higher compared to that of slan-positive CD16^{++} monocytes (36.8 $\pm$ 23 cells per microliter; n = 10); this was because nonclassical monocytes include a fraction of CD14$^+$ slan-negative CD16^{++} cells. However, the number of intermediate CD14^{++}CD16$^+$ monocytes (24.0 $\pm$ 11.2 cells per microliter) was lower compared to that of slan-negative CD16$^+$ monocytes (41.7 $\pm$ 24.1 cells per microliter; n = 10) because the slan-negative fraction included both CD14^{++} and CD14$^+$ monocytes. Intermediate vs nonclassical monocytes, together with slan-positive CD16^{++} vs slan-negative CD16$^+$ monocytes, were sorted using magnetic beads, and their transcriptional profiling was characterized via massive analysis of complementary DNA ends and RNA sequencing with an Illumina HiSeq2000.[1] Unique transcriptional signatures were identified using the CD14/CD16 and the slan/CD16 approach, with 676 and 385 differentially expressed genes, respectively, being identified (fold change cutoff, 1.2). Consistent with the role of carbohydrate (*N*-acetylglucosamine-6-*O*) sulfotransferase 2 (CHST2) in adding the slan residue on PSGL-1, CHST2 transcripts were upregulated in nonclassical vs intermediate monocytes.[1] A particularity of slan-positive CD16^{++} vs slan-negative CD16$^+$ monocytes was a ubiquitin C interactome[1] and the superior expression of purinergic receptor P2Y, G-protein coupled, 10 (P2RY10), a G protein–coupled receptor demonstrated to bind sphingosine-1-phosphate and lysophosphatidic acid.[8]

All lines of evidence provided by Hofer et al[1] clearly support the idea that slan-positive CD16^{++} monocytes represent a unique stage of monocyte differentiation. There remain many questions regarding the developmental origin of slan-positive CD16^{++} monocytes and their functional role.

Classical CD14^{++}CD16$^-$ monocytes lack slan expression.[1,3,7,9] However, a fraction of classical monocytes express a different epitope on PSGL-1: the cutaneous lymphocyte antigen (CLA),[9] a motif involved in cell recruitment into the skin via binding to P- and E-selectin.[7] If we consider that monocyte heterogeneity into the blood stream is the consequence of their differentiation from classical into intermediate and then into nonclassical monocytes, it is reasonable to assume that monocyte differentiation is associated with a switch in their trafficking potential from skin homing (via CLA) toward the ability to home into other lymphoid and/or nonlymphoid tissues (via slan). It was demonstrated that slan-positive cells fail to bind to P- and E-selectin,[7] but the ligand for slan remains unknown. Whether slan acts as a new "zip code" for monocyte migration into specific tissues remains to be clarified. In addition to slan, defining the role of P2YR10 and other specific markers in regulating slan-positive CD16^{++} monocyte trafficking and function remains an important line of research with potential clinical applications for the treatment of pathological conditions associated with the expansion of these cells, such as HIV infection.[10]

Monocyte differentiation depends on macrophage colony-stimulating factor (M-CSF) signals via the M-CSF receptor (CD115). The expression of CD115 is indeed upregulated on CD16$^+$ vs CD16$^-$ monocytes.[9] Consistent with the fact that M-CSF controls slan expression, Hofer et al[1] report that slan-positive CD16$^+$ monocytes are almost absent in the peripheral blood of subjects with HDLS. The authors consistently identified several signaling molecules downstream from CD115 being upregulated in nonclassical monocytes of healthy individuals.[1] In contrast to HDLS subsets, slan-negative, but not slan-positive, CD16$^+$ monocytes were expanded in subjects with sarcoidosis.[1]

In conclusion, Hofer et al[1] position slan on the expanding list of markers to be considered for the characterization of monocyte heterogeneity for immune monitoring studies. Of note, slan-positive and slan-negative CD16$^+$ subsets are still heterogeneous in terms of intensity of CD14 expression, with the functional relevance of such differences remaining to be explored. This new insight

will stimulate further investigations into the mechanisms regulating slan-positive monocyte development, the role of slan-positive monocytes in disease pathogenesis, and the possibility of using slan as a therapeutic target. On the basis of these studies, the recommendation that CD14 and CD16, together with slan, should be considered for the identification of functionally distinct monocyte subsets is now emerging (see figure).

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

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Ma et al, page 2611

The stop clock of platelet activation

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In this issue of *Blood*, Ma et al provide a novel explanation as to how regulators of G protein signaling proteins (RGSs) can be coordinated by both platelet agonists (eg, thrombin) or platelet inhibitors (eg, prostacyclin) to dampen persistent platelet activation that does not then become physiologically inappropriate.[1] There are ≥37 genes encoding RGS proteins in the human genome, and, in the context of hematology, RGS proteins have been described to control hematopoietic cell function (adhesion, migration, and granule release), a role during megakaryopoiesis (differentiation and platelet formation), and the control of platelet function.[2] Human platelets contain messenger RNA for at least RGS1, 2, 3, 6, 9, 10, 16, 18, and 19, with RGS10 and RGS18 being predominant. Thus far, the roles of RGS proteins in platelet function are poorly understood.[3]

As potent negative modulators of G protein coupling receptor (GPCR) signaling, RGS proteins bind to activated G protein α subunits (Gα) and accelerate the activity of GTPase, thus enhancing G protein deactivation via hydrolysis of Gα-bound GTP, allowing Gα to reform with Gβγ. The functional relevance of RGS10 and RGS18 proteins in platelet physiology is known, where they have been reported to bind to a phosphorylated scaffold protein spinophilin (SPL) and the Src-homology region 2 domain-containing phosphatase-1 (SHP-1).[4] Thrombin activates SHP-1 leading to dephosphorylation of SPL and the release of RGS10 and RGS18.[4] In another study, the interaction of platelet RGS18 with 14-3-3γ binding protein occurs in resting platelets and paradoxically increased when platelets become activated.[5]

It is in this context that Ma et al attempt to rationalize this apparent contradiction of free and bound RGS18 and have investigated how RGS proteins could be applied to act as a brake to platelet activation, but only when needed, thus acting in a dynamic and temporal manner to allow physiological function to occur in the first instance. They show that in resting platelets, free RGS18 levels are low, but increase on thrombin activation; contrarily, free RGS18 levels also rise when platelets are rendered resistant to activation by prostaglandin I_2 (PGI_2), which increases platelet cAMP. The mechanisms that raise free RGS18 and thus how RGS18 loses bound SHP-1, SPL, or 14-3-3γ are different after activation by agonist (thrombin) compared with active suppression by inhibitor PGI_2, or resting states, which is neatly summarized in the cartoon drawn by the authors of the present paper and reproduced here (see figure).[1]

Immediate questions that arise from this work are what is the role of the other predominant platelet RGS protein (RGS10)? Furthermore, there is a need to better understand the specific roles of the 14-3-3γ/RGS18 complex, as well as the SPL/RGS18/SHP-1 complex in modulating RGS18 activity. These interactions may be significantly dynamic on a temporal basis and so the interactions of these opposing complexes would make interesting study. The dependency of these complexes on the concentration of the primary agonist or, indeed specific agonists, is not known.

Further afield, RGS proteins have been described to control the actions of neutrophils, lymphocytes, and monocytes in functions that are pertinent to host defense.[6,7] Given the extraordinary advances being made currently uncovering the contribution of platelets also to host defense, inflammatory diseases, and cancer metastasis, it might be myopic to restrict the evaluation of RGS proteins on platelet function solely to hemostasis, because the activation of platelets during hemostasis is likely to be fundamentally different to the activation of platelets during host defense and inflammation, yet our knowledge of this is in its infancy.[8,9]

It is apparent that small molecule inhibitors of RGS proteins have been synthesized to demonstrate that these proteins are a "druggable" target elsewhere in the body.[10]