García-Ojeda et al have carefully structured their case to circumvent these problems. They have dissected the effects of Gata3 deletion by using highly defined precursor cell populations, efficient and quantitative precursor frequency assays, gene expression monitoring and T-cell receptor gene rearrangement analyses, and temporally controlled excision of Gata3 in cells that have already reached a definitive T-lineage developmental stage. Their results extend our understanding of both the roles of GATA-3 in T-cell developmental progression and the specific dependence of T-cell precursors on GATA-3 for B-lineage fate exclusion. The authors find that a loss of GATA-3 function at an early stage handicaps the generation of definitive DN2-stage, T-lineage progenitors from less-defined DN1 precursors. The inhibition is confirmed by the reduced expression of a suite of genes that normally turn on during the transition to T-lineage commitment (at DN2b stage) and just afterward, that is, Cd3e, Itk, Bell1b, Rag1, and Ets1. However, some DN2 cells are made, and these can be confirmed to be of early T-cell lineage through their robust expression of Tcfl7. Importantly, the GATA-3-deficient DN2 cells also show exaggerated, not reduced, expression of Notch-activated genes Dtx1 and Pten, proving that their Notch signaling is not inhibited. The appropriate cells exist, therefore, in which to ask whether GATA-3 is truly an intrinsic requirement for B-lineage exclusion.

When removed from Notch stimulation, Gata3−/− DN2 cells indeed display B-lineage potential along with reduced NK-lineage potential. B-cell precursor activity is detected at ~10% frequency (comparable to wild-type NK precursor frequency), in marked contrast to the complete lack of detectable B-cell potential in normal DN2 cells. The converted cells switch to a B-lineage transcriptional profile, eliminating any trace of previous T-lineage gene expression. However, at least some of them retain TCRβ gene rearrangements attesting to a T-lineage past. The concern that they might represent the outgrowth of an aberrant minority within the mutant DN2 population is countered by using an acute deletion system to remove Gata3 only after the cells have reached the DN2 stage with their GATA-3 function intact. Such cells should have had the full opportunity to activate GATA-3 as well as many of the T-cell genes shown to be dependent on GATA-3. Nevertheless, when Gata3 is removed, they, too, reveal B-cell precursor competence, a >10-fold increase in frequency from GATA-3–replete DN2 cells that lack detectable B-cell potential.

These experiments may still be affected by GATA-3 contributions to proliferation and survival; however, García-Ojeda et al make a strong case that GATA-3 has a nonredundant role in B-lineage exclusion specifically, once cells have entered the T-cell pathway. GATA-3 is not the answer for all aspects of T-lineage commitment: loss of GATA-3 reduces, rather than increases, access to the NK pathway, and the conditions used would not optimally reveal the role of GATA-3 in access to myeloid- or dendritic-cell fates. The steps in the pathway of lineage conversion, especially for cells starting from a normal DN2 stage, also remain to be defined.

Nevertheless, GATA-3 induction and the mechanisms that sustain its expression now appear to constitute the key with which common lymphoid precursors and any other uncommitted progenitors that enter the thymus are persuaded to lock the door to the B-lineage pathway.

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

REFERENCE

Comment on Zhi et al, page 1858

The platelet Fc receptor: a new role for an old actor

José A. López

In this issue of Blood, Zhi et al demonstrate an important role for Fcγ receptor IIa (FcγRIIa) in platelet functions dependent on integrin αIIbβ3 outside-in signals.

Being typecast is a problem not only for actors but also for biological molecules. For the former, a role may prove so convincing to an audience or be played so well that it is difficult for the audience to accept the actor in a different role. For biological molecules, the problem more often is associated with the names they carry. These names were usually assigned based on the first biological role with which the molecule was associated. Platelet-activating factor (PAF) is a case in point. This bioactive phospholipid was originally named for its ability to activate platelets but extensive subsequent works shows this to be only a minor component of PAF’s biological roles, which span the gamut from inflammation, to nociception, asthma, cell death, bone metabolism, and even sperm function.

Fc receptors have been similarly typecast, although their functions do relate more closely to the function for which they were named. Platelets contain 1 Fc receptor,
FCγRIIA, which mediates the activation of platelets exposed to immune complexes, to immunoglobulin-opsonized bacteria, and to autoantibodies that target a subset of platelet membrane proteins. This receptor has, however, also been implicated in the functions of other platelet receptors, in particular the glycoprotein (GP) Ib-IX-V complex and αIIbβ3 integrin. With respect to the former, it was shown that GP Ib-IX-V is in physical proximity to the population of FCγRIIA that mediates platelet activation by immune complexes because antibodies that target epitopes on the complex were effective in blocking immune complex–induced platelet activation.4 It was later shown that the association between the 2 receptors occurred primarily in lipid raft membrane microdomains, implying that the population of FCγRIIA that resides in rafts is the component that transmits activation signals produced by finding immunoglobulin Fc portions.3

FCγRIIA has also been shown to associate with αIIbβ3, the major integrin on the surface of the platelets, also with functional consequences, being involved in transmitting transmembrane “outside-in” signals produced when the activated integrin engages ligands such as fibrinogen or von Willebrand factor.3 These signals are necessary for the full responses of platelets to activation, responses that include granule secretion or clot retraction. The Fc receptor accomplishes this by virtue of having an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain, which becomes phosphorylated when ligands bind αIIbβ3, attracting protein tyrosine kinase Syk, which phosphorylates downstream targets, including phospholipase Cγ2. Boylan et al5 demonstrated that certain platelet functions that are dependent on outside-in αIIbβ3 signaling, including spreading on fibrinogen and clot retraction, could be inhibited by an antibody targeting the extracellular portion of FCγRIIA. They also demonstrated that the phosphorylation of FCγRIIA that accompanied these events required the β3 cytoplasmic domain. In a follow-up study reported in this issue of Blood, Zhi et al6 took advantage of the availability of a line of transgenic mice that express human FCγRIIA to study the effect of this receptor in several platelet functions that depend on integrin outside-in signaling. Wild-type mice do not express FCγRIIA, and the transgenic mice express the receptor at levels similar to those found on human platelets. Compared with their wild-type counterparts, the FCγRIIA transgenic platelets (1) spread more extensively on fibrinogen, (2) phosphorylated Syk, and PLCγ2 more robustly when adherent to fibrinogen, (3) retracted clots better, (4) formed larger thrombi ex vivo on a collagen-coated surface and in vivo in laser-injured mesenteric arterioles, and (5) displayed increased fibrin deposition in electrolytically injured femoral veins. Taken together, these data provide compelling evidence for an important role for FCγRIIA in αIIbβ3 outside-in signaling. Still, if this mechanism for integrin outside-in signaling is so important, one has to wonder how the mice get along so well without it. Perhaps the usual mechanism by which signals are transduced after integrin engagement in platelets is fundamentally different between mice and humans.

The mechanism through which FCγRIIA participates in these signaling events is still mysterious, as attempts by this group to directly demonstrate a physical association between the 2 receptors have been unsuccessful.5 Nevertheless, the fact that signals downstream of integrin engagement can be blocked by an antibody that targets the FCγRIIA extracellular domain suggests that, as with the association of FCγRIIA with the GPIb-IX-V complex, the association between the 2 receptors is induced by ligand engagement of the integrin, driving FCγRIIA into a location where it can participate in integrin-initiated signaling. Perhaps the clustering of the integrin provides the appropriate microenvironment. The authors elegantly and conclusively ruled out a role for immunoglobulin engagement in augmenting the signals by crossing the FCγRIIA transgenic mice with mice devoid of B cells (and therefore immunoglobulins) and demonstrating that the enhanced integrin-associated effects remained when the transgene was expressed.

The interesting findings reported in this article highlight once again that we should not be influenced too much by the names that biological molecules have been assigned in considering their potential involvement in biological phenomena. In this case, the demonstration that an ITAM-containing Fc receptor is involved in a vital function of an integrin has far-reaching implications beyond hemostasis and thrombosis, and suggests that such interactions can be targets not only in thrombosis, but also in such disparate disorders as cancer, inflammation, and wound healing.

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

REFERENCES

The platelet Fc receptor: a new role for an old actor

José A. López