PKD is for dense granule secretion

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binding that may elicit a preference for certain subpopulations/orientations of CD20 molecules, which was confirmed using 3-dimensional protein tomography and confocal microscopy. Moreover, these experiments also confirmed the well-known observation that type II mAbs bind approximately half as many CD20 molecules at the cell surface as type I mAbs.

How do we assimilate these new findings into a model of how type I and II mAbs bind, orientate, and induce their effector functions? The characteristic 2:1 ratio could, as the authors speculate, either reflect the relative abundance of different CD20 subpopulations recognized by each type of mAb or specific steric requirements that affect their binding. Given the almost universal 2:1 binding relationship on many different cell lines and cell types (which may well differ in their respective proportion of open and closed CD20 molecules), and the fact that type II mAbs apparently see both forms, it would seem that the latter possibility is more likely. However, to exclude the former hypothesis it would be helpful to assess the relative proportions of the different CD20 conformations in a wide variety of CD20-expressing cell types (both resting and after activation, say with II-4 or BCR stimulation) and correlate this with type I:II binding and function.

In contrast, constrained binding through Asn171 may explain the majority of current observations. First, it may force CD20 into the open conformation only seen with type II mAbs. Second, it may preclude subsequent binding of mAbs to adjacent CD20 molecules, thereby potentially explaining the reduced binding with type II, perhaps by favoring intra- rather than intertetramer binding (see figure). Third, the constrained binding and altered orientation may also explain the associated effector functions of type I and II mAbs as the resulting angle of orientation of the Fab regions could restrict the accessibility for Fc-binding to effector molecules. For example, type II mAb efficacy in CDC may be further diminished, not only because of a lack of raft redistribution and clustering, but also because of an unfavorable orientation of the presented Fc region. Interestingly, we have preliminary evidence that type I mAbs interact with the inhibitory FcγRIIB at the cell surface (resulting in its activation) whereas type II mAbs do not engage FcγRIIB or elicit its activation (Lim et al, submitted) which may imply that engagement of FcγR is also compromised by the type II binding mode (at least in this cis-interaction on the same cell surface). Furthermore, the open conformation CD20 binding evoked by type II mAbs may provide a clue toward their association with actin reorganization, homotypic adhesion and lysosomal cell death.

The next question that arises is what about ofatumumab? Ofatumumab is a fully human type I anti-CD20 mAb that binds a completely different, discontinuous epitope involving the smaller extracellular loop of CD20 and an amino-terminal region of the large loop at position 159-166. How then does it achieve its type I nature? Unfortunately, no mAb:CD20 structural information is available so we can only speculate that the type I nature of anti-CD20 mAbs is largely influenced by binding to the amino-terminal section of CD20. A secondary prediction is that it would bind only to closed conformers of CD20, but this remains to be seen.

In summary, just like in the time warp, type I mAbs take a jump to the left and type II mAbs take a step to the right, and this small distinction in binding appears sufficient to markedly change the orientation of binding and underpin the functional diversity evoked by these different mAbs. Hopefully, we will not need to go through another decades-long time warp before opening the next chapter in our understanding of this intriguing target molecule CD20.

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

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●●● PLATELETS & THROMBOPOIESIS

Comment on Konopatskaya et al, page 416

PKD is for dense granule secretion

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Platelet secretion of dense granule contents is important for human hemostasis, as is evident from the mucocutaneous bleeding associated with disorders of dense granule biogenesis and secretion. Dense granule secretion is also important for growth and stability of platelet thrombi in experimental models of thrombosis. Although patients with primary defects in platelet secretion make up a large proportion of those with hereditary platelet function disorders, the underlying molecular cause is rarely identified. Thus, understanding molecular mechanisms regulating platelet secretory function continues to be an important area of research.

It has been clear for years that the protein kinase C (PKC) family is important for platelet secretory function. PKCs are a family of serine-threonine kinases, at least 4 to 5 of which are expressed in human platelets (α, β, δ, θ and possibly υ). Of these, PKCα was recently shown to be particularly important for dense granule secretion; however, the substrates of PKCα and their specific roles in granule secretion have remained murky. In this issue of Blood, Konopatskaya et al identify protein kinase D2 (PKD2) as a substrate of...
PKCα in platelets and demonstrate the importance of this serine-threonine kinase for thrombus formation on collagen in a flow system.6

The human protein kinase Ds (PKD1, PKD2, and PKD3) share a common structure composed of a cysteine-rich domain containing 2 diacylglycerol (DAG) binding sites at the N-terminus, a central pleckstrin homology (PH) domain, and a C-terminal catalytic domain. In lymphocytes, activation of PKD is achieved by a combination of DAG binding to the cysteine-rich domain and phosphorylation of 2 serine residues in the catalytic domain, commonly termed 744 and 748 based on the sequence of PKD1.7 Thus, in PKD-expressing cells, liberation of DAG from the plasma membrane by phospholipase C may serve to colocalize PKDs at lipid membrane sites together with active PKCs, allowing activation by PKC-dependent phosphorylation of residues 744 and 748. Because phosphorylation of residues 744 and 748 (or the homologous residues 706/710 in PKD2 and 731/735 in PKD3) controls the kinase activity of the kinase,8 mutagenesis of these 2 sites renders the kinase inactive. By knocking out specific PKD isoforms in mice or knocking in the kinase-dead mutants of these isoforms, it has been established that PKD1 is important for embryogenesis and pathologic cardiac remodeling to stress stimuli,9 while PKD2 is required for optimal peripheral T lymphocyte responses.10 The results of the PKD3 knockout have not yet been published. By comparing platelets from mice expressing kinase-dead mutant isoforms of PKDs 1 or 2, Konopatskaya et al establish that PKD2 is the important PKD isoform for regulating platelet dense granule secretion and thrombus formation ex vivo.6 A graphic summary of the proposed role of PKD2 in platelet signaling and granule secretion is shown in the figure.

Stafford et al have previously shown that PKD becomes phosphorylated and activated after stimulation of human platelets with thrombin or convulxin, but the functional significance of its activation was not known.11 Here, Konopatskaya et al show that platelets from mice expressing the kinase-dead mutant form of PKD2 have suboptimal aggregation responses to thrombin and collagen-related peptide.6 Strikingly, ATP release after stimulation by either agonist is reduced by nearly half in the knockin platelets and thrombus growth on collagen-coated surfaces is also dramatically reduced. The defect in aggregation in the kinase-dead PKD2 knockin platelets was corrected by addition of exogenous ADP, suggesting that a deficiency in ADP secretion is in fact responsible for the aggregation defect. However, the loss of PKD2 activity only modestly affects aggregation, while the effect on thrombus formation under flow conditions is more robust; this may be because of the different contribution of secretion to these two models of platelet–platelet contact, but it remains formally possible that PKD plays an additional secretion-independent role in thrombus formation under flow.

This paper adds an important link in the chain of events leading from PKC activation in platelets to dense granule secretion. Many questions remain: Is some fraction of the many patients with unknown causes of mucocutaneous bleeding attributable to PKD signaling defects in platelets? Given that tail bleeding times were normal in the PKD2 kinase-dead mice, but thrombus growth was impaired, is PKD2 an attractive target for development of antithrombotic therapeutics? Experiments using PKCα−/− platelets clearly demonstrate that PKCα regulates phosphorylation of PKD after CRP stimulation, but there was little or no defect in PKD phosphorylation induced by thrombin in the PKCα−/− platelets: is another PKC family member compensating under
these conditions? More importantly, what are the substrates of PKD and how do they regulate the mechanics of dense granule trafficking and release? Answering these questions may give us new insights into the roles of PKD in the secretory processes of platelets and perhaps other cells.

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