phosphorylation, we validated the specificity of 33A12E10. When used to immunoprecipitate endogenous c-Myc, the 33A12E10 antibody enriches a band that reacts with both 33A12E10 and the polyclonal c-Myc antibody N262 (Figure 1A black arrow). We further found, by expressing point mutants of c-Myc in yeast and 293 cells, that 33A12E10 recognizes ectopic c-Myc only when S62 is not mutated, and this band overlaps with the band recognized by N262 (Figure 1B-C black arrow). These results demonstrate that 33A12E10 can specifically recognize c-Myc and is dependent on the S62 residue.

However, during our studies using the 33A12E10 antibody, we found that it strongly cross-reacts with a protein in FBS (Figure 1D gray arrow). This cross-reacting band is very similar in size to c-Myc, and substantial washing of cells with PBS is required to diminish its intensity (Figure 1E gray arrow). On further characterization of this cross-reactivity and the multiple 33A12E10-reactive bands, we found that the predominant lower molecular weight band recognized by c-Myc antibodies C19 and N262 is also recognized by 33A12E10 (Figure 1E bottom black arrow, F lanes 1-2), while the higher molecular weight serum protein is detected robustly by 33A12E10 and to a lesser degree by C19 and N262 (Figure 1F lanes 1,3 gray arrow). Importantly, washing multiple times reveals a persistent band that migrates slightly higher than the cross-reacting serum protein, visible with all 3 antibodies (Figure 1F lane 2 vs lane 1 and lane 3 top black arrow). While most of our previous studies used a validated, custom-generated polyclonal pS62 antibody, recently we examined the higher molecular weight c-Myc in breast cancer cell lines using the 33A12E10 antibody. We find that under serum-starved conditions and with ample PBS washing, this band can be manipulated with chemicals that alter c-Myc stability and with kinase inhibitors (X.Z., unpublished data, July 2009).

The cross-reactivity of the pS62 c-Myc antibody 33A12E10 with a serum protein is of particular concern when working with leukemia cell lines or other cells grown in suspension. As these cells require harvesting by centrifugation, the volume of PBS used during collection can dramatically affect the results generated with 33A12E10 and can potentially confound the study of this higher molecular weight c-Myc. We caution users to rigorously validate this antibody for cross-reactivity under their experimental conditions.

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References

To the editor:

Does the NO/sGC/cGMP/PKG pathway play a stimulatory role in platelets?

In their recent paper, Zhang and collaborators claim that they have identified a stimulatory role of soluble guanylyl cyclase (sGC) in platelets using platelet-specific sGC-deficient mice.1 These data are in contrast to numerous studies by many different investigators in the field of sGC research and to our recent publications. We studied both a global sGC knockout (KO) mouse2 and exactly the same platelet-specific sGC KO3 mouse model as that reported by Zhang et al.1 In our, and many other studies, it is clearly demonstrated that the NO/sGC/cGMP/PKG pathway plays an exclusively inhibitory role in platelets. We and others have never obtained evidence for any “stimulatory” role of sGC in platelets.

In the article by Zhang and collaborators, the key experiment presented in Figure 2A shows that thrombin increases platelet cGMP content, and proposes a “stimulatory” role of sGC in platelet activation.1 First, these data are not consistent with the...
In their discussion, Zhang et al state that the 2 major findings in their recent study are: first, that sGC plays a stimulatory role in platelet activation. This does not agree with several other studies as mentioned previously. Second, they reported that millimolar concentrations of SNP can mediate sGC-independent platelet inhibition. This was already described for human platelets by Marcondes et al in 2006, which was even cited by Zhang et al (reference 43 in Zhang et al), but for other reasons.

In summary, in our experiments we show that thrombin neither increases cGMP nor the subsequent cGMP-dependent VASP phosphorylation in platelets. Our data and those of many other groups do not support a “sGC platelet stimulatory concept.” In contrast, our present data and that of many other authors confirm the concept that the NO/sGC/cGMP/PKG pathway plays exclusively inhibitory roles in platelets.

Response

Yes, cGMP plays a stimulatory role in platelet activation

We are grateful for the opportunity to reply to Gambaryan et al who dispute conclusions of our recent publication. In our study, we have shown that soluble guanylyl cyclase (sGC) plays biphasic roles in platelet activation, a stimulatory role during platelet activation induced by low-dose platelet agonists and an inhibitory role when stimulated with high or pharmacologic concentrations of NO donors. Our finding extends our previous discoveries that the NO/cGMP/PKG signaling pathway plays biphasic roles in platelet...
Does the NO/sGC/cGMP/PKG pathway play a stimulatory role in platelets?

Stepan Gambaryan, Andreas Friebe and Ulrich Walter