QUANTITATIVE AND FUNCTIONAL STUDIES ON PLATELET GLYCOPROTEIN IV

To the Editor:

We read the recent report of Aiken et al. that showed the effects of OKM5, a monoclonal antibody to glycoprotein IV (GPIV), on platelet function with much interest. The capacity of OKM5 to induce platelet aggregation and secretion, as it has been also reported by others, does not appear to be specific of this particular antibody because we previously reported a similar effect using FA6-152, another monoclonal antibody to GPIV. The FA6-152 antibody was also used, in parallel with the OKM5 antibody, to immunoprecipitate GPIV in agarose gels and then to demonstrate a specific interaction with purified thrombospondin (TSP). The fact that GPIV immobilized with FA6-152 or OKM5 within those immune complexes could retain its capacity to react with TSP indicates that the epitopes for these antibodies were distinct from the binding site(s) for TSP on GPIV. These observations may well explain the relative failure of both antibodies to inhibit the surface expression of TSP on thrombin stimulation.

In the Aiken study, the fact that OKM5 induced extensive platelet aggregate formation does not help to clarify the physiologic role of GPIV that has been considered as either a receptor for thrombospondin or for collagen on platelets. Furthermore, the same effect also makes unreliable the estimation of the number of GPIV molecules on platelets because the antibody may not have had access to GPIV molecules hindered within the aggregates. This would particularly apply to those samples stimulated with thrombin because of the synergistic stimulatory effect observed by the investigators when thrombin is added to platelets in combination with OKM5. Unfortunately, the investigators did not report on the binding of the F(ab')2 fragment made from OKM5 that does not induce platelet aggregation. We have recently reported on the binding of FA6-152 antibody, either the intact IgG or its Fab fragment, and found approximately 20,000 IgG molecules and 30,000 Fab molecules bound per platelet under resting conditions. We attributed this difference to the smaller size of the Fab, which would facilitate its access to GPIV molecules in the plasma membrane, or to its monovalency, which would make it react with only one GPIV molecule when the divalent IgG would react with one or two GPIV molecules. Thus, our results point to a probable underestimation of GPIV molecules on platelets when using an intact IgG.

We are pleased that Dr Legrand found our study to be interesting and appreciate her comments.

We are aware of the study of Ockenhouse et al. published while our manuscript was under review which reported that OKM5 activated platelets (although these investigators followed a different course to characterize this effect). The study by Kieffer et al is also relevant because it showed that an anti-glycoprotein IV (GPIV) reacting with a different epitope also could induce platelet aggregation. Thus, these two publications, as well as our own report, expand on our preliminary report that an antibody to GPIV can activate platelets.

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penultimate sentence of our report: "Finally, while our data raise questions regarding the mechanism by which OKM5 might inhibit TSP surface expression on platelets, they do not exclude a role of GPIV as a TSP receptor."

Dr Legrand suggests that we may not have accurately estimated the number of OKM5 epitopes on platelets because, as we show, the OKM5 antibody also aggregates platelets. As our study demonstrates, OKM5-induced platelet aggregation is divalent ion and fibrinogen dependent. In Table 1 of our report, data are presented from OKM5 binding isotherms performed in the absence of divalent ions and fibrinogen such that aggregation would be minimized. The number of OKM5 binding sites was similar under all conditions, including those that would or would not support platelet aggregation. It is noteworthy that Yamamoto et al have recently reported a value of 10,300 ± 1,400 OKM5 binding sites per platelet. This value is very similar to ours (10,000 ± 400).

Finally, Dr Legrand indicates that we may have underestimated the number of GPIV molecules per platelet because we used OKM5 IgG rather than its Fab fragments. It is difficult to respond to this point because her manuscript reporting this observation is in press and because no standard deviations are provided on the numbers cited in her letter. Thus, it is unclear whether the value of 20,000 sites with intact Ig is significantly different from our values of 10,000 ± 500 on resting platelets and 10,000 ± 400 on stimulated platelets. Nor is it clear that the difference between 20,000 molecules of an Ig versus 30,000 molecules of a Fab bound per cell, which she cites, is statistically significant. Her group previously reported 25,400 ± 8,800 binding sites for their anti-GPIV antibody. Thus, it remains to be proven whether any of these differences are significant.

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REFERENCES


Quantitative and functional studies on platelet glycoprotein IV [letter; comment]

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