Titters of anti-E1/E2 HCV neutralizing antibodies (nAbs) were measured in plasma using pseudotype viruses expressing heterologous HCV envelope glycoproteins. Levels were unaffected by B-cell depletion. As others have noted, plasma IgM fell by more than 50% and continues to be depressed; IgG and IgA remain stable. Stimulated B cells account for most of the IgM circulating in plasma. Furthermore, circulating HCV in chronic infection is stable. Stimulated B cells account for most of the IgM circulating in plasma. Reduced HVR1 sequence diversity with depletion of B cells suggests that humoral immunity can exert immune selective pressure on HCV envelope.

These preliminary data are consistent with a significant effect of B cells on the control of plasma hepatitis C viremia.

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References


To the editor:

Are Erk, Btk, and PECAM-1 major players in GPIb signaling? The challenge of unraveling signaling events downstream of platelet GPIb

Several recently published studies in Blood have attempted to unravel the signaling events operating downstream of GPIb. One of these studies by Garcia et al examined the role of Erk in promoting VWF/GPIb-dependent activation of integrin αIbβ3 in platelets. These investigators analyzed changes in Erk phosphorylation in VWF/ristocetin-stimulated platelets and examined the effects of a range of pharmacological signaling inhibitors on Erk activation and integrin αIbβ3-dependent platelet aggregation. Based on these studies, the authors conclude that there is an important role for Erk in GPIb signaling and propose a model in which GPIb initiates a linear signaling cascade involving Src kinases → PLC → MEK → Erk → PLA2 that stimulates integrin αIbβ3 and platelet aggregation through an indirect mechanism dependent on the generation of TXA2. Although the results presented are consistent with such a model, we have some concerns with the definitive nature of these conclusions.

The main concern is fundamental and relates to the individual contributions of GPIb and integrin αIbβ3 to VWF-induced signaling. It is generally accepted that the VWF-GPIb interaction induces weak signals to initiate integrin αIbβ3 activation, and the subsequent VWF binding to activated integrin αIbβ3 in concert with released ADP and TXA2 triggers global platelet activation. Thus, many of the commonly used suspension-based functional assays to investigate signals downstream of soluble agonist receptors (ie, classical platelet aggregation, secretion, or ligand binding to activated integrin αIbβ3) are not ideal for analysis of signals derived exclusively downstream of GPIb. In particular, when VWF/ristocetin or VWF/botrocetin induces biphasic platelet aggregation the authors need to consider that output signals are derived from both GPIb and integrin αIbβ3, not solely from GPIb. These factors complicate the analysis of the findings presented by Garcia et al and also those by Liu et al investigating a role for Btk in GPIb signaling and Rathore et al examining PECAM-1 regulation of GPIb signals.

It should be acknowledged that dissecting signaling events downstream of GPIb is difficult, mainly because GPIb-induced signals per se are weak regardless of the experimental approaches used. Elucidating GPIb-specific signaling events independent of...
We agree with Jackson et al that caution should be exercised in interpreting our data demonstrating that Btk is required for GPIb-dependent signaling. We used multiple approaches to make interpreting our data uncomplicated. Not only did we use anti-αIIbβ3 Ig or EDTA to prevent aggregation in the bt/VWF suspension system, we confirmed our results using platelets from mice deficient in normally functional Btk, the prediction being that if Btk is required for early events in GPIb-elicited αIIbβ3 activation, TxA2 would not be produced, and therefore αIIbβ3 would not be activated. Those are the data we obtained. Finally, we used the ferric chloride carotid artery injury system to provide an in vivo demonstration of the physiological significance of Btk function in GPIb signaling. The results were unequivocal; Btk was required for stable thrombus formation in this GPIb-dependent system. The in vivo results confirmed the GPIb signaling data obtained using an in vitro system. Although it could be argued that the ferric chloride injury model may not reflect normal physiologic interests, we used the ferric chloride carotid artery injury system to provide an in vivo demonstration of the physiological significance of Btk function in GPIb signaling.

Measuring calcium flux in vitro remains an important approach, but the ultimate touchstone of physiological significance is an in vivo test. Therefore in view of our in vivo data, it is clear that Btk plays an important role in GPIb-dependent signaling.

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