Retroviral infection and selection of culture-derived platelets allows study of the effect of transgenes on platelet physiology ex vivo and on thrombus formation in vivo.

Abstract:
Background- We recently reported the development of culture-derived (CD) platelets with the aim to express any protein of interest in these platelets. We now report a specific protocol of retroviral infection into the progenitor cells and subsequent selection, which allows to generate large amounts of highly homogenous transgene-expressing CD platelets and to study transgene function rapidly and reliably at large-scale ex vivo and in vivo settings. METHODS AND RESULTS: After retroviral infection and selection, the activation-dependent expression profile of surface markers, aggregation, and granule release were investigated. The function of transgene-expressing CD platelets, the precursor cells of which had been retrovirally infected, compared well to noninfected CD platelets or freshly isolated platelets. Hence, the retroviral infection protocol did not alter platelet physiology. In contrast, adenoviral infection of precursors to CD platelets resulted in marked functional alterations that obviated their use in analytic experiments. Additionally, sufficient amounts of selected CD platelets were generated to warrant intravenous injections into living mice. This approach permitted study of their adhesive profile at endothelial lesions and their effect on thrombus formation in vivo by intravital videofluorescence microscopy. CONCLUSIONS: The
novel selection method allowed us to produce recombinant transgene-expressing platelets in sufficient amounts to study genetically modified platelets in vitro and in vivo.