

Isolation and functional analysis of reticulated platelets

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Introduction

The platelet population is a heterogeneous mix of various sub-populations, potentially each with distinct biological capabilities. Of particular interest is the reticulated sub-population, so called because of the presence of RNA in their cytoplasm. This subpopulation is estimated to account for roughly 10% of the total platelet population and has been reported to have a higher thrombotic potential than the non-reticulated population.

Methods

Flow cytometry was used to identify reticulated platelets within platelet rich plasma following staining with thiazole orange. The thiazole orange stained platelets with the highest mean fluorescence intensity (top 10%) was selected as the 'reticulated population', the middle 50% as the 'intermediate population' and the bottom 30% as the 'non-reticulated population'. mRNA content in each of the subpopulations was determined using qRT-PCR. Platelet aggregation and P-selectin expression, in response to various agonists, was also assessed using imaging flow cytometry. Comparisons were made using a one-way ANOVA or t-test where appropriate.

Results

Reticulated platelets were confirmed to contain more mRNA than either the intermediate or non-reticulated populations (average Ct value: PF4, 23.9 vs 27.9 vs 29.9; TUBB1, 25.7 vs 30.4 vs 32.8; ITGA2B, 27.0 vs 31.9 vs 33.9 $p < 0.05$ $n = 3$). For each agonist used in the aggregation study, the percentage of single reticulated platelets remaining in the overall single platelet population after stimulation (i.e. platelets that had not responded) was significantly reduced compared to that in control conditions (AA 1 mM, 2.4% vs 9.4%; ADP 20 μ M, 6.2% vs 9.6%; collagen 1 μ g/mL, 6.0% vs 9.1%; TRAP6 25 μ M, 1.3% vs 9.1% $p = 0.05$ $n = 6$). In addition, reticulated platelets had a significantly higher fold change in the expression of cell surface P-selectin (CD62P) than nonreticulated platelets following exposure to three concentrations of TRAP-6 (2.5 μ M, 2.6 \pm 0.4 fold greater; 10 μ M, 3.2 \pm 0.5 fold greater; 25 μ M, 2.7 \pm 0.4 fold greater $p < 0.05$ $n = 6$). Image analysis also indicated that reticulated platelets were more often present at the centre of platelet aggregates.

Conclusion

Reticulated platelets, i.e. those containing more mRNA, are more avidly recruited to the forming aggregates than non-reticulated platelets, as demonstrated by the greater proportional drop within the overall single platelet population. Reticulated platelets also localise to the cores of aggregates, indicating that they are particularly recruited at the early stage of platelet aggregate formation. Thus reticulated platelets are more reactive than nonreticulated platelets and may be early drivers of thrombus formation.