

Abstract

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A monoclonal antibody against platelet glycoprotein IIb/IIIa was obtained from 8-weeked BALB/c mice immunized with platelet derived from normal human blood group O, by hybridoma technology. The immune spleen cells were fused with X63Ag8.653 myeloma cells at a ratio of 5:1. Indirect sandwich ELISA method was performed to screen hybrid cells secreted antibodies against homemade purified gpIIb/IIIa. Ten hybrids were found to secrete antibodies against purified gpIIb/IIIa. Hybrids were cloned by 2 rounds of limiting dilution. Platelets, K562 and U937 cell lines were used to detect antibody specific to gpIIb/IIIa and its related antigen by Platelet suspension immunofluorescent technique (PSIFT) and Indirect immunofluorescent technique, respectively. It was found that only two gave positive to all cell tested. PY-13, one of them was studied to determine the epitope on platelet membrane by immunoprecipitation and it showed 2 bands at 97 and 240 kD. For further characterization, PY-13 was immobilized onto proteinG IgG and used to isolate the platelet gpIIb/IIIa from platelet extract. The result indicated that PY-13 could pull down gpIIb/IIIa molecule by expressing the 140 kD in non-reduced gel and two bands at 110 and 120 kD in reduced gel. The isotype was also performed and the result was IgG₁kappa. In conclusion, by mean of all results, it can be indicated that PY-13 is the monoclonal specific to platelet gpIIb/IIIa.