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PRODUCTION OF HUMAN MONOCLONAL ANTIBODIES SPECIFIC FOR PLATELET MEMBRANE GLYCOPROTEIN IIIa. Kenichi Furihata (1), Diane J. Nugent (2), Amy L. Bissonette (1), Elizabeth Vokac (1) and Thomas J. Kunicki (1). The Blood Center of Southeastern Wisconsin, Milwaukee, WI, USA (1) and Fred Hutchinson Cancer Research Center, Seattle, WA, USA (2).

Human monoclonal antibodies specific for platelet membrane glycoproteins (GPs) are potentially important reagents for studies of the immunogenicity of membrane glycoproteins. A human monoclonal autoantibody, 5E5, reactive with platelet GPIIIa has been developed (Nugent, et al., Blood, 1987, in press). In this report, we describe the production of additional human monoclonal antibodies specific for GPIIIa. Peripheral blood lymphocytes from one patient with post-transfusion purpura (PTP) and one woman who had delivered an infant with neonatal alloimmune thrombocytopenic purpura (NATP) were used as a source of antigen-specific lymphocytes. A B-lymphocyte-enriched population was transformed with Epstein Barr virus, strain B95-8, and cultured in microtiter plates. After two weeks, culture supernatants were screened by an antigen-capture ELISA wherein murine monoclonal antibody specific for the GPIIb-IIIa complex was used to hold corresponding antigen from a lysate of normal platelets. B-lymphoblastoid cell lines producing IgG and/or IgM antibodies were expanded and either cloned by limiting dilution technqiue or hybridized with a HAT-sensitive, ouabain-resistant heterohybrid fusion line, F6, using polyethylene glycol. Hybridomas were selected in medium containing HAT and ouabain. After two weeks, hybridomas producing anti-GPIIb and/or anti-GPIIIa antibody were cloned by limiting dilution. Culture supernatants from cloned B-lymphoblastoid cell lines and cloned hybridomas were rescreened by ELISA wherein affinity-purified GPIIIa or other platelet GP were directly conjugated to microtiter plates. One IgM antibody produced by a cloned B-lymphoblastoid cell line (CH16) and two IgG antibodies produced by cloned hybridomas (Del5.19 and Del5.23) were shown to react with GPIIIa but not other GP. Further characterization of these human monoclonal antibodies, produced continuously in culture now for four months, is in progress.

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STUDIES ON THE INVOLVEMENT OF GPIIb/IIIa COMPLEX IN PLATELET AGGREGATION WITH TWO MONOCLONAL ANTIBODIES. J.J. Ryckewaert, O. Valiron, I.A. Newton, E. Concord, M. Prenant and R. Berthier DRF/Laboratoire d'Hématologie,/INSERM U217, CEN.G, 85X, 38041 Grenoble.

The involvement of the glycoproteins IIb/IIIa in the binding of fibrinogen to stimulated platelets and in the aggregation of these cells is well documented. Monoclonal antibodies (MoAb) directed against these two glycoproteins are useful probes in functional studies. Two MoAbs have been obtained against the two glycoproteins in their heterodimer form. Both MoAbs immunoprecipitate the complex from platelet lysates and only bind to platelets if the complex of the two glycoproteins is not dissociated. Studies on the effect of the two MoAbs on platelet function have been performed. Firstly CS9, a murine IgG 2a, inhibited the binding of fibrinogen to stimulated platelets and prevented platelet aggregation. Fab CS9 obtained by papain digestion had a similar activity. The binding of I^{125} Fab CS9 to resting platelets was similar to that of ADP-stimulated platelets (30 000 MoAbs/platelet). Similar behaviour of MoAbs against GPIIb/IIIa has been repeatedly reported and is considered as evidence that the fibrinogen receptor is the GPIIb/IIIa complex. The second MoAb (CS3), an IgG_1 subtype had a different action on platelet function. Contrary to all other known antibodies directed against the GPIIb/IIIa complex, CS3 or (Fab'2)CS3 induced the binding of fibrinogen to platelet in the absence of any platelet stimuli. Washed platelets in the presence of CS3 underwent immediate and extensive aggregation, even in the absence of fibrinogen. The stimulatory activity of MoAb CS3 on platelet can be eliminated by PGE_1. Monovalent CS3 (Fab fragment) failed to exhibit the properties of the parent antibody and had no effect on platelet function. The most likely explanation of the action of CS3 on platelet function may be that crosslinking of two GPIIb/IIIa complex mediated by the antibody leads to platelet stimulation. Whether or not this mechanism is evocative of a physiological event when platelets are stimulated remains to be demonstrated. When both MoAbs are used in combination, the binding of fibrinogen and platelet aggregation are prevented by CS9 despite the stimulatory activity of CS3. Thus both MoAbs are able to bind to the GPIIb/IIIa complex at the same time.

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DISTRIBUTION OF GLYCOPROTEINS (GP) IIb AND IIIa AND THEIR COMPLEX ON ADHERENT/ACTIVATED PLATELETS. J.C. Lewis[1], R.R. Hantgan[1], N. Kieffer[2], A. Nurden[2] and J. Breton-Gorius[2]. [1]Bowman Gray School of Medicine, Winston-Salem, NC USA; [2]INSERM U-91 and U-150, Cretéil, France.

Redistribution of GP IIbIIIa has recently been demonstrated for platelets activated in suspension in the presence of either fibrinogen or specific monoclonal antibodies, and it has been suggested that redistribution is important for normal platelet function. Reported here is the distribution of GPIIb an GPIIIa following adhesion, and event focal to the hemostatic process. Human platelets isolated by centrifugation from heparinized blood and washed in Hank's Salts, pH 6.5 with and without EDTA (3%) were activated by adhesion to carbon-stabilized formvar grids. Subsequent to activation/adhesion, GP's were localized by immunoelectron microscopy on whole-mount cells using colloidal gold particles of different sizes. Primary antibodies included mouse monoclonal antibodies C17 (anti IIIa) and P112 (anti IIb), rabbit polyclonal antibodies aIIb, aIIIa and antibodies HpL2 and IgGL directed against IIb and the complex. Upon activation with pseudopod extension the Gp's were colocalized with combined immunochemistry (C17 & aIIb, P112 & aIIIa). Greatest density was observed along pseudopods and the elaborating hyalomere. In addition to colocalization, discrete pools of either IIb or IIIa were interspersed with the complexes over the hyalomere. The pools, ranging in diameter to 0.3 µM were accentuated with hyalomere development; corresponding with release, both the pools and the complexes migrated centripetally to the granulomere. This resulted in an absence of Gp over the hyalomere. During hyalomere Gp migration, the label at pseudopods and the cell margin remained intact. Both centripetal movement and pool sizes were accentuated at 37°C. Immunodetection over the granulomere was minimal following release, but small pools of individual Gp's were again observed randomly over the hyalomere. Both clustering and relocation of Gp occurred in the presence of EDTA. Verification of individual Gp clusters and IIbIIIa complex clusters was achieved by double labeling using an antibody against the complex in conjunction with antibodies against IIb or IIIa. The complex always colocalized with the individual Gp's; however, in all combinations the individual Gp's were also observed as separate pools.

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THE SOLE USE OF MAGNESIUM CHLORIDE FOR BLOOD ANTICOAGULATION: PRESERVATION OF PLATELET SURFACE-BOUND "PROTEINACEOUS HALO"! N. Crawford (1), M. Crook (1), J. Dawes (2) and C. R. W. Gray (1) Department of Biochemistry, Royal College of Surgeons of England, London, U.K. (1) and M.R.C. and S.N.B.T.S. Blood Components Assay Group, Edinburgh, U.K. (2).

European Patent No. 83901336 [C.R.W. GRAY & N. CRAWFORD] refers to the use of bivalent cations for blood anticoagulation and the preparation of plasma and cellular products. Both $CaCl_2$ and $MgCl_2$ are effective as sole anticoagulants although the concentration of Ca^{2+} to maintain blood fluid for >7 days exceeds the osmolarity limits for good preservation of cellular integrity. With $MgCl_2$, however, [at final concentrations 25-30 mM and suitable blood/$MgCl_2$ volume ratios] red cells show good preservation, granulocytes phagocytose well and platelets are discoid and respond to all conventional agonists [although higher than normal doses are required]. The concentrations of ß thromboglobulin (ßTG) in "$MgCl_2$-plasma" are substantially lower than with Ca^{2+} complexing anticoagulants containing theophylline and PGE_1. Thrombospondin [TSP] another major granule protein, unlike ßTG, binds after release to the activated platelet surface in the presence of physiological $[Ca^{2+}]$[1] RIA assays of TSP has revealed that "Mg^{2+} platelets" have ~ 3 times more surface bound TSP than "CPD-platelets". Subpopulation profiling by continuous flow electrophoresis shows that formol-fixed platelets from Mg_2 anticoagulated blood are substantially less electronegative than "CPD platelets" similarly processed. We believe that platelets from blood in which the extracellular $[Ca^{2+}]$ is undisturbed carry a surface-associated "halo" of non-specific [electrostatically-associated] and Ca^{2+}-linked adsorbed proteins. Released TSP is an example of the latter association. Since plasma ßTG levels are influenced by kidney clearance kinetics and other factors, the measurement of surface bound TSP on platelets from $MgCl_2$-anticoagulated blood may have clinical value as an index of in vivo release events and particularly in vascular disorders accompanied by renal damage where the findings with ßTG have been equivocal[2]

1. George, J. N. et al. (1986) J. Clin. Invest. 78, 340-348.

2. Guzzo, J. et al. (1980) J. Lab. Clin. Med. 96, 102-113.