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A MONOCLONAL ANTIBODY TO VIII:C PRODUCED BY A MOUSE HYBRIDOMA. H.P. Muller, N.H. van Tilburg, R.M. Bertina, J. Derks and E. Klein-Breteler. Haemostasis and Thrombosis Research Unit, Department of Medicine and Department of Human Genetics, Leiden University Hospital, Leiden, The Netherlands.

Spleen cells of a Balb-c mouse immunized with VIII:C (isolated by affinity chromatography) were fused with mouse myeloma cells (MOPC-21 derivative). After the fusion 12/32 wells produced an inhibitor to VIII:C. After subclonation (3 x) a stable hybridoma line was obtained. The antibody in the supernatant was detected with a modified VIII:C inhibitor technique. The supernatant of in vitro cell cultures of the hybridoma cells contained anti-VIII:C titers (Bethesda) of about 0.3-1.0 units/ml. Injection of the hybridoma cells in pristane pretreated Balb-c mice results in anti-VIII:C titers of 5,000-10,000 units/ml ascites.

Analysis of the produced immunoglobulin demonstrated the presence of one band after isoelectric focussing, which contained heavy chains both of IgG_1 and IgG_{2B} subclass. Because of the unusual kinetics of the monoclonal antibody with VIII:C extensive characterisation of the nature of its VIII:C neutralising properties was necessary.

The monoclonal antibody does not bind ^{125}I-fibrinogen or isolated VIIIR:AG, it reacts with isolated VIII:C and can be used in a two-site immunoradiometric assay for VIIICAG. The epitope against which the antibody is directed is not present on 'serum-VIIICAG'.

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ANTIBODIES AGAINST PLATELET MEMBRANE GLYCOPROTEINS: EFFECT ON RISTOCETIN-INDUCED PLATELET AGGREGATION. E.F. Ali-Briggs, C.S.P. Jenkins and K.J. Clemetson. Departments of Hematology, Wilhelmina Gasthuis, Amsterdam, and Montefiore Hospital, Bronx, NYC, and Theodor Kocher Institute, Berne, Switzerland.

Some membrane glycoproteins (GPs) have been isolated by lectin-affinity chromatography and antibodies towards them have been raised. Platelets that have lost glycocalicin no longer respond to ristocetin-human VIII:WF, bovine VIIIR:WF, or to anti-glycocalicin or anti-GPs Ia and Ib antibodies but are still agglutinated by anti-GPs IIb and IIIa antibodies. Anti-GPs Ia and Ib and anti-glycocalicin antibodies, IgG and Fab' fragments inhibited ristocetin-human VIIIR:WF- and bovine VIIIR:WF-induced aggregation of fixed, washed platelets and of platelets in plasma while anti-GPs IIb and IIIa antibodies were without effect.

Crossed immunoelectrophorectic studies showed that glycocalicin was present on whole platelets in only trace amounts; anti-glycocalicin antibodies, however, recognized a slower migrating component. Platelets incubated in an EDTA-free medium no longer respond to ristocetin-human VIIIR:WF. Membranes isolated from such platelets contained glycocalicin which cross-reacted with a remnant of the slower migrating component. Anti-GPs Ia and Ib antibodies gave more complex patterns but it was possible to identify the slower moving component recognized by the anti-glycocalicin antibodies.

These results show that glycocalicin is not normally found as such on whole platelets but is present as a precursor which is most likely GP Ib. On degradation of this precursor, glycocalicin is released from the membrane and VIIIR:WF-receptor activity is lost.