Wednesday, July 15, 1981

Oral Presentations

Coagulation – X

Monoclonal Antibodies to Factors VIII and IX

08:00–09:30 h

Coagulation – XI

Factor VIII/von Willebrand, Factor IX

09:45–11:00 h

Cinema 2

0503

08:00 h

A MONOCLONAL ANTIBODY TO FACTOR IX

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We describe a mouse monoclonal antibody with high affinity for a functional site on coagulation factor IX.

Mice were hyperimmunised to factor IX by repeated intraperitoneal injections of between 15 and 20 µg of purified factor IX in complete Freunds adjuvant, over a period of 5 months. Three days after a final boost with 15 µg of factor IX in saline, the spleen was removed from a mouse that had a serum anti factor IX activity of 200 NIH units measured in the standard coagulation assay. The spleen cells were fused with the HAT sensitive, non-secreting mouse myeloma cell line, P3-NSI/1-Ag4-1 (donated by C. Milstein) using polyethylene glycol 1500 (40% w/w). After plating at 1 x 10^6 cells/ml in 2 ml multiwell culture plates (211 cultures) hybrid cells were selected from the mixed cell population by culturing in the presence of HAT medium for a period of 33 days. Hybrid clones of cells appeared from day 10 onwards and culture supernatants were tested for the production of antibody to factor IX in a coagulation assay.

One of the cultures that produced an inhibitory effect in this assay has been subcloned twice and established as a monoclonal cell line that can be maintained in continuous culture or can be grown as an ascites tumour in syngeneic mice. This cell line secretes an $IgG_{1(k)}$ antibody designated RFF-IX/1, that binds to the coagulation site of factor IX. RFF-IX/1 inhibits only factor IX and no other coagulation factor. A solid-phase radiometric assay is also described that detects the presence of RFF-IX/1 by its ability to bind to factor IX linked to polystyrene beads and that can be used to screen for production of similar antibodies by hybrid cell lines.

0504

08:15 h

A MONOCLONAL ANTI HUMAN FACTOR IX PRODUCED BY A MOUSE HYBRIDOMA.

R.M. Bertina, I.K. van der Linden, H.P. Muller, J. Derks and E. Klein-Breteler. Haemostasis and Thrombosis Research Unit, Leiden University Hospital, Leiden, The Netherlands.

Spleen cells of a Balb-c mouse immunized with purified human FIX were fused with mouse myeloma cells (MOPC-21 derivative). Among the fusion products one hybridoma was found producing an inhibitor of factor IX procoagulant activity. After subclonation (5 x) a stable hybridoma was obtained. In vitro cell culture of the hybridoma cells gives anti-FIX titers (Bethesda units) of about 0.8 units/ml. Injection of the hybridoma cells in pristane pretreated mice results in anti-FIX titers of 600-1000 units/ml ascites. Analysis of the produced immunoglobulin demonstrated the presence of one main band after iso-electric focussing, which contained heavy chains both of IgG_1 and IgG_{2B} subclass.

Monoclonal anti-factor IX was isolated from ascites liquid using affinity chromatography on protein-A-Sepharose. Using the purified antibody a radioimmunoassay was developed for the epitope on factor IX, against which this antibody is directed. The epitope is present both on FIX and activated FIXa; however, the affinity of the antibody for binding to FIXa is at least 10 times less. The antibody has no significant affinity for binding to the isolated heavy and light chain of FIXa.

About 27 different genetic variants of factor IX (from haemophilia B patients) were tested for the presence of this epitope. All FIX-variants possessed the epitope. At least 2 variants demonstrated a reduced affinity for binding to the antibody.

0505

08:30 h

MURINE MONOCLONAL ANTIBODY TO PORCINE FACTOR VIII:C.

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Mice were immunized with factor VIII complex and boosted with partially purified VIII coagulant (VIII:C). These mice produced antisera which caused inhibition of VIII:C activity in clotting assays. Spleen cells from the antibody positive mice were fused with NS-1 plasmacytoma cells, aliquoted into 96 cultures, and 68 culture wells positive for hybrid cell formation were assayed for anti-VIII:C antibodies. An immunoradiometric assay using ^{125}[I]-labelled human factor VIII:C inhibitor was used to identify anti-VIII:C antibodies. Twelve hybrid supernatants mediated significant binding of the radiolabelled probe in the presence of partially purified VIII:C. Of these, 8 were shown to be positive through the binding of porcine Willebrand factor, rather than direct factor VIII:C binding. Three hybrid supernatants bound factor VIII:C from a preparation of dissociated low molecular weight coagulant, could not be dissociated from the antigen with 0.25 M calcium chloride and bound no detectable porcine Willebrand factor. One of these hybrids was subcloned and grown as an ascitic tumor in mice. After labelling with ^{125}[I], the monoclonal antibody could be used to measure VIII:C bound to polystyrene in an RIA. The antibody shows negligible inactivation of VIII:C. The antibody coupled to agarose, quantitatively removes coagulant from solutions of dissociated VIII:C but will not remove VIII:C activity from plasma (or other sources of undissociated factor VIII complex). The VIII:C absorbed onto the antibody beads is still active. Less than 10% of input activity is retrievable from the beads using chaotropic ions, but 60% of the VIII:C activity is obtained with organic solvent elution. When analyzed by SDS gel electrophoresis, the active protein is indistinguishable from that eluted in inactive form, at low pH, from human inhibitor columns. The porcine VIII:C activity isolated using the antibody shows a 2 to 3 x 10^5-fold purification based on units VIII:C per A_{280}.