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IMPORTANCE OF CONFORMATIONAL CHANGE OF FIBRINOGEN INDUCED BY SURFACE ADSORPTION. **Lu He (1) (2),Mc Mirshahi (1). J. Soria (3), C. Soria (1), M. Mirshahi (3), A. Thomaidis (1), C. Boucheix (4), J.P. Caen (1). Haematology Department Hôpital Lariboisière, Paris, France (1), University of Medicine n° 2 of Shanghai, People's Republic of China (2), Laboratories of Professors Adolphe, Bernadou and Samama, Hôtel Dieu, Paris, France (3), INSERM U. 268, Villejuif, France (4).**

Concerning the problem of blood - foreign surface interaction, it is a great importance to know whether fibrinogen adsorbed on a solid surface is conformationally modified.

Therefore, we tested the reactivities of soluble and immobilized fibrinogen against monoclonal antibodies obtained by mouse immunisation with fibrin derivatives. by an immunoenzymological assay using a monoclonal antibody which recognized an epitope available in the D domain of the molecule, but which is poorly exposed on soluble undegraded fibrinogen, we found that the epitope became accessible to this monoclonal antibody after the binding of fibrinogen to polystyrene. We concluded that fibrinogen undergoes conformational change when absorbed to a solide phase.

Conformational change of fibrinogen induced by immobilization on polystyrene may modify the biological properties of fibrinogen molecule. We have shown that after its adsorption, fibrinogen allows plasminogen activation by tissue type plasminogen activator (tpA) in a similar manner as fragment D and fibrin. Therefore, this modification of fibrinogen structure may allow the exposure of the epitope which is masked on native fibrinogen and demasked on fragment D and fibrin and which is essential for tpA-induced plasminogen activation.

These results might be very useful in the screening of synthetic material used for grafts and extracorporeal circulation.

A similar conformational change occurs when fibrinogen binds to ADP-treated platelets. Further work however will be required to determine whether conformational change is essential for ADP-induced platelet aggregation.

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SPECIFICITY OF MONOCLONAL ANTIBODIES (MABS) TO FIBRINOGEN/FIBRIN MOLECULE USING THE AVIDIN-BIOTIN IMMUNOPEROXIDASE STAINING OF FROZEN SECTIONS OF HUMAN FIBRIN.
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To study the effect of Factor XIIIa mediated cross-linking of fibrin on the specificity of MAb/TD-1 (specific for the D-dimer of the DD/E region of human cross-linked [XL] fibrin, MAb/T2G1s (specific for the amino terminal region of ß chain, ß 15-42 and Fibrin II [des AABB fibrinogen]) and MAb/1-8C6 (specific for fibrinogen, Fibrin I [des AA fibrinogen] and Bß 1-42) an Avidin Biotin Immunoperoxidase staining of frozen sections of human fibrin was used. The three monoclonals were applied as first antibodies in the Vectastain ABC Kit (Vector Laboratories, Burlingame, CA). Clots were prepared from Imco fibrinogen using 0.015 M Na_2HPO_4, 0.075 M NaCl, pH 6.35. Where applicable, EDTA 0.01 M was used in the buffer. Bovine thrombin 1 u/ml was added and the clots were incubated overnight at 20°C. Frozen sections were prepared and stained. Kabi grade (L) fibrinogen was also used, but failed to clot in the presence of EDTA. The results of the comperative study were as follows:

ANTIBODY	EDTA clot	Normal Clot
MAb/T2G1s	Positive	Positive
MAb/TD-1	Very weak	Positive
MAb/1-8C6	Negative	Negative

The positive reaction with T2G1s in both clots preparations is due to the presence of the neoepitope in cross-linked and noncross linked Fibrin II. The specificity of TD-1 for cross-linked fibrin is supported by a positive reaction in the normal clot, while the reaction was very weak in the presence of EDTA. Due to the cleavage by thrombin of Bß (Arginine14-Glycine15) MAb/1-8C6 was negative in both clots. These results indicate, that the Avidin-Biotin Immunoperoxidase staining of frozen sections of human fibrin, is useful for the evaluation of monoclonal antibodies specificity to fibrinogen/fibrin molecule.

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IMMUNO-VISUALIZATION OF FIBRINOGEN Aa-CHAIN HETEROGENEITY IN NORMAL PLASMA COMPARED TO PLASMA FROM PATIENTS WITH DIC.
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Citrated plasma from healthy individuals or from patients with disseminated intravascular coagulation(DIC) was diluted, and electrophoresed on 11% SDS-PAG slabs after reduction.
Subsequently, the electrophoretic pattern was Western-blotted onto nitrocellulose and,-after blocking with gelatine-, reacted with either:a)polyclonal antibodies to human fibrinogen,b)polyclonal antibodies to FPA, or c)monoclonal antibody to non-released FPA (gift from Dr.Nieuwenhuizen,Leyden,Holland).
Visualization of fibrinogen-related material was then done with peroxidase-conjugated secondary antibodies.
In normal plasma a heterogeneity of the Aa-chain which was substantially more pronounced than hitherto described was noted. This pattern of fibrinogen Aa-chain heterogeneity was even more noticeable in plasma from patients with DIC. The presence/absense of all appropriate fibrinolytic inhibitors during sampling of blood had no effect upon the Aa-chain heterogenity patterns described above. This picture of heterogeneity was also seen when unreduced plasma was electrophoresed on SDS-PAG(2.5%)/AGAROSE(0.5%) slabs.
When comparing the patterns produced by the three different antibodies used for identification/visualization, it could be deduced that: 1)The pattern observed was mainly due to Aa-chain heterogeneity, and that the N-terminus of almost all species of Aa-chains were intact, confirming earlier observations that cell- or plasmin -mediated proteolysis of this chain is mainly C-terminal; 2) The present technique allows extremely sensitive characterization of both reduced and non-reduced plasma fibrinogen in the presence of all other plasma proteins.

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IMMUNO-VISUALIZATION OF FIBRINOGEN AND FIBRIN IN GELS PRDUCED BY GELATION OF PLASMA WITH ETHANOL.
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The Ethanol Gelation Test(=EGT) is a well-documented,simple,specific and frequently used test to detect plasma soluble fibrin(Godal &Abildgaard:Gelation of soluble fibrin in plasma by ethanol.Scand. J.Haemat.3,342,1966).If soluble fibrin present in plasma amounts to 1% or more of the plasma-fibrinogen conc.,the admixing of 0.15 ml 50% ethanol to 0.5 ml plasma in a test tube will-(subsequent to incubation for 10 min at 20°C)-upon tilting the test tube semi-horizontally produce a characteristic,(upwardly) convex gel.
Although earlier studies have confirmed the validity and specificity of EGT as a means to detect soluble fibrin,we found it of interest to see to which degree such soluble fibrin is FXIII-stabilized EGT-positive(from patients with Disseminated Intravascular Coagulation(DIC) and EGT-negative plasma was studied as follows:The EGT test was performed as above,and the entire content of the test tube emptied upon a nylon micro-meshed membrane.Applying slight suction underneath the nylon membrane the fluid was removed,leaving the ethanolprecipitated material,which was immediately dissolved in SDS (1%)-urea(5M)-Tris-HCl(0.15M,pH8.6).After incubation at 100°C for 1 min the material was SDS-electrophoresed on flat-bed agarose(2%) Subsequent to Western-blotting onto nitrocellulose and gelatine-blocking,the fibrin(ogen)-related pattern was reacted with either: a)polyclonal antibodies to fibrinogen,b)plyclonal antibodies to FPA or c)monoclonal antibody to FPA(gift from Dr.Nieuwenhuizen,Leyden, Holland).Then,the fibrin(ogen)related pattern was developed using peroxidase-conjugated secondary antibodies.From the specificity of the primary antibodies used,it could be deduced that:1)A substantial amount of the soluble fibrin content of DIC-plasma was present in an oligomeric form(up to 6-mers).2)These oligomers contained fibrinogen,i.e. thus representing FXIII-linked fibrinogen/fibrin hybride molecules.3)Even normal plasma contained some detectable oligomers(up to 3-mers).4)Collecting blood with all appropriate thrombin- and FXIII-inhibitors did not change the patterns obtained and described above.It may be concluded that soluble fibrin occurs mainly in a FXIII-stabilized,oligomeric form which contains fibrinogen Due to the nature of the polymerization process,the fibrinogen moiety of these hybride molecules must be end-located,representing a physiological means to inhibit further polymer growth.