520

MONOCLONAL ANTIBODY IDENTIFICATION OF FIBRIN DEPOSITS IN RENAL DISEASE. A. Bini (1), V. D'Agati (1), C. Pirani (1), B. Kudryk (2), K.L. Kaplan (1). Departments of Medicine and Pathology, Columbia University (1), and The New York Blood Center (2), New York, NY, USA.

Glomerular and vascular "fibrin" deposition has frequently been reported in human and experimental renal diseases. The biochemical form of this "fibrin" has not been well defined. We studied 16 renal biopsies (Bouin's fixed paraffin embedded) with the ABC-immunoperoxidase technique using monoclonal antibodies (MAbs); MAb I8C6 (Bβ1-42) to fibrinogen and fibrin I; MAb T2G1 (β15-42) to fibrin II; and MAb GC4 to fragment D or D-D. Polyclonal antisera to fibrinogen, albumin and IgG were used as controls. Renal biopsy specimens included 9 cases of microangiopathy (Group I: 6 hemolytic uremic syndrome (HUS), 1 scleroderoma, 2 acute humoral rejection) and 7 miscellaneous cases of other renal disease (Group II: 2 IgA nephropathy, 2 minimal change disease, 2 membranous GN, 1 acute interstitial nephritis). Fibrinogen and fibrin I were present on the glomerular (glom) endothelial cells in 8/9 Group I and 7/7 Group II cases, but was present in glom capillary lumens in Group I only. Staining of the endothelial aspect of interstitial capillaries, arterioles and arteries was also observed in both groups. Fibrin II was present in most glom and interstitial capillaries in both groups. However, intense staining for fibrin II was observed in arterioles and arteries in Group I only. Staining for fragments D and D-D was observed in glom capillaries in 6 Group I cases (6 HUS) and 3 Group II cases (2 IgA nephropathy, 1 membranous GN) but was most diffuse and intense in Group I. Traditional histochemical stains for fibrin (Lendrum and PTAH) were positive in 4 Group I cases only, indicating that the ABC technique is far more sensitive. Controls (14 needle biopsies of non-renal tissue) showed no vascular ractivity for fibrinogen, fibrin I, II and fragments D and D-D, suggesting that the vascular staining observed in renal biopsy tissue is not caused by the biopsy procedure itself. These findings indicate that 1) Fibrin formation and lysis occur in many renal diseases of both vascular and non-vascular origin. 2) Fibrinolytic activity is higher in the glom capillaries than in the larger vessels. 3) Damaged renal endothelium may be an active participant in these processes.

521

DIFFERENCES IN PEPTIDE MAPS OF α POLYMERS FROM FIBRIN PRODUCED IN THE PRESENCE AND ABSENCE OF ERYTHROCYTES.
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α chain polymerisation during clot formation is accelerated in the presence of erythrocytes. This effect is abrogated if the erythrocytes are obtained from patients with various haemoglobinopathies. Enzyme digests of the α polymers produced in the presence or absence of erythrocytes were prepared to further define any differences between them.

Clots were produced from citrate/EACA plasma samples or plasma/erythrocyte mixtures by the addition of thrombin and calcium. After five hours, clots were washed in 8 M urea until traces of haemoglobin were removed. After reduction and alkylation clots were dissolved in 0.5% SDS. α polymers were purified on sephacryl S-300 and protein concentrations were adjusted to 0.5 mg/ml. These were digested with S. Aureus V8 protease (150 mg/ml), papain (50 mg/ml) or chymotrypsin (100 mg/ml) at 37°C at sequential time intervals.

After the addition of 2% SDS samples were analysed on 15% SDS polyacrylamide gels.

In all cases digestion of α polymers from clots formed in the absence of erythrocytes took place more rapidly and contained peptide bands not apparent in other digests.

The observations suggest that α polymers formed in the presence or absence of erythrocytes exhibit differing kinetics of response to proteolytic cleavage and indicate that erythrocytes may influence the primary and/or quartenary structure of the polymers studied.

522

EARLY α CHAIN CROSSLINKING OF PARTIALLY DEGRADED FIBRIN(OGEN) MOLECULES. J.H. Sobel, C.A. Thibodeau and R.E. Canfield. Department of Medicine, College of Physicians and Surgeons of Columbia University, New York, NY, USA.

Previous immunochemical studies have demonstrated that the introduction of α chain crosslinks by Factor $XIII_a$ begins immediately after fibrin aggregation. While there are data to indicate that regions Aα #241-476 and Aα #518-584 are specifically involved in this early crosslinking process, identification of the exact glutamine and lysine residues that participate in the reaction remains to be determined. In this study monoclonal antibodies, whose specificity for these two regions has been defined (anti-Aα #259-276, F-103; anti-Aα #540-554, F-102), were used to isolate preparations of partially degraded fibrinogen molecules which could then be characterized for COOH-terminal Aα chain heterogeneity and crosslinking capacity to obtain a more precise localization of residues involved in early crosslinking. In order to assess the relative contribution of sites within the COOH-terminal region that includes 1 potential GLN at Aα #563 and 4 potential LYS at Aα #556, 562, 580 and 583, fibrinogen molecules that were missing these residues were isolated from preparations of purified fibrinogen using F-102-Sepharose immunoaffinity adsorption. Characterization of the partially degraded material (i.e., non-binding) by Western Blotting using F-102 and F-103 confirmed the presence of Aα remnants (29K-66K) that shared the structure Aα #1-276 but differed in the extent to which regions between ∿#276 and ∿#539 were preserved. When the crosslinking capacity of these partially degraded molecules was examined, using Western Blotting to monitor the appearance of crosslinked α chains during in vitro clotting, effective formation of early crosslinked species (∿100K, ∿200K) as well as eventual accumulation of α polymers, could be demonstrated. These findings indicate that at least one early α chain crosslink involves activity on the part of LYS and GLN residues located proximal to Aα #540. While these data do not rule out the existence of additional crosslinking sites situated more distally (i.e., within Aα #540-584), they do indicate that degraded fibrinogen molecules, circulating under pathophysiologic conditions, may undergo adequate fibrin stabilization despite a loss of at least 70 COOH-terminal Aα chain residues.

523

MODIFICATION OF FIBRIN POLYMERIZATION INDUCED BY MONOCLONAL ANTIBODIES AGAINST FRAGMENT D DOMAIN OF FIBRIN/OGEN DEGRADATION PRODUCTS. **M. Mirshahi, (1), J. Soria (1), C. Soria (2), S. Mirshahi (2) J.Y. Perrot (1), C. Boucheix (3) A. Bernadou (1), Laboratoires des Professeurs Adolphe, Bernadou et Samama, Hôtel Dieu, Paris, France. (1). Département d'hématologie Professeur Caen, Unité INSERM 150, LA 334 CNRS, Hôpital Lariboisière,Paris, France (2), INSERM U. 268, Villejuif, France (3).**

Monoclonal antibodies (McAb) are often used to detect the domains of a molecule involved in an important function of a biological system.

In this work, the effect of 20 McAb directed against the D domain of fibrin/ogen degradation products (FbDP) were analyzed on fibrin polymerization. Two McAb which react with fragment D1 but not with fragment D3 inhibited both thrombin and reptilase-induced fibrin formation. It is therefore suggested that these 2 McAb recognize an epitope in the vicinity of the polymerization site "a" located in the γ 374-396 domain in fibrin/ogen molecule. (This sequence is only present in D1 and not in D3.

An inhibitory effect on thrombin and reptilase-induced fibrin formation was also induced by 2 other anti D McAb which recognize an epitope present in both D1 and D3 fibrinogen-degradation products. Therefore, these 2 McAb do not react directly with the polymerizing site "a". A possible mechanism of this inhibition could be a conformational change induced by the binding of these McAb, leading to the masking of the polymerizing site "a". Another possible mechanism of the inhibitory effect is that thrombin and reptilase-induced fibrin formation may involve other sites on the fibrinogen molecule. It should therefore be suggested that unknown domains could be important for fibrin polymerization. These domains may act either directly or indirectly by inducing a three dimensional structure of the native molecule required for the expression of polymerizing domains.