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DEMONSTRATION OF C_{3d} AND C_{3b} IN MESANGIAL IgA-GLOMERULONEPHRITIS AND HENOCH-SCHÖNLEIN PURPURA NEPHRITIS

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Summary

Glomerular deposits of C_{3b} and C_{3d} were visualized by immunofluorescence using specific antibodies against C_{3d} and C_{3c} (the latter detecting tissue bound C_{3b}) in biopsy specimens of 23 patients with IgA nephritis and two with Henoch-Schönlein purpura nephritis. C_{3b} and C_{3d} deposits were identified in all locations where IgA deposits were present. C_{3d} staining was more intense than C_{3b} staining in 11 of 25 patients; in addition, staining for C_{3d} was positive in locations where C_{3b} could not be detected (capillary pattern for C_{3b} in six of 25 patients; for IgA in 11 of 25; for C_{3d} in 17 of 25). These findings indicate local complement activation.

Introduction

Mesangial IgA glomerulonephritis (IgA-GN) is one of the most frequent forms of glomerulonephritis. It is characterized by mesangial or mesangioperipheral deposits of IgA (often together with smaller amounts of IgG and/or IgM) and of 'C_3'-antigens [1]. It is not known whether preformed IgA polymers and/or IgA immune complexes (IgA-IC) [2] home into the mesangium or whether immune complexes are formed in situ. Aggregated IgA and/or IgA-IC can activate the complement system via the alternative pathway [3,4]. Detection of 'C_3' antigens in mesangial IgA deposits cannot be interpreted as evidence of local (in situ) complement activation because such deposits could also be caused by IgA-IC having incorporated activated complement components in the circulation. Consistent demonstration of 'C_3' at glomerular sites with no staining for IgA but adjacent to IgA deposits would argue for local C_3 activation with local scattering of C_3 reaction products. Therefore, we compared the staining for IgA, C_{3c} and C_{3d} in biopsy specimens of patients with IgA-GN and Henoch-Schönlein nephritis.

Materials and methods

Twenty-five kidney biopsies were evaluated which showed the pathognomonic extensive granular IgA deposits with purely mesangial (14 of 25) or mesangio-peripheral pattern (11 of 25). Twenty-three patients had IgA-GN and two Henoch-Schönlein purpura nephritis. Clinical features were gross haematuria (11 of 25) or microscopic haematuria (9 of 25) with or without proteinuria at first presentation; two cases presented exclusively with proteinuria, one with hypertension; Henoch-Schönlein nephritis patients presented with purpura. The IgA-GN cases were classified histologically as minimal glomerular lesions (MGL: 7 of 23), focal segmental glomerulonephritis (FSGN: 5 of 23), mesangio-proliferative glomerulonephritis (MPGN: 8 of 23), endo- and extracapillary glomerulonephritis (EEGN: 2 of 23) and diffuse glomerular sclerosis (GS:1 of 23). One of the Henoch-Schönlein cases was classified as FSGN, the other as MGN.

Immunofluorescence microscopy Acetone fixed cryostat sections were incubated with IgA antibodies, unlabelled rabbit-anti-C_{3c} (for staining of C_{3b} deposits) or rabbit-anti-C_{3d} (all from Dakopatts), diluted 1:20 with PBS containing 10% normal swine serum. At this dilution, both antisera (C_{3c} and C_{3d} respectively) caused staining of similar intensity with C_{3b}- or C_{3d}-thiol-sepharose prepared according to Ross [5]. The second antibody was FITC-labelled swine-anti-rabbit-IgG (Dakopatts). The sections were examined in a Zeiss Standard 18 fluorescence microscope (epi-illuminator; HBO 50 lamp, excitation filter 485 + 10nm, barrier filter 515nm). Two independent investigators assessed 1) localization of glomerular deposits, and 2) fluorescence intensity of the deposits, i.e. weak (1+), marked (2+) or extensive (3+).

Results

IgA-, C_{3b}- and C_{3d}-deposits were found in all patients. There was a close correspondence of the localization pattern, i.e. mesangial versus mesangio-peripheral, for IgA on the one hand and C_{3b} on the other hand ($p<0.002$). In contrast, C_{3d} was found in a mesangioperipheral pattern in 17 of 25 cases whereas a mesangioperipheral pattern for C_{3b} was found in only six of 25 and for IgA in 11 of 25 cases. In particular, six cases with purely mesangial IgA had C_{3d} in the capillary loops. One such case is shown in Figure 1. Elution experiments in six cases (data not shown) showed that C_{3d} and C_{3b} were covalently bound. In 11 of 25 cases fluorescence was more intensive with anti-C_{3d} than with anti-C_{3c}. This was observed particularly in cases with MGL and FSGN.

Discussion

In this study, different patterns of localization were found for binding of C_{3c} and C_{3d} antibodies in patients with IgA-GN or Henoch-Schönlein purpura nephritis. C_{3b} was detected with an anti-C_{3c} antibody which reacts with C_3,

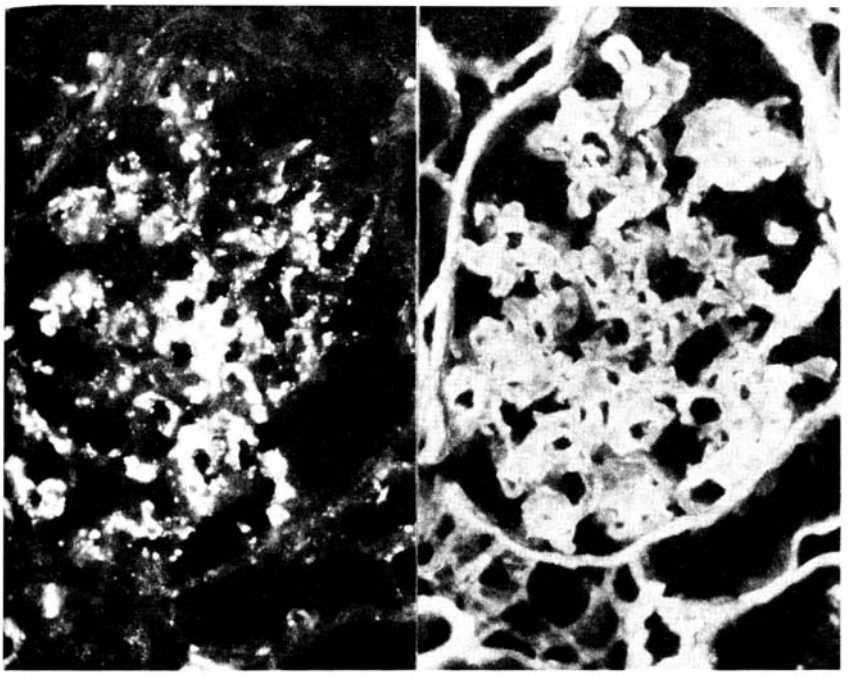

Figure 1. Comparison of immunofluorescence staining for C_{3b} (left) and C_{3d} (right) in a patient with IgA-GN. Note demonstration of C_{3d} staining with high intensity in a pseudolinear pattern, in sites which do not stain for C_{3b}

C_{3b}, iC_{3b} and C_{3c} as previously shown by immunoblotting [6]. Anti-C_{3d} reacted with C_3, C_{3b}, iC_{3b}, C_{3d} and $C_{3d\text{-}g}$, although the reaction with the C_3 and C_{3b} was faint. Such weaker reactivity (even in dot-blot analysis using native C_3 and C_{3b}) suggests that deposits identified in the tissue with anti-C_{3d} primarily represented the metabolites C_{3d} and $C_{3d\text{-}g}$. Since elution experiments failed to demonstrate indications for unspecific adsorption of 'C_3'-antigens, it must be assumed that we detected mainly covalently attached C_{3b} and iC_{3b} or C_{3d} and $C_{3d\text{-}g}$ respectively. In the present study we found C_{3d} in glomerular sites which did not stain for either C_{3b} or IgA. As indicated above, elution studies suggested covalent binding and chess-board titration using C_{3b}- or C_{3d}-thiol-sepharose [5] suggesting comparable detection thresholds for anti-C_{3c} and C_{3d} respectively. Upon activation of C_3 an internal thioester group is activated on the alpha-chain of C_{3b}. The activated peptide binds within fractions of a second to reactive groups (SH, OH or primary aminogroups) [7]. In the presence of factor I and H (or CR-1 on cell membranes) such C_{3b} is rapidly cleaved into C_{3d} (or $C_{3d\text{-}g}$) which remains covalently attached, while fragments bearing the C_{3c} epitopes are released [5]. Tissue bound C_{3d} is more resistant than C_{3b} against further degradation.

The discrepancy between the localization of IgA and C_{3d} deposits suggests the following chain of events: activation of C_3 by alternative pathway C_3-convertases, locally induced by IgA-IC, generates an excess of C_{3b}. Even within the short time frame while the thioester group is reactive, some activated C_{3b} molecules may escape into adjacent glomerular structures where they are rapidly cleaved to C_{3d}. Detectable C_{3d} despite no IgA is presumably not an artefact of low IgA-antibody sensitivity, since similar findings were obtained with a variety of different IgA antibodies. In other conditions involving IgA deposition in the tissue, e.g. linear IgA dermatosis, strict co-localization of IgA and C_{3d} was noted. The above findings in IgA-GN contrast also with those in other forms of glomerulonephritis, e.g. membranous GN [8–10] and anti-glomerular basement membrane GN [9–10] where immunoglobulins and C_{3d} were found in identical sites.

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