DEMONSTRATION OF C₃d AND C₃b IN MESANGIAL
IgA-GLOMERULONEPHRITIS AND HENOCH-SCHÖNLEIN
PURPURA NEPHRITIS

E W Rauterberg, Anne-Margaret Wingen, H-M Lieberknecht, E Ritz

Institute of Immunology and Serology, Internal Medicine,
University of Heidelberg, FRG

Summary

Glomerular deposits of C₃b and C₃d were visualized by immunofluorescence using specific antibodies against C₃d and C₃c (the latter detecting tissue bound C₃b) in biopsy specimens of 23 patients with IgA nephritis and two with Henoch-Schönlein purpura nephritis. C₃b and C₃d deposits were identified in all locations where IgA deposits were present. C₃d staining was more intense than C₃b staining in 11 of 25 patients; in addition, staining for C₃d was positive in locations where C₃b could not be detected (capillary pattern for C₃b in six of 25 patients; for IgA in 11 of 25; for C₃d 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 mesangiothelial deposits of IgA (often together with smaller amounts of IgG and/or IgM) and of ‘C₃’-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 ‘C₃’ 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 ‘C₃’ at glomerular sites with no staining for IgA but adjacent to IgA deposits would argue for local C₃ activation with local scattering of C₃ reaction products. Therefore, we compared the staining for IgA, C₃c and C₃d 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 (EEN: 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-C₃c (for staining of C₃b deposits) or
rabbit-anti-C₃d (all from Dakopatts), diluted 1:20 with PBS containing 10%
normal swine serum. At this dilution, both antisera (C₃c and C₃d respectively)
caused staining of similar intensity with C₃b or C₃d-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 glome-
ricular deposits, and 2) fluorescence intensity of the deposits, i.e. weak (1+),
marked (2+) or extensive (3+).

Results

IgA-, C₃b- and C₃d-deposits were found in all patients. There was a close cor-
respondence of the localization pattern, i.e. mesangial versus mesangio-peripheral,
for IgA on the one hand and C₃b on the other hand (p<0.002). In contrast,
C₃d was found in a mesangio-peripheral pattern in 17 of 25 cases whereas a
mesangio-peripheral pattern for C₃b was found in only six of 25 and for IgA
in 11 of 25 cases. In particular, six cases with purely mesangial IgA had C₃d
in the capillary loops. One such case is shown in Figure 1. Elution experiments
in six cases (data not shown) showed that C₃d and C₃b were covalently bound.
In 11 of 25 cases fluorescence was more intensive with anti-C₃d than with anti-
C₃c. This was observed particularly in cases with MGL and FSGN.

Discussion

In this study, different patterns of localization were found for binding of C₃c
and C₃d antibodies in patients with IgA-GN or Henoch-Schönlein purpura
nephritis. C₃b was detected with an anti-C₃c antibody which reacts with C₃,
C₃b, iC₃b and C₃c as previously shown by immunoblotting [6]. Anti-C₃d reacted with C₃, C₃b, iC₃b, C₃d and C₃d-g, although the reaction with the C₃ and C₃b was faint. Such weaker reactivity (even in dot-blot analysis using native C₃ and C₃b) suggests that deposits identified in the tissue with anti-C₃d primarily represented the metabolites C₃d and C₃d-g. Since elution experiments failed to demonstrate indications for unspecific adsorption of ‘C₃’-antigens, it must be assumed that we detected mainly covalently attached C₃b and iC₃b or C₃d and C₃d-g respectively. In the present study we found C₃d in glomerular sites which did not stain for either C₃b or IgA. As indicated above, elution studies suggested covalent binding and chess-board titration using C₃b- or C₃d-thiol-sepharose [5] suggesting comparable detection thresholds for anti-C₃c and C₃d respectively. Upon activation of C₃ an internal thioester group is activated on the alpha-chain of C₃b. 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 C₃b is rapidly cleaved into C₃d (or C₃d-g) which remains covalently attached, while fragments bearing the C₃c epitopes are released [5]. Tissue bound C₃d is more resistant than C₃b against further degradation.
The discrepancy between the localization of IgA and C3d deposits suggests the following chain of events: activation of C3 by alternative pathway C3-convertases, locally induced by IgA-IC, generates an excess of C3b. Even within the short time frame while the thioester group is reactive, some activated C3b molecules may escape into adjacent glomerular structures where they are rapidly cleaved to C3d. Detectable C3d 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 C3d was noted. The above findings in IgA-GN contrast also with those in other forms of glomerulonephritis, e.g. membranous GN [8–10] and antiglomerular basement membrane GN [9–10] where immunoglobulins and C3d were found in identical sites.

References

1 Berger J, Hinglais N. J Urol Nephrol 1968; 74: 694
3 Miller GW. J Immunol 1976; 117: 1374
6 Martinez J, Rauterberg EW. Immunobiol 1985: submitted
7 Law SK, Lichtenberg NA, Levine RP. J Immunol 1979; 123: 1388