'IN SITU' IMMUNE COMPLEX NEPHRITIS AND BASIC PROTEINS

A Vogt, H U Schmidt, H Takamiya, S Batsford

University of Freiburg I. Br., FRG

Summary

This investigation was designed to clarify the potential role of basic antigens in immune complex nephritis. The GBM is negatively charged and binds cationic substances. We studied 1) whether chemically cationised proteins can bind to the GBM, act as planted antigen, and induce 'in situ' immune complex formation or 2) if a pre-formed cationic antigen antibody complex can localise in the GBM. Results obtained with 5 chemically cationised proteins support the first possibility.

Introduction

For more than thirty years, the idea that glomerular deposition of soluble immune complexes (IC) was the precipitating event in immune complex nephritis, was virtually dogma. A riddle in this conception is to explain why such complexes deposit in the GBM, as preformed IC have little or no affinity for this structure [1]. Observations on the Heymann model of nephritis reopened the discussion concerning the general validity of this concept [2-4]. Since then a growing body of evidence has accumulated which supports the view that 'in situ' formation of immune complexes may induce glomerulonephritis (for a detailed review see [1]). In this case the problem lies in explaining why antigens, rather than IC, bind to the GBM.

We postulated that basic proteins could play a crucial role in IC nephritis since it is known that the GBM is negatively charged and has affinity for cationic substances. A basic protein which had bound to the GBM (planted antigen) could act as a target for circulating antibody and produce an 'in situ' IC. On the other hand a soluble IC involving a basic antigen might also be able to fix to the GBM by electrostatic attraction.

We tested both these possibilities with the aid of chemically cationised, purified proteins.
**Materials and methods**

Experiments were performed in Male Wistar rats of body weight between 100 and 120g.

The test material was injected under ether narcosis either into the tail vein or directly into the left renal artery.

The following proteins were used: Ovalbumin (Sigma, A-5503), HSA (Behringwerke, FRG), Hu IgG (Bergglobin, Behringwerke, FRG), Ferritin was prepared from horse spleens [5], Hu IgM was isolated from Myeloma serum by combined ε-globulin precipitation and gel filtration. Proteins were cationised by substituting carboxyl groups with amino groups [6]. The isoelectric points (PI) of the products were determined with the aid of slab gel isoelectrofocussing (IEF).

Antisera were prepared by immunising rabbits with the appropriate native antigen in Freund’s complete adjuvant; the IgG fractions were labelled with FITC or TRITC.

The Fab fragment of rabbit anti HSA IgG antibody was prepared by Papain digestion [7].

**Results**

With the aid of immunofluorescent staining we investigated the influence of molecular size and overall net charge of protein antigens on binding to the glomerular basement membrane. The results are shown in Table I.

<table>
<thead>
<tr>
<th>Cationised Antigen*</th>
<th>Isoelectric point</th>
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<tr>
<td></td>
<td>7.5–8.5</td>
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<tr>
<td>Ovalbumin</td>
<td>0</td>
</tr>
<tr>
<td>HSA</td>
<td>0</td>
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<tr>
<td>Hu IgG</td>
<td>0</td>
</tr>
<tr>
<td>Ferritin</td>
<td>++†</td>
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*up to 5mg cationised protein was injected intravenously into rats. Kidneys were removed 30 minutes later
† mainly mesangial
‡ partially mesangial

For a given protein, the binding increased with increasing cationisation. At a given PI, binding increased with molecular size; between 500 000 and 900 000 daltons there appears to be a cut off, since even highly cationised IgM localised mainly in the mesangium. The fluorescent staining pattern of highly cationised Hu IgG is shown in Figure 1.
Figure 1. Fluorescent micrograph depicting the binding of highly cationised Hu IgG in a largely linear pattern along the glomerular capillary wall. This rat was given 1mg of modified antigen intravenously, and sacrificed after 15 minutes. Section stained with FITC labelled anti-Hu IgG . (x 320)

The degree of persistence of highly cationised proteins is shown in Table II. Maximum binding occurred within minutes; with cationised proteins capable of binding to GBM persistence increased with molecular size.

TABLE II. Correlation between molecular size and persistence of cationised antigens in the GBM

<table>
<thead>
<tr>
<th>Cationised Antigen*</th>
<th>Time elapsed after injection</th>
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<tr>
<td></td>
<td>30 minutes</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>++</td>
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<tr>
<td>HSA</td>
<td>++</td>
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<tr>
<td>Hu IgG</td>
<td>+++</td>
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<tr>
<td>Ferritin</td>
<td>+++</td>
</tr>
<tr>
<td>Hu IgM</td>
<td>+++†</td>
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*5 to 10mg of highly cationised antigens (I.P. > 10.0) per rat were injected
†mainly mesangial localisation

To test the accessibility of the planted antigens (Ovalbumin, HSA, Hu IgG, ferritin and Hu IgM) we injected the appropriate specific antibody one to three hours following antigen administration. This led to an intensive deposition of the antibody in the same pattern as the antigen (Figure 2).

In further experiments we studied the glomerular localisation of preformed immune complexes consisting of highly cationised antigen and antibody. Firstly we prepared complexes consisting of highly cationised Hu IgG and the IgG fraction
of the antiserum in an antigen-antibody ratio of 10 to 1. This produced largely soluble complexes showing, in IEF, a net charge of pH between 8.5 – 10.0 (Figure 3). We administered up to 15mg of the mixture per animal into the renal arteries. Binding within the glomerulus was weak and appeared largely within the mesangium (Figure 4). As a control a mixture of cationised antigen and normal rabbit Ig was employed, only staining for antigen was positive.

In a second experiment we used Fab fragments of anti-HSA instead of antibody. This was mixed with cationised HSA in a molar ratio of 14:1. The resulting complexes consisted largely of one antigen molecule bound to one antibody molecule and had an PI of 8.0 to 9.5 (Figure 3). Before injection the free Fab molecules were removed by gel filtration on Sephadex G 100. No free antigen was present. Up to three mg of this material was injected into the renal arteries. Clear positive staining for both antigen and antibody along the capillary wall was seen (Figure 5), the intensity of staining was however not comparable with that seen with 'in situ' formation. No staining was noted when HSA plus Fab fragments of anti HSA or modified HSA plus Fab fragments of normal rabbit IgG were administered as controls.

Discussion

These experiments demonstrate the potential role of the anionic binding sites of the GBM in the pathogenesis of immune complex nephritis. Cationic moieties bound to these anionic sites, provided their molecular weight did not exceed a critical value lying between 500 000 and 900 000 daltons. The critical PI was between 8.5 and 9.5. Preferential accumulation and persistence within the peripheral GBM of highly cationised protein appears to be favoured when the mole-

616
Figure 3. Slab gel isoelectrofocusing; 1) IgG fraction of rabbit anti-Hu IgG; 2) Complex of cationised Hu IgG and anti-Hu IgG. The complexes lie between pH 8.5 and 10.0. Free antigen (pH > 10.0) is present. 3) Cationised Hu IgG. 4) Fab fragment of rabbit anti-HSA. 5) Complex formed from cationised HSA and Fab fragment of rabbit anti-HSA. The isoelectric point of the complex lies between pH 8.0 and 9.5. 6) Cationised HSA

Figure 4. Fluorescent micrograph of rat glomerulus after injection of 15mg of cationised Hu IgG-rabbit anti-Hu IgG complex directly into the left renal artery. Stained with TRITC labelled anti-rabbit IgG. Staining is mainly confined to the mesangial region. (x 320)
Figure 5. Fluorescent micrograph of rat glomerulus after injection of 3mg of cationised HSA-rabbit anti-HSA (Fab fragments) into the left renal artery. Stained with TRITC labelled anti-rabbit IgG. Deposition of complex along the glomerular capillary wall is apparent. (x 320)

cellular size is about 150 000 to 500 000 daltons. Smaller molecules disappear more rapidly whilst larger molecules accumulate within the mesangium additionally.

On the basis of these observations we were able to select conditions to test the two pathogenic mechanisms, involving basic antigens, postulated in the introduction.

In the first case we clearly demonstrated that a highly cationised protein can act as a planted antigen with which circulating antibody can react to form an ‘in situ’ immune complex. Provided the antigen was administered directly into the renal arteries, ‘in situ’ immune complex formation led to a severe GN with massive subepithelial dense deposits and marked proteinuria (results to be published in detail elsewhere). Our attempts to localise a preformed soluble basic antigen-antibody complex were only partially successful. In spite of intrarenal injection of large quantities of complex only weak immunofluorescent staining was seen in the glomerular capillary wall, and then only when small complexes were given (HSA plus Fab fragment). Larger complexes localised preferentially in the mesangium. We did not observe proteinuria following administration of these complexes.

These preliminary results suggest that if basic antigens are involved in the pathogenesis of immune complex nephritis, then they act as a planted antigen rather than as part of a cationic, soluble IC.

Other reports have provided evidence that ‘in situ’ IC formation is a relevant pathomechanism in GN. In Heymann nephritis it has been shown that the GBM contains an antigen similar to FX1A present in the proximal tubules (either as intrinsic or as a planted antigen) which acts as a target for circulating antibody [2–4], resulting in a membranous GN. A similar mechanism has been claimed to
operate in man [7]. The finding that DNA has an affinity for GBM preparations led to the suggestion that lupus nephritis is also an example of 'in situ' immune complex formation [8]. Golbus and Wilson [10] demonstrated that the lectin Concanavalin A could bind to the endothelial side of the GBM and act as a planted antigen, and subsequent injection of anti-Con A antibody caused transient proteinuria.

To date the relevance of basic antigens as nephritogenic agents in man is purely speculative. Based on our results it would however be worthwhile to look for basic antigens in cases of IC nephritis, particularly when infectious agents are involved.

References

1. Couser WG, Salant DJ. *Kidney Internat* 1980; 17: 1

Open Discussion

STRUYVENBERG (Chairman) This is an excellent demonstration of one of the ways in which the glomerular basement membrane can be damaged and has very important clinical implications I think.

BOULTON-JONES (Glasgow) Did proteinuria occur after you planted the cationic antigen but before antibody had arrived at the basement membrane?

VOGT No, we did this control experiment of course and we did not observe any proteinuria. Usually in our experiments when we injected antigen followed by antibody the proteinuria starts after 1–3 days and lasts about 3–4 weeks.

BOULTON-JONES Did you do any biopsies at say day 2 to see if there was foot process fusion as a result of the cationic protein planted in the GBM?

VOGT If you inject very high doses of highly cationised antigen then you may see fusion of the epithelial cells, as in the amino-nucleoside nephrosis.

ROSSMANN (Prague) From the presentation it seemed that the time lapse between the administration of antigen and the assembling of tissue was up to 10 days. This is the time for acute glomerulonephritis to appear. Did you observe any changes evoking the immunological picture of acute glomerulonephritis, like proliferation, leucocytic reaction or platelet aggregation?
VOGT We have only done a little histology. As you know in the rat acute glomerulonephritis is not characterised by proliferation and the main feature is a thickening of the basement membrane and dense deposits in the subepithelial area and changes in the epithelial foot processes.

ROSSMANN From the quantitative point of deposition was there any difference between the various antigens cited, like ferritin?

VOGT Yes, we obtained the best results with highly cationised human IgG and so we restricted ourselves mainly to this antigen, but you also can get immune complex nephritis using cationised ferritin.

NYULASSY (Co-Chairman) Is it known what the anionic sites in the glomerular basement membrane are?

VOGT The negative structures of the glomerular basement membrane contain sialic acid residues, and they are mainly located in the subepithelial region because it is known that anionic dyes bind mainly to this region.

BROWN (Sheffield) Are you absolutely certain there was no remaining free circulating antigen before you injected the antibody?

VOGT I can give you a preliminary answer. We labelled the cationised antigen with $^{125}$Iodine and no radioactivity could be observed in the circulation at the time of antibody administration.