Virchows Arch. A Path. Anat. and Histol. 384, 103–107 (1979)

Virchows Archiv A

Glomerular Localization of Preformed Immune Complexes in Nephrotoxic Serum Nephritis

S. O'Regan

Department of Pediatrics (Nephrology Division), University of Montreal, Hôpital Sainte-Justine, Centre de Recherche Pédiatrique, 3175, Chemin Côte Sainte-Catherine, Montreal, Quebec, Canada H3T 1C5

Summary. Pre-formed immune complexes solubilized in antigen excess were infused into rats with nephrotoxic serum nephritis and normal control animals. Complex localization was evident in seven of ten nephrotoxic animals suggesting that the presence of immune mediated glomerular injury makes the kidney susceptibility to further immune injury.

Key words: Nephrotoxic nephritis – Immune complexes.

Introduction

Gross abnormalities of glomerular structure and function are associated with immune complex injury to the kidney. Thus anatomical changes as well as increased glomerular permeability to protein may be associated with immune complex deposition in the kidney [2]. Also glomerular injury may be associated with antibody to glomerular basement membranes antigens with similar alterations to that induced by circulating immune complexes [3]. Previous studies using macromolecules, e.g., aggregated IgG, suggested that deficient macromolecule clearance by the glomerular mesangium may result in abnormal uptake of these macromolecules. As it is known that pre-formed immune complexes do not localize in the glomeruli of normal rats [9] we have infused these complexes to determine if glomerular localization of pre-formed immune complexes might result in animals with Masugi nephritis. This allowed us to determine whether deranged mesangial clearance of immune complexes resulted from initial immune injury and thus allowed for possible propagation of immune injury by trapping of complexes.

Materials and Methods

Male adult rats of the Royal Victoria Hospital strain were used. Pre-formed immune complexes were prepared as follows: For production of antiserum a goat was immunized and appropriately boosted with Human Cohn Fr II (The American National Red Cross, Blood Research Laboratory,

Supported by Grant MA-6122, Medical Research Council of Canada

0340-1227/79/0384/0103/$01.00

Bethesda, Maryland) in Freund's complete adjuvant. Globulins were precipitated from serum using saturated ammonium sulphate at 4° C. The globulins from the accumulated antisera were then pooled. A quantitative precipitin curve was produced and the amount of antibody precipitated at equivalence was measured using the Lowry-Folin method [6] for protein determination. The concentration of specific antibody in the purified pooled globulin fraction to human Cohn Fraction II was 7.1 mg/ml. After washing in cold saline, 0.15 M, the precipitated complexes were redissolved to achieve an antibody concentration of 14.2 mg/ml in thirty times antigen excess, this being the minimum degree of antigen excess capable of solubilizing the complexes. The volume of solution containing complexes and antigen was then adjusted by the addition of saline to the required concentration for infusion.

Nephrotoxic serum was produced by repeated injections into rabbits of sonicated rat glomerular basal membrane prepared by the method of Krakower and Greenspon [5] in complete Freund's adjuvant. By this method a high titre pooled anti rat basement membrane antiserum was produced. Each animal was given half of 1 ml intravenously of the nephrotoxic serum 24 h prior to the study, this dose of serum had already been verified to produce severe nephrotoxic serum nephritis.

To ensure access to the circulation, under Pentothal anesthesis a PE 50 catheter was introduced into the external jugular vein of each of the experimental animals. 10 mg of antibody in 30 times antigenic excess was infused over 2 min to the rats and a half of 1 ml of 0.15 M saline was infused into the line to insure complete flushing of all complexes into the rats circulation. Ten rats were given this dose of immune complexes. For control purpose five rats similarly treated were given 2 ml of normal saline via external jugular cutdown.

Twenty four hours after infusion of immune complexes all animals were sacrificed and a sample of kidney tissue was cut and snap frozen in pre-cooled isopenthane. A sample of tissue was also taken for light microscopy. This was embedded in parafin and stained with HPS, PAS and other selected stains for examination by light microscopy.

Four micra sections from both experimental and control animals' renal tissue were stained for rabbit immunoglobulin utilizing a fluorescein isothrocyanate (FITC) [8] labelled anti rabbit IgG antiserum. Similarly all tissues were stained for goat antibody to indicate the presence of infused immune complexes utilizing an FITC rabbit antigoat IgG antiserum.

When positive staining for goat antibody, indicating the presence of infused immune complexes, was observed sections from the same tissue block were then pre-incubated with a non FITC labelled rabbit anti-goat IgG antiserum with subsequent staining with the previously labelled FITC conjugated rabbit anti-goat IgG antiserum.

Results

All rats had deposition of rabbit antibody as indicated by positive staining with the FITC labelled goat anti-rabbit IgG. Thus the nephrotoxic serum utilized was readily detectable along the glomerular basement membrane. Of the 10 animals given an infusion of pre-formed immune complexes seven showed localization of goat antibody indicating the presence of the pre-formed immune complexes. In all seven animals localization was mainly mesangial grade 1+ to 2+ (Fig. 1a). In two animals a granular pattern of complex localization was evident along the glomerular base capillary walls indicating that the increased permeability of the capillaries allowed for localization of complexes in this area (Fig. 1b). Verification that these complexes consisted of the goat antibody with antigen was made by elimination of staining with prior incubation with a non FITC labelled rabbit anti-goat antiserum. There was no immunofluorescent microscopic evidence of complex localization in control animals.

Light microscopic changes typical of Masugi nephritis were evident in tissue from both experimental and control animals.

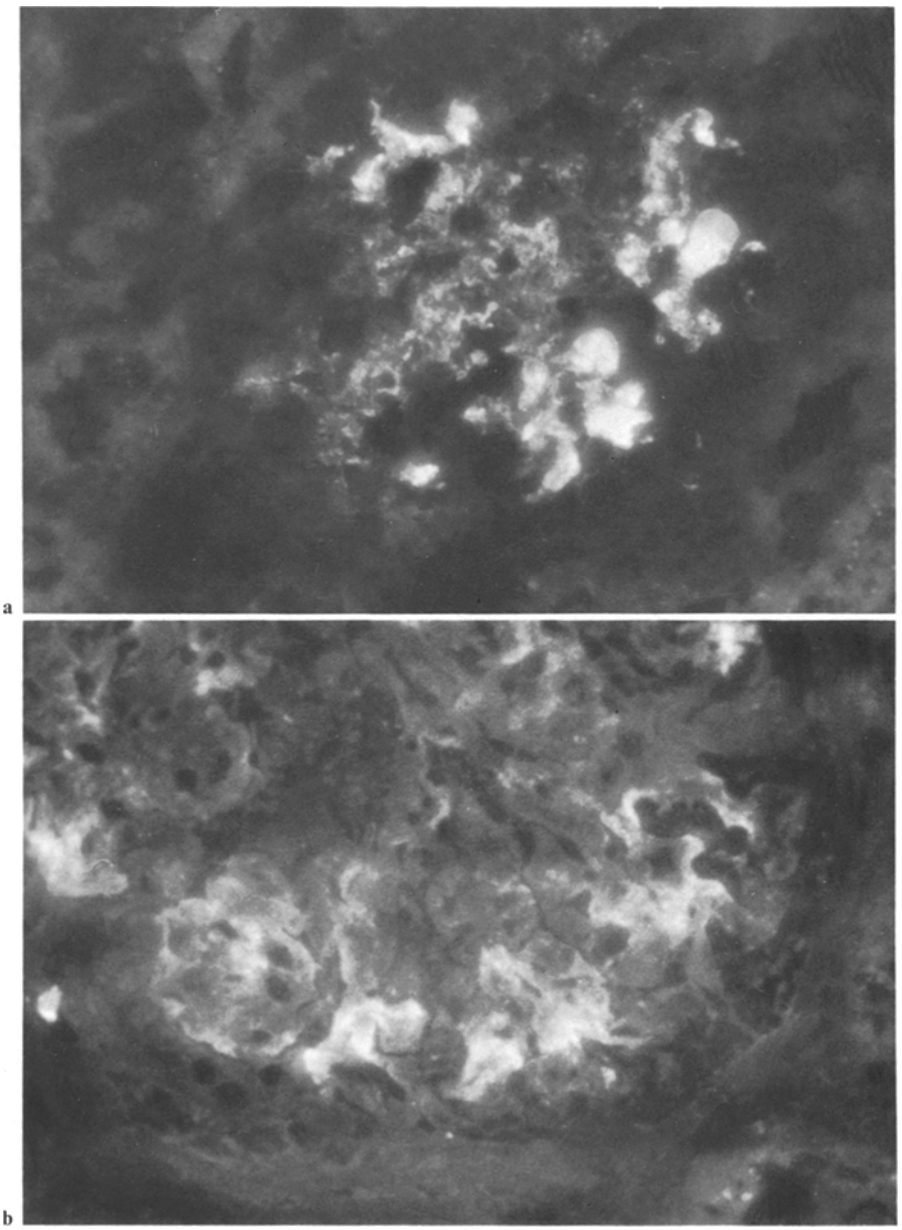

Fig. 1 a and b. Glomerular (**a**) mesangial and (**b**) capillary wall localization of infused immune complexes in nephrotoxic nephritis

Discussion

Previous studies indicated that infusion of solubilized pre-formed immune complexes in normal rats did not result in glomerular localization of these macromolecules. Previous studies utilizing aggregated IgG radiolabelled with ^{125}I injected into rats resulted in mesangial localization of these complexes [7]. However in these studies animals were pre-treated with promazine, a drug with antihistamine proprieties. Since the effect of histamine in influencing complex localization has been well recognized, it might be surmized that the antihistamine effects of the promazine might have influenced the aggregated IgG localization [1]. Animals were sacrificed 24 h after administration of nephrotoxic serum in order that the effect of the rat immune response to the injected foreign protein (the nephrotoxic serum) would be minimized. Thus the rat immune response to the nephrotoxic serum would not influence infused immune complex localization and thus complex deposition would only be due to the injurious effect of the nephrotoxic serum binding to the glomerular basement antigens with associated complement activation, histamine release from platelets [2], lysosomal enzyme release from migrating polymorphonuclear leucocytes [4], etc.

The absence of complex localization in controls indicated that in the absence of immune injury to the glomerulus, mesangial ability to clear deposited immune complexes is normal. However in the animals with nephrotoxic serum nephritis, i.e., immune injury to the glomerulus, localization of complexes was observed. Though deposition was mainly mesangial, in two animals peripheral localization was also evident indicating that increased permeability of the glomerulus allowed for localization of these macromolecules along the glomerular capillary wall. The accumulation of circulating immune complexes in the mesangium in previously immunologically injured glomeruli would suggest that the presence of any immunologic injury might allow accumulation of these nocuous macromolecules with potential for further destruction of the glomerulus. Thus one might speculate that initiation of immunologic mediated glomerular injury leads to a self propagating mechanism for further glomerular destruction. Such an evolution of nephritic injury might also occur in other animal models of immune complex glomerular injury.

Acknowledgement. Supported in part by Grant MA-6122: Medical Research Council of Canada. FITC conjugated antisera were obtained from Cappel Labs. Inc., Thudridge Farm, Cochranville, PA 19330, U.S.A.

References

1. Bolton, W.K., Spargo, B.A., Lewis, E.J.: Chronic autologous immune complex glomerulopathy effect of cyproheptadine. J. Lab. Clin. Med. **83**, 695–704 (1974)
2. Cochrane, C.G.: Mechanisms involved in the deposition of immune complexes in tissues. J. Exp. Med. **134**, 75s–89s (1971)
3. Germuth, F.G., Rodriguez, E.: Immunopathology of the renal glomerulus. Boston: Little, Brown and Co. 1972

4. Henson, P.M.: Interaction of cells with immune complexed, adherence, release of constituents and tissue injury. J. Exp. Med. **134**, 114s–135s (1971)
5. Krakower, C.A., Greenspon, S.A.: Localization of the nephrotic antigen within the isolated glomerulus. Arch. Pathol. **51**, 629–639 (1951)
6. Lowry, O.H., Rosebrough, N.J., Farra, L., Randall, R.J.: Protein measurement with the Folin-phenol reagent. J. Biol. Chem. **193**, 265–275 (1951)
7. Mauer, S.M., Fish, A.J., Blau, E.B., Michael, A.F.: The glomerular mesangium. I Kinetic studies of macromolecular uptake in normal and nephrotic rats. J. Clin. Invest. **51**, 1092–1101 (1972)
8. Michael, A.F., Drummond, K.N., Good, R.A., Vernier, R.L.: Acute poststreptococal glomerulonephritis immune deposit disease. J. Clin. Invest. **45**, 237–248 (1966)
9. O'Regan, S., Smith, M., Drummond, K.N.: Immune complex infusion in the rat. Renal functional and morphological changes. Clin. Exp. Immunol. **24**, 110–115 (1976)

Received December 6, 1979