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Passively transferred anti-brush border antibodies induce injury of proximal tubules in the absence of complement

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SUMMARY

In active Heymann nephritis, antibodies directed against the brush border membrane of proximal tubules are able, when deposited *in vivo*, to cause substantial damage to the tubule epithelium. Prominent features of the lesion include fragmentation and loss of microvilli and proliferation of epithelial cells. Passive transfer of anti-brush border serum to appropriate proteinuric recipients also leads to proximal tubule pathology. In experiments reported here, full expression of the damage was observed in complement deficient recipients of passively transferred anti-brush border serum. A complement-independent process initiated by cross-linking of membrane determinants, which is analogous to the stimulation of B cell proliferation following cross-linking of Ig receptors by appropriate ligands, could account for the pathogenicity of anti-brush border serum.

Keywords Heymann nephritis brush border renal immunopathology decomplementation

INTRODUCTION

Immunization of some strains of rat with an extract prepared from rat kidney cortex produces an autoimmune disease known as Heymann nephritis (Heymann *et al.*, 1959; Edgington, Glassock & Dixon, 1968). Autoantibodies in the sera of rats with Heymann nephritis react *in vitro* with an antigen that is present in large amounts in the brush border of proximal tubules (Grupe & Kaplan, 1969) and has also been demonstrated to be associated with epithelial cells of the glomerular capillary wall (Kerjaschki & Fahrquhar, 1983). An accumulation of autoantibodies and complement *in vivo* at the epithelial side of the glomerular basement membrane leads to the membranous glomerulopathy that is characteristic of Heymann nephritis.

The increased glomerular permeability of Heymann nephritis has been shown, in both active and passive forms of the disease, to be complement-dependent (Salant *et al.*, 1980; Noble *et al.*, 1984). With significant proteinuria circulating anti-brush border antibodies are able to reach the proximal tubules (Mendrick *et al.*, 1980). Deposition of those antibodies *in vivo* along the brush border membrane causes substantial disruption of the normal architecture of the proximal tubules in both active Heymann nephritis and appropriate passive transfer experiments (Mendrick *et al.*, 1980; Noble *et al.*, 1981a). The investigation described in this report was undertaken with the objective of evaluating the role of complement in the pathogenesis of antibody-dependent injury to proximal tubules.

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MATERIALS AND METHODS

Preparation of anti-brush border serum. A pool of serum with high antibody titre to brush border was obtained from female LEW rats (Charles River Breeding Laboratories, Wilmington, Massachusetts, USA) immunized with an extract of kidney cortex (Fx1a) prepared according to the method of Edgington, Glassock & Dixon (1967). Details of the immunization schedule used in our laboratory to produce Heymann nephritis have been described in earlier publications (Mendrick *et al.*, 1980; Noble *et al.*, 1981a). Titres were measured by means of indirect immunofluorescence tests on frozen, acetone fixed sections of normal rat kidney (Mendrick *et al.*, 1980). The antiserum pool used in these experiments, which had a titre of 1:256, was stored at −70°C until the time of passive transfer. To inactivate complement the serum was heated to 56°C for 30 min.

Recipients of anti-brush border serum. As in passive transfer experiments performed earlier, rats with proteinuria resulting from chronic serum sickness served as recipients of anti-brush border serum (Noble *et al.*, 1981a). To produce chronic serum sickness glomerulonephritis female LEW rats were immunized with bovine serum albumin (Cohn Fraction V, Miles Laboratories, Kankakee, Illinois, USA) according to a scheme described in detail in previous publications (Arisz *et al.*, 1978; Noble *et al.*, 1981b). Individuals used as recipients had exhibited abnormal urinary protein excretion (> 50 mg/24 h) for 2–3 weeks. For the determination of urinary protein excretion, urine was collected overnight in metabolism cages in which rats were allowed access to water but not to food. A biuret test was performed to determine the concentration of protein in urine specimens from which the 24 h urinary protein excretion was calculated.

Depletion of complement. Cobra venom factor (CVF) treatment, employed to deplete rats of complement, has been used in a variety of studies to evaluate the contribution of complement activity to immunopathology (Bartolotti & Peters, 1978; Salant *et al.*, 1980; Capron *et al.*, 1982). The methods used in these experiments were similar to those described by others. Purified CVF used in these experiments was the gift of Dr Richard Pickering, Albany, New York, USA. Haemolytic assays to measure complement activity (CH_{50}) of rat serum were performed in the Laboratory of Clinical Immunology, Veterans Administration Medical Center, Buffalo, New York, according to standard methods (Mayer, 1961). A single intravenous injection of 0·2 ml of the CVF preparation reduced CH_{50} values of all normal rats and of all rats with chronic serum sickness from a CH_{50} of > 30 units/ml to undetectable concentrations for 5 days. Urinary protein excretion of rats with chronic serum sickness was found not to be significantly influenced by CVF treatment (before CVF, mean = 153 mg/24 h, after CVF, 149 mg/24 h).

Protocol of passive transfer experiments. Recipients were divided into four groups. Group A ($n = 5$) was given a total of 4·5 ml of heat-inactivated normal rat serum administered intravenously in 0·5 ml doses over a 24 h period according to a schedule that has been described in detail before (Noble *et al.*, 1981a). Group B ($n = 5$) received 0·2 ml CVF 24 h before 4·5 ml of normal rat serum was given exactly as to group A. Group C ($n = 7$) was given 4·5 ml of heat-inactivated anti-brush border serum and group D ($n = 16$) received 0·2 ml CVF 24 h before the transfer of 4·5 ml of anti-brush border serum was started. Animals in all groups were killed 48 h after the start of intravenous injections of normal or anti-brush border serum. Tissues taken at autopsy were processed for examination by light, immunofluorescence and electron microscopy.

Immunofluorescence microscopy. Kidney tissue obtained by right unilateral nephrectomy when the rats were killed was tested for the presence of rat immunoglobulin (Ig) and complement (C3) according to methods described in detail elsewhere (Mendrick *et al.*, 1980). FITC conjugated rabbit antisera were purchased from Cappel Laboratories, Cochranville, Pennsylvania, USA. The extent of deposition was estimated on a scale from 0 to 3 (0 = no fluoresence along brush border, 1 = up to 30% of proximal tubules positive; 2 = 30–70% of proximal tubules positive, 3 = more than 70% of proximal tubules positive). The pattern and extent of deposition of immune reactants in glomeruli was also evaulated and scored on a scale from 0 to 3 (Noble *et al.*, 1981b).

Light microscopy. To achieve optimal preservation of the morphology of proximal tubules, the left kidney was fixed *in situ* by perfusion with 1% glutaraldehyde in modified Tyrode buffer (Mendrick *et al.*, 1980). Kidney tissue was embedded in Epon 812-Araldite, cut to 0·5 μm thickness

and stained with methylene blue. A semi-quantitative estimation of the extent of proximal tubule brush border damage in individual rats was made from examination (at a magnification of × 450) of tissue sections prepared from at least four randomly chosen kidney fragments.

Damage of the proximal tubules: in each of the four sections examined, approximately 25 proximal tubules were judged for loss of brush border, expressed as the percentage of brush border missing or severely damaged, in an individual tubule. Four categories were distinguished: (1) more than $\frac{3}{4}$, (2) $\frac{1}{2}$–$\frac{3}{4}$, (3) $\frac{1}{4}$–$\frac{1}{2}$ and (4) less than $\frac{1}{4}$ of brush border completely absent or severely damaged.

Cells in the lumen of proximal tubules: in each of the four sections, intraluminal cells were counted in five high power fields; the mean number per field was calculated.

To determine whether sections of tissue embedded in plastic were representative of the whole kidney, additional larger pieces of cortical tissue from each kidney were studied.

Statistics. The Mann–Whitney U-test was used for statistical evaluation of data obtained by light microscopy.

RESULTS

Immunofluorescence tests (Table 1)

Deposition of Ig and C3 in the glomeruli of rats in groups A and C was typical of chronic serum sickness (Fig 1a). Heavy accumulations of both immune reactants were present along the glomerular capillary wall. Only recipients of anti-brush border serum (group C and D) had extensive accumulations of rat Ig in proximal tubules as well as glomeruli. The pattern of distribution of antibodies along the luminal border of the tubule epithelium was identical to that seen in both passive transfer experiments and active Heymann nephritis (Mendrick *et al.*, 1980; Noble *et al.*, 1981a). Pre-treatment with CVF did not influence the deposition of passively transferred Ig in the tubules, nor did it affect greatly glomerular deposits of Ig. However, C3 was found to be entirely undetectable in glomeruli as well as tubules of rats given CVF 3 days earlier (Fig. 1b).

Histopathology of proximal tubules (Table 2)

A detailed semiquantative evaluation of the proximal tubules of rats in group A was not made for this study. In experiments reported earlier, tubules of rats with moderate chronic serum sickness have been shown to be normal (Mendrick *et al.*, 1980; Van Liew, Brentjens & Noble, 1983). Furthermore, the normal appearance of proximal tubules of rats in group B (Fig. 2a) demonstrated that neither transfer of normal serum nor treatment with CVF caused any detectable alteration in

Table 1. Effect of CVF on distribution of Ig and C3 in kidneys of rats with chronic serum sickness given anti-brush border serum

Group	Number of rats	CVF	Passive transfer of	Extent of deposition* Ig		C3	
				GCW	BB	GCW†	BB‡
A	5	no	normal serum	+++	0	+++	0
B	5	yes	normal serum	+++	0	0	0
C	7	no	anti-brush border serum	+++	++	+++	+
D	16	yes	anti-brush border serum	+++	++	0	0

* Median score for each group, determined by immunofluorescence tests.

† GCW = glomerular capillary wall.

‡ BB = brush border of proximal tubules.

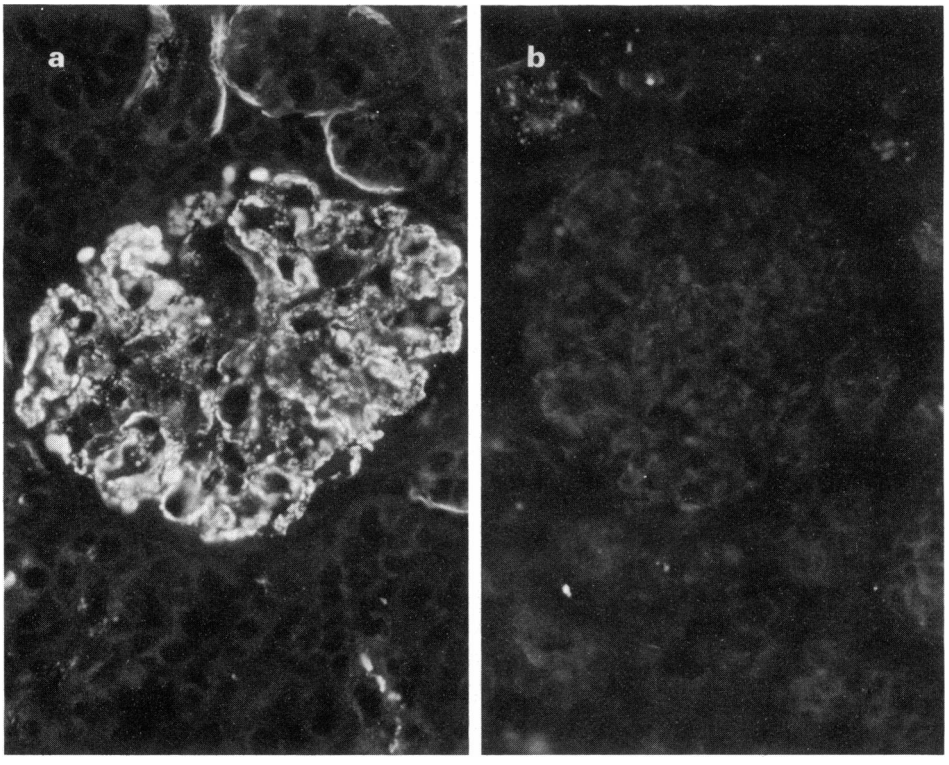

Fig. 1. (a) Frozen kidney section, from a rat with chronic serum sickness that was given anti-brush border serum (group C), stained by the direct immunofluorescence technique for rat C3. Heavy granular deposits of C3, typical of chronic serum sickness, are present in the glomerular capillary wall. In addition, an interrupted linear pattern of staining for C3 is seen along the basement membrane of some tubules. A similar staining pattern may be observed in normal kidneys (magnification × 500). (b) Frozen kidney section, from a rat with chronic serum sickness given anti-brush border serum after CVF treatment (group D), stained for C3. Reaction of the tissue with FITC labelled anti-C3 is completely negative after CVF treatment (magnification × 500).

Table 2. Aspects of proximal tubule damage after passive transfer of anti-brush border serum to rats given CVF

Group	*n*	CVF	Passive transfer of	Percentage of tubules with various degrees of brush border loss				Cells in tubule lumen
				More than $\frac{3}{4}$	From $\frac{1}{2}$ to $\frac{3}{4}$	From $\frac{1}{4}$ to $\frac{1}{2}$	Less than $\frac{1}{4}$	
B	5	yes	normal serum	0	2±1	3±1	95±2	0·5±0·3
C	5	no	anti-brush border serum	19±10*	32±5	23±7	30±8	2·3±0·6
D	12	yes	anti-brush border serum	23±8	29±8	21±4	27±5	2·5±0·8

* Mean ± s.e.

proximal tubule histology. Recipients of anti-brush border serum suffered severe damage to proximal tubules (Fig. 2b). The appearance of tubules of rats in group C was identical to that seen in other passive transfer experiments (Noble *et al.*, 1981a) and in active Heymann nephritis (Mendrick *et al.*, 1980). Proximal tubules of rats in groups D had the same lesions. The most conspicuous abnormality was complete loss and/or severe destruction of the brush border. A significant loss of

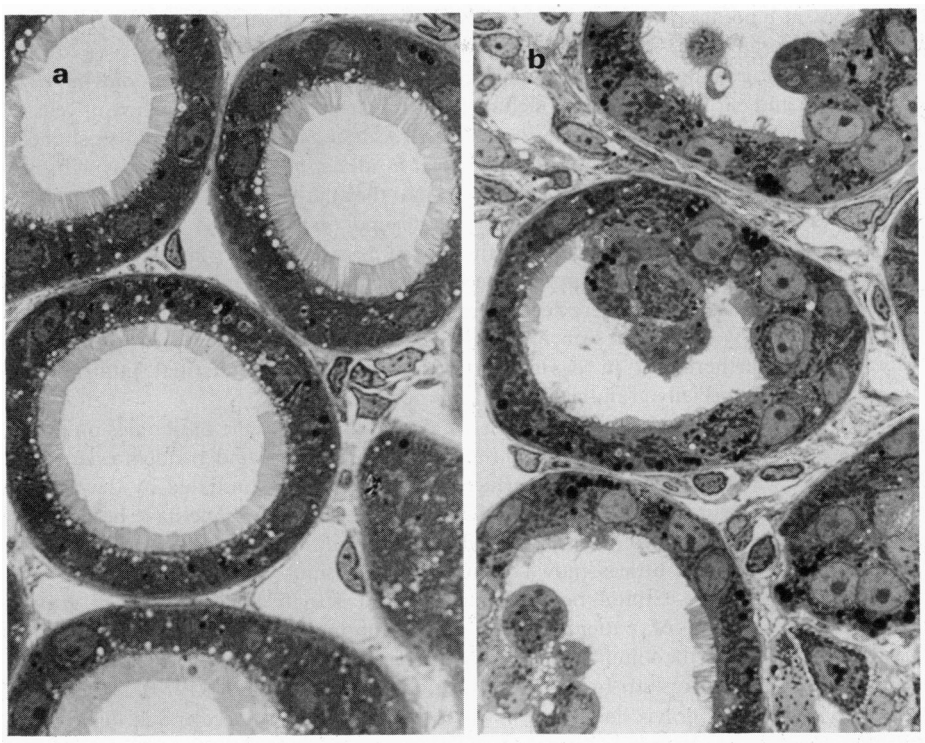

Fig. 2. (a) Light micrograph of a kidney from a rat with chronic serum sickness given normal rabbit serum after CVF treatment (group B). The structure of the proximal tubules, shown here in profile, appears completely normal (methylene blue, magnification × 900). (b) Light micrograph of a kidney from a rat with chronic serum sickness given anti-brush border serum after CVF treatment (group D). There is considerable disappearance of brush border from proximal tubule cells. In addition, proliferation of epithelial cells has led to the accumulation of clusters of cells that partially occlude the tubule lumen (methylene blue, magnification × 900).

microvilli ($P < 0{\cdot}05$) was sustained by rats in groups C and D when compared to those in group B. Floccular debris, consisting of fragments of plasma membrane, was present in the lumen of many tubules. Many epithelial cells had a flattened shape and were greatly deficient in basal infoldings as well as luminal microvilli. Epithelial cell proliferation, which has been described to be associated with the damage mediated by anti-brush border antibodies (Mendrick *et al.*, 1980; Noble *et al.*, 1981a), was observed again in group C and also in group D. Large clusters of cells apparently derived from the tubule epithelium were found in significant numbers ($P < 0{\cdot}05$) in the lumen of many tubules from groups C and D. Other cells appeared to be in the process of 'pinching' away from the epithelial cell layer into the lumen. An increased frequency of mitotic figures was evident. Cuboidal cells of the epithelial layer were replaced in many cases by rather round, pale cells lacking many of the specialized features of mature proximal tubule cells, including microvilli, pinocytotic vesicles and basal infoldings.

DISCUSSION

In previous studies of animals with active Heymann nephritis and in passive transfer experiments, antibody-mediated damage to proximal tubules did not appear to be closely linked to local activation of complement (Mendrick *et al.*, 1980; Noble *et al.*, 1981a). Deposits of complement were much less extensive than deposits of immunoglobulin along the luminal membrane of the tubules

and complement binding was not correlated with either the overall severity or with any particular aspect of tubule injury.

The most striking changes found to result from the deposition of specific antibodies on the brush border in these and earlier experiments include loss of microvilli, and proliferation of cells of the epithelium (Mendrick *et al.*, 1980; Noble *et al.*, 1981a). As lesions detected in complement deficient recipients of anti-brush border serum were identical in all aspects to those in rats with normal concentrations of complement, it may be concluded that the ability of anti-brush border antibodies to damage proximal tubule epithelium does not depend on the action of complement. The pathology produced by anti-brush border antibodies in passive transfers and in active Heymann nephritis did not have the typical appearance of classical complement-mediated injury. Frank necrosis, cell lysis and cell ghosts were never observed. Discontinuities in the plasma membrane of proximal tubule cells were also not seen, although the brush border was often greatly diminished or entirely absent. Furthermore, in passive transfer experiments, interstitial infiltration with inflammatory cells, especially granulocytes, did not occur.

The histopathology that is associated with the deposition of specific antibodies on the brush border membrane of proximal tubules could result from complement-independent events that are the consequence of cross-linking of cell surface antigens. Processes initiated by the reaction of antibodies or other ligands with macromolecules in the plasma membrane have been studied in detail using cells, especially lymphocytes, suspended in tissue culture medium (Warner, 1974; Braun & Unanue, 1980). Relatively little is known of the potential of similar phenomena to cause changes in complex tissues *in vivo*. Stimulation of thyroid gland activity and thyroid cell growth by immunoglobulins in sera of patients with thyroid autoimmunity have been attributed to mechanisms analogous to the stimulation of B cell differentiation and proliferation that results from interactions with antigens or anti-Ig reagents (Roitt, Doniach & Bottazzo, 1979). Neuromuscular weakness in myasthenia gravis has been shown to be caused by an accelerated degradation of acetylcholine receptors occurring after cross-linking of those receptors by autoantibodies (Drachman *et al.*, 1978). Cross-linking by antibodies of plasma membrane antigens has been associated with endothelial cell damage in an animal model of lung immunopathology (Barba *et al.*, 1983).

Binding of ligand to the immunoglobulin receptor on the surface of B lymphocytes leads to extensive changes in cell morphology and physiology involving microfilaments of the cytoskeleton (Braun & Unanue, 1983). Those changes do not depend on the action of complement. Receptor–ligand complexes rearrange to form microaggregates on the cell surface; they may migrate to form a cap at one pole of the cell (Taylor *et al.*, 1971). After cap formation, complexes may be internalized or shed into the surrounding medium (Engers & Unanue, 1973). Microaggregate formation *in vivo* has been demonstrated to occur on the membrane of endothelial cells in the lung as a result of the reaction of antibodies to angiotensin converting enzyme with specific antigens (Barba *et al.*, 1983). The coarse and clumpy distribution of Ig bound to the brush border *in vivo* (Mendrick *et al.*, 1980; Noble *et al.*, 1981a) is consistent with the possibility that microaggregates form on the brush border following antibody fixation. Indirect immunofluorescence tests to detect brush border antigens in *vitro* always show a more regular and uniform distribution of Ig on the cell membrane. Small brush border membrane fragments, visible in the lumen of proximal tubules after antibody deposition *in vivo* (Noble *et al.*, 1981a), may be counterparts of receptor–ligand complexes shed *in vitro*.

In the case of B lymphocytes, ligand–receptor interactions at the cell membrane lead to blast transformation and proliferation (Warner, 1979; Braun & Unanue, 1980). Antibody deposition on the brush border is also followed by a rapid and dramatic increase in the rate of epithelial cell proliferation that results in an extensive alteration of the normal tubule architecture. Round, pale cells lacking the specialized organization of proximal tubule cells take the place of normal cells; large clusters of undifferentiated cells partially occlude the lumen of some tubules.

In the experiments described in this report, CVF administration was associated with depletion of C3 from immune complex deposits in glomeruli of recipients with chronic serum sickness nephritis. A similar observation has been made by Bartolotti & Peters (1978) in acute serum sickness of rabbits. Turnover of the C3 associated with antigen–antibody deposits could lead to the

continuation and progression of complement-dependent glomerular lesions long after new antigen–antibody deposits cease to be formed. On the other hand, the finding that proteinuria of chronic serum sickness was not reduced by depletion of C3 from glomeruli indicates that complement activation is not required to maintain increased permeability in this model of nephritis.

In conclusion, complement does not appear to be essential for the damage to proximal tubules that results from deposition of anti-brush border antibodies on the plasma membrane of tubule cells. Several features of tubule lesions, in particular clumping and shedding of membrane fragments and enhanced proliferation, are highly reminiscent of complement-independent events that occur *in vitro* as the consequence of interactions of cell membrane determinants with specific ligands. To evaluate that hypothesis, passive transfer experiments using F(ab) fragments of anti-brush border immunoglobulins are planned.

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