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Brush Border Antibodies of Heymann Nephritis of Rats in Normal Human Serum (39591)

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Heymann and associates (1) in 1959 first reported the experimental production of a nephrotic syndrome in rats by repeated injections of a mixture containing rat kidney cortex and complete Freund's adjuvant (CFA). This disease subsequently has been shown to be an immunologically induced membranous glomerulonephropathy and is identical histologically and immunohistologically to the idiopathic membranous glomerulonephropathy (IMGN) in humans (2–4). The immunized rats produce autoantibodies to several renal tubular antigens, (5, 6) and one of the main disease-producing autoantibodies seems to be the brush border antibody (BBAb) (2, 5, 7). This antibody is directed against an antigen (BBAg) present in the brush border region of the epithelial cells of the proximal renal tubules (2). BBAg has been recovered from rat kidneys as an insoluble, large molecular weight lipoprotein (7), and is also present in small amounts in the circulation of normal rats (3). In an immunized rat, it (BBAg) combines with its autoantibody (BBAb) to form soluble immune complexes, which are then trapped in the glomerular capillary walls, produce immune injury, and lead to the development of glomerulonephropathy.

The etiology and pathogenesis of the IMGN in humans is not known. Japanese investigators (8) have reported the presence of BBAg in the glomerular immune deposits in some cases of human IMGN. Is BBAg present in the circulation of normal humans, and does BBAb have any role in the pathogenesis of IMGN? The answers to these questions are not known, and the pathogenesis of IMGN is open to conjecture. This report describes the detection of BBAb in normal human serum.

Materials and Methods. Three hundred fifty-eight normal adult human sera (age 22–38 years) obtained from blood donors and six laboratory personnel were examined. The six laboratory personnel were healthy and had normal urinalyses. Fresh, normal, Sprague-Dawley (SD) rat, rabbit, and guinea pig kidneys were quickly frozen in liquid nitrogen, and 2- to 4-μm sections were cut in a cryostat. These sections were used to detect BBAb in test sera by an indirect-immunofluorescence technique previously described (6). Fluorescein-isothiocyanate-labeled rabbit and horse anti-human IgG were obtained from Behring Diagnostics (American Hoechst Corporation, Route 202–206, North Somerville, N.J. 08876). The specificity of these was proven by double-diffusion gel precipitation and by immunoelectrophoresis.

Some of the positive sera were absorbed with a lyophilized human or rat renal cortical fraction, similar to the fraction F × IA described by Edgington and associates (7). The cortical fraction was prepared by a modification of Edgington's method. Rat cortical fraction was prepared from rat kidneys (SD) perfused *in vivo* with physicological saline. The capsule was stripped; renal cortex was separated from medulla, and the cortex was chopped with a razor blade almost to a thick paste-like consistency. It was gently pushed through a 60-mesh stainless steel wire cloth with the hlep of a spatula, and the cortical suspension was collected in a beaker placed in an ice bath. For each gram of cortical tissue, approximately 30–40 ml of physiological saline was required to obtain a uniform cortical suspension. It was centrifuged in cold (4°) at 200*g* for 3 min and washed three times with cold normal saline to obtain a clear supernatant. The sediment was saved for isolation of glomeruli. The supernatant from the initial centrifugation and subsequent washings were pooled and centrifuged in cold at 100,000*g* for 1 hr. The supernate and sediment (cortical fraction) were saved and lyophilized. Human cortical fraction was pre-

pared in a similar manner from unperfused kidneys obtained at autopsy from patients dying of nonrenal causes. The protein content of the lyophilized cortical fractions was determined by Lowry's method. Expressed as fraction of the dry weight, the human cortical fraction contained 83% protein, and rat cortical fraction contained 75% protein. For absorption studies, measured quantities of test serum and cortical fraction were allowed to react at room temperature for 18 hr under continuous shaking as previously described (6). The biological activity of rat and human cortical fractions was proven by their ability to induce Heymann nephritis (HN) in SD rats.

Autopsied renal tissue from patients dying of nonrenal causes and renal biopsy specimens from patients with minimal change disease were used as a source of

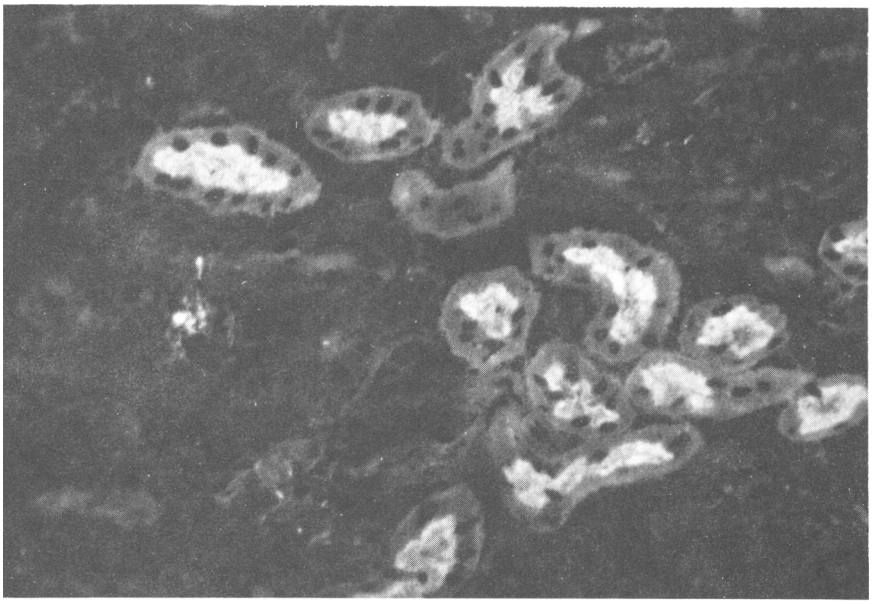

FIGURE 1

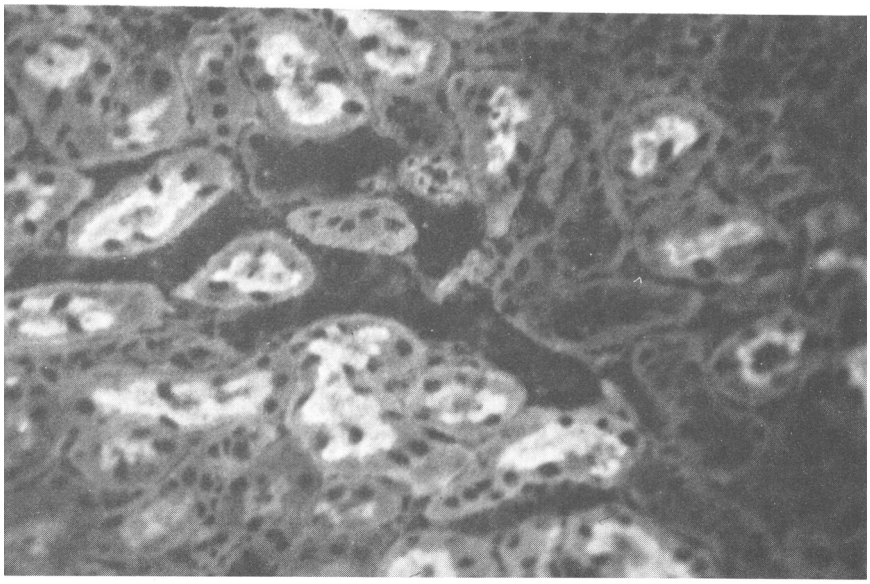

FIGURE 2

human 2- to 4-μm frozen sections. Fresh sections cut from the biopsy specimens within an hour of the biopsy were used for test sera on the same day.

Results. Thirty-six percent (128/358) of the sera examined were positive for BBAb when tested on normal SD rat kidney sections (Figs. 1 and 2). The staining was primarily localized along the brush border in the majority of the cases; however, with four sera a faint staining along the tubular basement membrane of the proximal tubule was also observed (Fig. 3). Appearance of staining along the brush border of the proximal renal tubules by normal human serum and serum from HN rats was indistinguishable. Controls using direct staining of SD rat, rabbit, guinea pig, and human kidney sections with fluorescein-labeled anti-human IgG without normal human serum were negative on every occasion. Of the 271 sera from males, 94 (34.7%) were positive, and of the 87 females, 34 (39.1%) were positive, suggesting no significant sex difference. An attempt was made to see if there was a correlation between blood groups and positive sera (Table I). Even though the sample size for blood group AB was too small, when the data for all four blood groups were analyzed by Chi-square test, it was found that persons with blood group B were significantly less prone to have BBAb in their sera ($P < 0.001$, chi-square value 15.8), and that there was no difference among blood groups O, A, and AB. To find a correlation with the Rh-factor, persons positive for BBAb were divided into Rh-positive and Rh-negative groups. Among Rh-positive subjects, 110/317 were positive for BBAb, and among Rh-negative, 13/41 were positive for BBAb. This difference was not significant; $P = 0.247$. Positive sera were also tested on human, guinea pig, and rabbit kidneys; sera showing a positive reaction on rat kidney also gave a positive reaction on guinea pig kidney, but not human or rabbit kidney. The explanation for this species difference is not apparent. Although the normal human serum did not give a positive reaction with human kidney sections, the BBAb present in the serum were partially absorbed with human renal cortical fraction. However, the rat renal cortical fraction was more effective in absorbing the

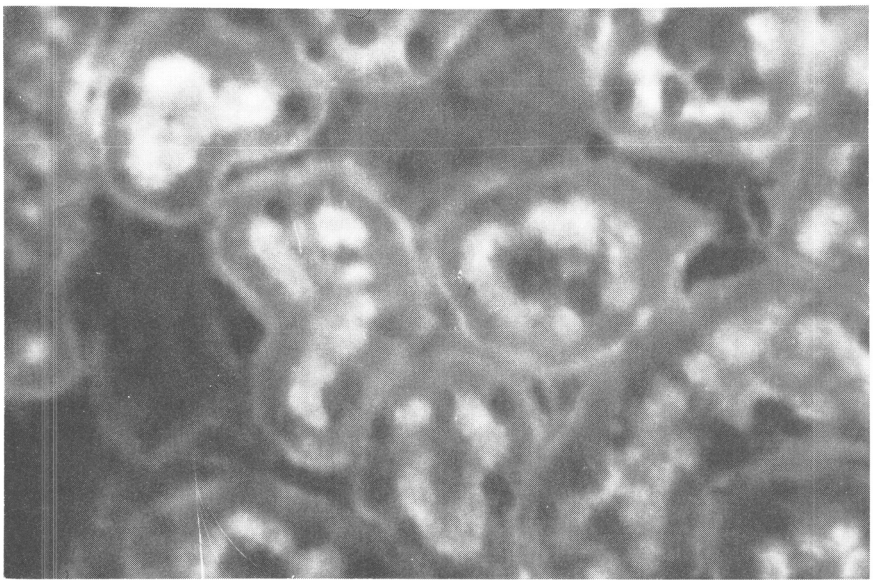

FIGURE 3

FIGS. 1, 2, AND 3. Fluorescent photomicrographs showing brush border antibodies in normal human serum by indirect immunofluorescence technique. Frozen sections from normal Sprague–Dawley rat kidney were first reacted with a normal human serum and then stained with fluorescein-isothiocyanate-labeled anti-human IgG. Bright fluorescence along the brush border region of the proximal renal tubules is seen. A part of the glomerulus seen in the right lower corner of Fig. 1 does not show any staining. Another normal human serum shows staining along the tubular basement membrane of proximal renal tubules (Fig. 3).

human BBAb (Table II). Absorption of a positive serum with other rat tissues, such as liver, spleen, and muscle, was ineffective in abolishing or reducing the positive reaction.

Sera from two patients (ages 16 and 14 years) with IMGN were also examined. Both children were found to have BBAb in their sera. The titer of BBAb in one patient was 1:8 and in the other 1:16; these titers were similar to those detected in normal human sera.

Discussion. This study has demonstrated BBAb in normal human serum reacting with rat and guinea pig kidney. However, these antibodies were not found in all human sera that were tested, and therefore, to find if only a certain group of individuals was susceptible to develop these antibodies, we attempted to see if there was a correlation between positive sera and major blood group or Rh-antigens. The Rh-factor seemed to have no correlation with the presence of BBAb; however, among the major blood groups a significantly lower incidence of BBAb was found in the B-group individuals. Why persons with blood group B have less chance of developing BBAb in their sera cannot be answered from this study. If there is any relationship between the presence or absence of BBAb in an individual's serum and the susceptibility to develop IMGN, it may prove useful to look at the blood group distribution among patients with IMGN.

Two possible explanations can be considered for the presence of these antibodies in some human sera. The first possibility is that the positive sera represent a subclinical IMGN and that 36% of the normal people are carrying a subclinical IMGN. Since urinalyses were performed on only 6/128 positive persons and renal biopsies were obtained on none, it is not possible to know if indeed these persons had subclinical IMGN. The second possibility is that the BBAb present in the normal human serum is a nonspecific heterophile antibody and is unrelated to the human IMGN. Whatever the explanation, the observation that BBAb is present in over 1/3 of normal human sera is important to keep in mind because many investigators are looking for BBAb in the sera of patients with IMGN, and if one finds BBAb in the sera or cryoprecipitates of these patients, it will not necessarily establish a causal relationship of BBAb with human IMGN.

It is of interest to note that in rats that are highly susceptible to the experimental production of HN, the author was unable to find BBAb in 1080 normal rat sera. Based on this finding it may be speculated that those humans who do not have BBAb in their sera are more susceptible to develop

TABLE I. RELATIONSHIP OF BBAb WITH BLOOD GROUPS.

	Major blood groups			
	O	A	B	AB
Positive for BBAb (%)	71 (41.3)	51 (38)	5 (11.3)[a]	1 (12.5)
Total	172	134	44	8

[a] Persons with blood group B are less likely to have BBAb in their serum; $P < 0.001$.

TABLE II. ABSORPTION OF BBAb WITH A RENAL CORTICAL FRACTION.[a]

		Absorption with rat cortical fraction (mg /ml)			Absorption with human cortical fraction (mg/ml)		
	BBAb titer	1	10	20	1	10	100
Human serum	1:16	Partial	Complete	Complete	None	None	Partial
Heymann nephritis rat serum	1:32	None[b]	Partial[c]	Complete[d]	None	None	Partial

[a] All absorptions were performed on undiluted sera. The degree of absorption was assessed by an estimate of the reduction in the intensity of immunofluorescence.

[b] None: no change in the intensity of fluorescence.

[c] Partial: considerable (about 50%) reduction in the intensity of fluorescence.

[d] Complete: no immunofluorescence observed (100% reduction).

IMGN and vice versa.

It is suggested that further studies be carried out to understand the significance of these antibodies. These should include examination of many more normal adult human sera, normal newborn human sera, sera of patients with IMGN, sera from patients with other chronic glomerulonephritides, and, if nephrectomy specimens from IMGN patients become available, immunoglobulins should be eluted from these specimens and tested on normal rat and human kidney sections. It would be of interest to know if the percentage of positive sera increases with age, with infants having the lowest and older people the highest incidence.

Summary. Brush border antibody of Heymann nephritis of rats was detected in 36.0% of normal human sera by indirect immunofluorescence technique. Persons with blood group B were less likely to develop this antibody. Its existence in the serum may or may not be related to the genesis of idiopathic membranous glomerulonephropathy in humans. Many investigators are looking for BBAb in the sera of patients with renal disease; thus, it is important to know that BBAb can exist in the sera of normal human controls who do not have any clinical evidence of renal disease. Further studies on this subject will be needed to understand the significance of this observation. Possibilities to explain this observation are presented.

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