BK PAPOVAVIRUS IMMUNE COMPLEXES IN GLOMERULO-NEPHRITIS

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Summary

Kidney biopsies from 98 patients were studied for BK virus (BKV) antigens by indirect immunofluorescence. Intense fluorescent staining was observed in 11/12 cases of lupus nephritis, 11/12 cases of membranous nephropathy, 21/23 cases of IgA mesangial glomerulonephritis, 3/4 cases of membrano-proliferative Glomerulonephritis and 5/12 cases of exudative glomerulonephritis. Antisera to different viruses did not react with any kidney sample. Deposition of BKV was strictly related to the presence of immunoglobulins in renal glomeruli. The specificity of reaction for antigens related to BKV was demonstrated by absorption of sera with different substances and BKV. Absorption of rabbit anti-BKV serum with human immunoglobulins completely abolished glomerular fluorescence.

We conclude that the fluorescence obtained in kidney biopsies by staining with anti-BKV serum was a false positive reaction dependent on common antigenic determinants present in BKV capsid proteins and human immunoglobulins.

Introduction

BK virus (BKV) is a human papovavirus first isolated from the urine of a renal transplant recipient [1]. Although BKV is frequently detected or isolated from immunodeficient or immunosuppressed patients [2-4] it has not yet been aetiological related to any common human disease. Serological surveys have shown that BKV is common and widespread in normal human beings producing a long-lasting, inapparent, persistent infection [5,6]. Sera from 60-80 per cent of normal adults contain complement-fixing or haemagglutination-inhibiting antibodies to BKV and 20 to 40 per cent of positive sera show high antibody titres [7]. This observation suggests that exogenous superinfections or endogenous reactivations of the persistent infection occur frequently. During these recurrences viral antigens might be produced in excess and react with antibodies to form soluble immune complexes which could be deposited within renal glomeruli [8,9].
On the other hand, although some nephropathies, such as membranous glomerulonephritis and IgA nephropathy, are certainly immune complex diseases [10,11], the nature of the antigen in immune complexes is unknown in more than 80 per cent of cases [11], suggesting that identification of the antigen is needed. Panem et al [12] claimed that BKV antigens were present in renal glomeruli in several cases of immune complex glomerulonephritis.

We report here our investigation into the presence of BKV antigens in the renal tissue of patients suffering from glomerulonephritis.

Material and methods

Preparation of anti-BKV serum

BKV was grown in Vero cells and purified onto a cushion of a saturated KBr solution followed by two cycles of equilibrium density gradient centrifugation in CsCl. Two purified virus preparations were pooled. The purified virus pool had a titre of $5.1 \times 10^5$ haemagglutinating units per ml. Specific sera to BKV structural antigens were produced by inoculating rabbits subcutaneously with purified BKV mixed with complete Freund adjuvant [13,14]. The rabbit serum used had a haemagglutination-inhibiting titre of 80000 for BKV and an immunofluorescent titre of 640 on BKV-infected Vero cells.

Absorption of sera

Anti-BKV rabbit serum, diluted 1:32 and goat anti-rabbit fluorescein-isothiocyanate-conjugated (FITC) immunoglobulins (Behring Diagnostics), diluted 1:5, were absorbed with either of the following reagents: acetone-dried guinea-pig liver powder, lyophilised normal human kidney, lyophilised human gammaglobulins (30mg/ml), packed human red blood cells (0.5ml), human plasma-proteins polymerised with 2.5% glutaraldehyde [14,15], Vero cells, and purified preparations of ectromelia virus, herpes virus Type 1, Sendai virus, Vaccinia virus and BKV (100µg each).

The presence of cross-reaction with human immunoglobulins was controlled by testing anti-BKV serum with indirect immunofluorescence on mouse liver previously incubated with high titres of anti-nuclear factor human antibodies and on the livers of mice previously injected with aggregated human immunoglobulins [16].

Elution of specific antibodies

Elution of specific antibodies was performed on kidney tissue obtained at autopsy from three patients: one with membranous nephropathy, one with IgA nephropathy and one patient without kidney disease. The preparation was made according to Woodroffe and Wilson [17]. The eluates were tested by indirect immunofluorescence on monolayers of BKV-infected Vero cells.
Microscopical procedures

Renal biopsies were obtained by left percutaneous biopsy from 98 patients with various nephropathies and immediately processed for light, immunofluorescence and electron microscopy as described elsewhere [18]. The renal frozen sections were stained by the direct method with FITC anti-human IgM, IgA, IgG, C₃, C₄, fibrinogen (Behring Diagnostics). Kidney sections of all these biopsies were also tested by indirect immunofluorescence using either serum anti-BKV before and after absorption or rabbit pre-immune serum. Sera to herpes simplex, polio, vaccinia and influenza viruses were also tested on the same kidney samples. Fluorescence was evaluated from trace to 4+ according to the intensity of the pattern observed.

Results

When renal biopsies were tested by immunofluorescence with unabsorbed rabbit antiserum to BKV structural antigens, fluorescent material was found strictly localised to glomeruli along the glomerular basement membrane and within the mesangium. Fluorescence was generally intense, ranging from 2+ to 3+ (Figure 1).

Figure 1. Evidence of cross-reaction between human immunoglobulins and BKV.
A) Glomerulus stained with anti-human IgG serum (direct IF).
B) The same case stained with anti BKV serum (indirect IF).
C) Mouse liver previously incubated with human anti-nuclear factor. Intense fluorescence of nuclei with anti-BKV serum (indirect IF).
D) Mouse liver previously inoculated with aggregated human gamma globulins. Intense sinusoids fluorescence with anti-BKV serum (indirect IF)
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Direct immunofluorescence</th>
<th>Indirect immunofluorescence</th>
<th>Anti-BKV serum absorbed with: human immunglobulins or polymerised plasma proteins</th>
<th>Anti-BKV serum absorbed with purified BKV</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgA</td>
<td>IgM</td>
<td>Unabsorbed anti-BKV serum and anti-BKV serum absorbed with: human red cells or guinea-pig liver or human kidney or Vero cells or other human viruses</td>
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<td>Lupus Nephritis</td>
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<td>9/12 (4+)</td>
<td>8/12 (4+)</td>
<td>11/12 (3+)</td>
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<td>11/12 (3+)</td>
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<td>IgA Nephropathy</td>
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<td>21/23 (4+)</td>
<td>5/23 (2+)</td>
<td>21/23 (3+)</td>
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<td>2/4 (2+)</td>
<td>2/4 (4+)</td>
<td>3/4 (3+)</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Exudative</td>
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<td>3/12 (3+)</td>
<td>3/12 (3+)</td>
<td>5/12 (3+)</td>
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<td>Normal Kidney</td>
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</tbody>
</table>

* Number of positive/Number tested (intensities of fluorescence: 1+ to 4+)

† This group includes: Nephroangiosclerosis, haemolytic-uraemic syndrome, Schönlein-Henoch nephropathy, segmental focal glomerulosclerosis, diabetic nephropathy, renal amyloidosis
No fluorescence was observed outside the glomeruli. Positive fluorescence with anti-BKV serum was detected in nearly all biopsies from lupus nephritis, membranous nephropathy and IgA nephropathy; whereas biopsies from other nephropathies did not react to anti-BKV serum (Table I). No fluorescence was found in normal kidneys.

The glomerular fluorescence obtained with anti-BKV serum had the same pattern as that produced by staining with FITC anti-human immunoglobulins (IgM, IgA and IgG). No anti-BKV fluorescence was observed in specimens which were negative for anti IgM, IgA or anti IgG fluorescence (Table I).

Absorption of rabbit anti-BKV serum with guinea pig liver powder, human red cells, Vero cells and lyophilised human kidney did not decrease fluorescence from monolayers of BKV-infected Vero cells or from kidney biopsies. Likewise, fluorescence was not removed when anti-BKV serum was absorbed with ectromelia, vaccinia, herpes and Sendai viruses, while fluorescence was completely removed when serum was absorbed with BKV. No fluorescence was observed when the same kidney specimens were tested with a panel of sera to the most common and ubiquitous human viruses.

<table>
<thead>
<tr>
<th>Substrates used for controls</th>
<th>Direct immunofluorescence</th>
<th>Indirect immunofluorescence</th>
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<tr>
<td></td>
<td>FITC conjugated anti human immunoglobulins serum</td>
<td>Unabsorbed anti-BKV serum and anti-BKV serum absorbed with: human red cells or guinea-pig liver or human kidney or Vero cells or other human viruses</td>
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<tr>
<td>Mouse liver obtained after i.v. inoculation of heat aggregated human immunoglobulins</td>
<td>4+</td>
<td>3+</td>
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<td>Mouse liver sections previously incubated with anti nuclear factor positive serum</td>
<td>4+</td>
<td>3+</td>
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<tr>
<td>Mouse liver sections obtained from normal animals</td>
<td>NEG.</td>
<td>NEG.</td>
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</table>
Material eluted from renal tissue, taken at autopsy from a case of membranous nephropathy and from a patient with IgA nephropathy and a normal subject, did not react to monolayers of BKV-infected Vero cells.

Absorption of rabbit and anti-BKV serum with either human immunoglobulins or polymerised human plasma proteins completely removed glomerular fluorescence in all biopsies of membranous and IgA nephropathy and produced a strong reduction of fluorescence from 4+ to 1+ in lupus nephritis.

Conclusions

An intense fluorescent staining (3+) was obtained when anti-BKV serum was reacted by indirect immunofluorescence on mouse-liver slices coated with anti-nuclear factor human immunoglobulins. Omission of anti-nuclear factor human immunoglobulins from the test completely abolished fluorescence, suggesting that the positive reaction was not due to anti-nuclear antibodies present in anti-BKV rabbit serum. This result was confirmed by positive fluorescent staining mediated by anti-BKV serum on mouse-liver containing aggregated human immunoglobulins (Table II).

On the basis of the evidence of cross-reaction between BKV structural antigens and human immunoglobulins, we conclude that the fluorescence obtained in kidney biopsies by staining with anti-BKV serum was a false positive reaction dependent on common antigenic determinants present in BKV capsid proteins and human immunoglobulins.

References

1. Gardner SD, Field AM, Coleman DV, Hulme D. Lancet 1971; ii: 1253
4. Shah KV, Daniel RW, Zeigil RF, Murphy GP. Transplantation 1974; 17: 131
5. Gardner SD. Br med J 1973; i: 77
Open Discussion

STRUYVENBERG (Chairman) You say in the abstract that you got positive results in 51 out of 63 patients in which you determined the immunoglobulins. Do you, yourself suspect that this is a chance finding or that it has anything to do with the disease, because of the large number of positive results?

DONINI Because of the large cross reaction between immunoglobulin staining and BKV antigen staining I suspected that the initial results were not associated with the disease. I think further work with virus or bacterial antigens on kidney biopsies must be accompanied always by controlled readings.