Cytomegalovirus is Not Specifically Associated With Immunoglobulin A Nephropathy¹,²

Jung Sik Park,³ Jae-Hoon Song, Won Seok Yang, Soon Bae Kim, Yoo Kyum Kim, and Changgi D. Hong

ABSTRACT
Cytomegalovirus (CMV) has been suspected to be involved in the pathogenesis of IgA nephropathy (IgAN). Whether CMV is present in renal tissue of IgAN, however, remains controversial. To determine the presence of CMV in IgAN, compared with other glomerulonephritis (GN) as disease control, polymerase chain reaction amplifying a 159-base-pair fragment of the immediate early gene of CMV and indirect immunofluorescence staining with anti-CMV monoclonal antibody were performed on 10 IgAN and 14 non-IgAN GN renal tissues. CMV DNA was detected in 6 of 10 IgAN tissues and 10 of 14 other GN by polymerase chain reaction, whereas no CMV antigen was detected in all renal tissues by immunofluorescence. This frequent observation of CMV DNA in various types of GN as well as in IgAN would suggest that CMV is not specifically associated with the pathogenesis of IgAN seen in endemic areas of CMV infection.

Key Words: Cytomegalovirus, immunoglobulin A nephropathy, polymerase chain reaction, indirect immunofluorescence

Although immunoglobulin A (IgA) nephropathy (IgAN) has become the most common primary nephropathy in many parts of the world, particularly in the Orient (1), its pathogenesis is largely unknown (2). Recently, the involvement of virus, including Epstein Barr virus, hepatitis B virus, and cytomegalovirus (CMV), has been suggested. Although Epstein-Barr virus has been thought to play a pathogenetic role (3), evidence for mesangial deposition is not available. Hepatitis B virus antigens have been detected in the mesangium of patients with IgAN from endemic areas (4), but it is unlikely that they play a pathogenetic role in other geographic areas (5). The presence of CMV antigen or DNA in the kidney tissue of IgAN and its possible role in IgAN pathogenesis are still controversial since the first report by Gregory et al. (6) in 1988.

In this study, we investigated whether CMV DNA and antigen are present in the renal tissues of the IgAN patients who have been infected with CMV. The tissues from patients with various glomerulonephritits (GN) other than IgAN were used as disease controls. We used polymerase chain reaction (PCR), which specifically amplified a 159-base-pair (bp) fragment of the immediate early gene of human CMV, and an indirect immunofluorescence (IF) technique, which used anti-CMV monoclonal antibody in previously obtained paraffin-embedded renal biopsy tissues.

METHODS
A total of 24 paraffin-embedded renal biopsy specimens that were histopathologically diagnosed as IgAN (N = 10), membranous GN (N = 5), minimal change lesion (N = 3), membranoproliferative GN (N = 1), lupus nephritis (N = 2), crescentic GN (N = 1), mesangial proliferative GN (N = 1), and focal segmental GN (N = 1) were used for PCR and IF. In all cases, IgG and IgM antibodies to CMV were tested by enzyme-linked immunosorbent assay. Histologically proven CMV colitis tissues and CMV AD 169 strain were used as positive controls, and three normal kidney tissues were used as negative controls. PCR was carried out with primers from the sequence of

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CMV immediate early gene flanking a 159-bp fragment. The oligonucleotide primers were synthesized by the use of an Applied Biosystems DNA synthesizer (Model 391; Foster City, CA) and had the following sequences: IE 1 (5'-CCA CCC GTG GTG CCA OCT CC-3') and IE 2 (5'-CCC GCT CCT CCT GAG CAC CC-3'), corresponding to nucleotides 2418 to 2437 and 2560 to 2579, respectively. Another oligonucleotide, corresponding to nucleotides 2447 to 2486 (5'-CTG GTG TCA CCC CCA GAG TCC CCT GTA CCC GCC ACT ATC C-3'), was used as a probe in the subsequent Southern blot analysis of the amplified DNA.

Fifty cycles of amplification were carried out in an automated thermal cycler (Hybaid; Teddington, Middlesex, United Kingdom) consisting of preincubation for 7 min at 94°C, denaturation for 25 s at 95°C, annealing for 15 s at 42°C, and polymerization for 60 s at 72°C. The DNA fragment amplified by PCR was identified by Southern blot hybridization with the 32P-labeled internal probe. Before these experiments, the primers used in this study were confirmed to be specific for CMV with the following viral strains: CMV AD 169 strain (ATCC VR 538), CMV Towne strain (ATCC VR 977), herpes simplex type I strain F (ATCC VR 733), herpes simplex type II strain G (ATCC VR 734), and varicella zoster strain Ellen (ATCC VR 586).

IF analysis was carried out with anti-CMV monoclonal antibody to the early and late antigens of CMV (DAKO-CMV; Dakopatts, Copenhagen Denmark) and fluoroscein isothiocyanate-labeled secondary antibody (Cappel, Organon, Teknika, Durham, NC).

RESULTS

The specificity of the primers used in PCR was tested against DNA from various viral strains. Amplification products of the expected size (159 bp) were obtained only from the extracts of two CMV strains (AD 169 and Towne) and DNA from the well-documented CMV colitis tissues, but not from the extracts of other viruses (data not shown).

Analysis of the PCR products by Southern blot hybridization with the 32P-labeled internal probe showed that 6 of the 10 IgAN specimens and 10 of the 14 non-IgAN GN specimens were positive for CMV DNA (Figure 1). The non-IgAN GN that were positive included membranous GN (three of five), minimal change lesion (two of three), membranoproliferative GN (one of one), lupus nephritis (two of two), crescentic GN (one of one), and mesangial proliferative GN (one of one).

IF with monoclonal antibodies to the early and late antigens of CMV showed no positive glomerular staining in the specimens from the patients with IgAN as well as other GN. The results of the PCR, IF, and serologic status of anti-CMV antibody were not congruent, as would be expected if CMV is a pathogenetically important factor (Table 1).

DISCUSSION

In this report, we showed the presence of the CMV DNA in many renal biopsy specimens from patients with different types of GN, including IgAN, whereas IF with anti-CMV monoclonal antibody failed to show the presence of the CMV antigens in all renal tissues, regardless of their pathologic type. These results are somewhat different from those of previous studies. The association of CMV and IgAN was first reported by Gregory et al. (6), who showed deposition of the CMV antigen in the mesangium by IF staining using polyclonal antisera. This observation, however, could not be reproduced in the subsequent studies using monoclonal antibodies that consistently showed negative results (7–9). These conflicting data may have been the result of technical problems, such as lack of specificity of the antibody and sensitivity of the IF method. Further attempts to detect CMV DNA in the renal biopsy specimens of patients with IgAN by in

Figure 1. Autoradiograph from Southern blot hybridization with 32P-labeled internal probe. Amplification products of 159 bp were obtained from 50 cycles of PCR with one pair of oligonucleotide primers located in the immediate early gene of human CMV. Lane 1, positive control (CMV AD 169 strain ATCC VR 538); Lanes 2, 27, and 28, negative controls (2, distilled water and 27 and 28, normal kidney tissues, respectively); Lanes 4 to 9, 12, 16, 17, and 22, IgAN; Lanes 3, 11, 14, 15, and 18, membranous GN; Lanes 10, 19, and 24, minimal change lesion; Lane 13, focal segmental GN; Lanes 20 and 23, lupus nephritis; Lane 21, mesangial proliferative GN; Lane 25, membranoproliferative GN; Lane 26, crescentic GN.
TABLE I. Comparison of the CMV positivity among PCR, IF, and serology

<table>
<thead>
<tr>
<th>Tests</th>
<th>IgAN (N = 10)</th>
<th>Other GN (N = 14)</th>
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<tbody>
<tr>
<td>(+) PCR</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>(+) IF</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(+) serum IgG</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>(+) serum IgM</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

situ hybridization (10) or PCR (11–13) also showed contradictory results. These negative results by the other investigators using PCR in CMV DNA detection in the renal tissues of IgAN may be because of differences in the primers and the specimens and the degradation of small amounts of CMV DNA by the fixation procedures. In addition, we amplified the immediate early gene because this has been reported to be more sensitive in detecting the CMV in clinical isolates compared with the late gene (14).

In this study, CMV DNA was detected in 6 of 10 IgAN as well as 10 of 14 other GN. Recently, Muller et al. reported similar results with PCR, in which 14 of 19 frozen kidney specimens with IgAN and 4 of 18 normal kidney tissues were positive for CMV DNA (15). Kaderelt et al. reported the presence of CMV DNA in renal tissues from patients without IgAN; 8 of 26 non-IgA renal biopsy specimens and 3 of 4 IgAN were positive for CMV DNA by PCR (16).

If CMV is present in the renal tissues of IgAN as well as in other types of GN, a role for this virus in the pathogenesis of IgAN is unlikely. IgAN may result from an exaggerated mucosal immune response to a foreign substance, including infectious agents. The specific antigens involved may differ in different geographic locations, and the inciting agents may be present in complexes without replicating in the kidney (17). Renal or tonsillar tissues from IgAN patients can generate cytopathic effects when added to cultured cells, suggesting viral involvement (18). Recently, several reports have described the presence of IgAN in human immunodeficiency virus (HIV) infection (19–21). Kimmel et al. demonstrated the presence of the circulating anti-HIV antibodies and immune complexes containing IgA and recovered the identical material from the renal tissue. They also demonstrated the presence of HIV genome in renal parenchyma. These findings suggest the role of virus in the pathogenesis of IgAN (21).

Although our results further confirm the presence of CMV in the renal tissues with IgAN, they do not indicate a pathogenetic role of this virus in the development of the disease in the geographical region from which our clinical specimens were collected. Another possible factor that may influence the presence of CMV DNA in the renal tissues from various sources is the high prevalence of CMV infection in certain areas. In endemic areas such as Korea, where almost all healthy adults show evidence of past CMV infection, the nonspecific presence of CMV DNA in various GN would be expected.

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REFERENCES