HUMAN GLOMERULONEPHRITIS AND PERSISTENT NON-POLIO ENTEROVIRUS INFECTION

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Summary

In a prospective work, we have studied the non-polio enterovirus (NPEV) excretion in spot urine and stools by four appropriate cell cultures, in four different groups: 20 exposed controls (GI), 88 patients with renal biopsy ‘proven GN’ (GII), 38 cases with ‘proven nonGN’ (GIII), and 9 with ‘probable nonGN’ (GIV). The positive excretion in stools and/or urine is respectively 0 and 5% in Group I, 14 and 33% in Group II, 6 and 19% in Group III, and 13 and 22% in Group IV. Viruria, the consequence of a viraemia, is therefore associated with ‘proven GN’ (p < 0.05). In the majority of patients with positive NPEV excretion, we have made an additional but similar study, 3–12 months later. Persistent excretion was confirmed in 22/30 cases in Group II (73%) versus 0/8 in Group III–IV (p < 0.001). These data concerned patients with membranoproliferative GN [5], membranous GN [6], mesangial IgA GN [6], endocapillary GN [2] or minimal lesions [3].

Thus we have demonstrated a significant relation between persistent NPEV urine/stools excretion and the occurrence of active GN in humans. Such persistent viral infections may represent the cause of some GN, probably mediated by an immune complex mechanism with viral antigens.

Introduction

Renal damage during the course of or associated with a non-polio enterovirus (NPEV) infection in humans is rare [1–18]. We have recently reported [14] persistent NPEV excretion in urine and/or stools in 9 patients with well documented glomerulonephritis (GN). We suggested various explanations: a chance association in an epidemic area, a viral infection secondary to chronic renal failure, which is an immunosuppressed state, a direct viral replication in kidney cells with cytopathic effect, or specific viral antigens causing an immune complex nephritis.

In this paper, we have studied the frequency of NPEV infection in a large spectrum of well documented renal diseases, in order to determine the possible causative role of such viruses in glomerulonephritis.
Material and methods

The controls and the patients

We have prospectively studied four different groups of subjects, all connected with our Nephrology unit.

Group I is composed of 20 controls selected from staff members of our department (mainly nurses).

Group II is composed of 88 patients with renal biopsy proven glomerulonephritis. This group included 34 mesangial IgA GN (28 idiopathic and 6 Schönlein Henoch Purpura), 19 membranoproliferative GN, 14 membranous GN, 11 nephrotic syndrome with minimal lesions or segmental/focal hyalinosis, 6 proliferative endocapillary ± extracapillary GN and 4 miscellaneous cases.

Group III is composed of 38 patients without GN, but well diagnosed renal diseases: diabetic glomerulosclerosis (4), interstitial nephritis (8), nephrosclerosis and/or hypertension (9), different uropathies and miscellaneous cases (11).

Group IV is composed of 9 patients with renal disease which was probably not a GN.

The methods

For all these subjects, a urine and a stool specimen were collected in sterile bottles and delivered to the virology laboratory. Each specimen was then cultured (either immediately for urine or after freezing to 20°C for stools) on four different cell cultures, selected for appropriate enterovirus growth: primary monkey kidney cells (Cercopithecus aethiops), MRC5, HeLa, and RD. Three subcultures were systematically performed. The results of these different cultures were generally obtained in 2 to 3 months.

For all subjects with NPEV isolated from urine or/and stools we asked for a new specimen collection, obtained 3 to 12 months after the first check.

Results

The results of the first viral screening

These are given in Table I. In the group of ‘biopsy proven GN’ (group II), the NPEV excretion in urine is significantly greater than in controls (p<0.05). There is a regular increase in the following order in the frequency of NPEV urine or/and stool excretion: control group, proven non GN, probable non GN and proven GN. Details of the results in group II are given in Table II.

Results of further study in patients with NPEV excretion

Most of the patients with positive NPEV excretion underwent an additional study (38 out of 46: 83%) involving 30 out of 34 in group II, 5 out of 8 in group III and 3 out of 3 in group IV. None of the patients from groups III and IV were still excreting a virus. But in group II, 22 patients had persistent NPEV excretion (73%);
### TABLE I

<table>
<thead>
<tr>
<th>Groups of Subjects</th>
<th>Positive urine</th>
<th>Positive stools</th>
<th>Positive urine and/or stools</th>
<th>Repeat viral study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects retested</td>
<td>Positive urine and/or stools</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I Control</td>
<td>1/20 (5%)</td>
<td>0/20 (0%)</td>
<td>1/20 (5%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>II Proven GN</td>
<td>28/84 (33%)*</td>
<td>11/79 (14%)</td>
<td>34/88 (39%)*</td>
<td>30/34 (88%)</td>
</tr>
<tr>
<td>III Proven non GN</td>
<td>7/36 (19%)</td>
<td>2/32 (6%)</td>
<td>8/38 (21%)</td>
<td>5/8 (63%)</td>
</tr>
<tr>
<td>IV Probable non GN</td>
<td>2/9 (22%)</td>
<td>1/8 (13%)</td>
<td>3/9 (33%)</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td></td>
<td>0/5 (0%)</td>
<td>0/3 (0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05 versus controls  
** p < 0.001 versus group III + IV

### TABLE II

<table>
<thead>
<tr>
<th>Group II ‘Biopsy proven GN’</th>
<th>Positive urine</th>
<th>Positive stools</th>
<th>Positive urine and/or stools</th>
<th>Subjects retested</th>
<th>Positive urine and/or stools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative GN endo ± extra (6)</td>
<td>4/5 (80%)</td>
<td>1/6 (17%)</td>
<td>4/6 (67%)</td>
<td>4/4 (100%)</td>
<td>2/4 (50%)</td>
</tr>
<tr>
<td>Minimal lesions or segmental hyalinosis (11)</td>
<td>6/11 (55%)</td>
<td>2/10 (20%)</td>
<td>6/11 (55%)</td>
<td>6/6 (100%)</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>Membranous GN (14)</td>
<td>5/13 (38%)</td>
<td>1/11 (9%)</td>
<td>6/14 (43%)</td>
<td>6/6 (100%)</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>Membranoproliferative GN (19)</td>
<td>5/18 (28%)</td>
<td>2/18 (11%)</td>
<td>7/19 (37%)</td>
<td>5/7 (71%)</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Mesangial IgA GN (34)</td>
<td>8/33 (24%)</td>
<td>5/30 (17%)</td>
<td>11/34 (32%)</td>
<td>9/11 (82%)</td>
<td>6/9 (67%)</td>
</tr>
<tr>
<td>Miscellaneous (4)</td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 88</td>
<td>28/84 (33%)</td>
<td>11/79 (14%)</td>
<td>34/88 (39%)</td>
<td>30/34 (88%)</td>
<td>22/30 (73%)</td>
</tr>
</tbody>
</table>
the difference is highly significant ($p < 0.001$) and the details are given in Table I. The NPEV infection is persistent mainly in the subgroups of membranous GN and membranoproliferative GN (Table II).

**Discussion**

Most of the studies in search of viral infections in human glomerulonephritis were based on non-oriented serological titres, often inadequate and of low sensitivity. These failed to demonstrate any significant difference in comparison with matched controls [15] with one exception [16].

We designed a protocol for direct isolation and identification of enteroviruses in urine and stools. This group was chosen, because we are a reference WHO laboratory for enteroviruses. The existence of 67 different enteroviruses does not allow routine screening for all specific antibodies.

Enterovirus infections are very common in the population under 14 years of age and especially in young children (less than 5 years) [17]. Our study concerns only adults.

The enteroviruses are usually isolated from stools [18]. In our study NPEV excretion in stools was high but not significant. In contrast, in our renal patients, we found a high incidence of NPEV isolation from urine, which became significant for the glomerulonephritis group. This unexpected finding may be related to the presence of renal lesions, raising the possibility of a ‘renal leak’ for viruses. But viruria does not correlate with the selectivity index of proteinuria.

In fact, viruria is associated with viraemia [13, 19–21]. It is more likely that viruria is the consequence of a viraemia [22], because enterovirus replication in human kidney cells has not been demonstrated, in contrast with monkey kidney cells which offer an appropriate growth culture.

Persistent NPEV infections have never been demonstrated in individuals without immunological disorders [13]. The usual and normal response to enteroviruses includes intestinal viral replication soon after ingestion, followed by a transient viraemia around the tenth day. Immune protection against enteroviruses is mainly humoral by protective antibodies [18].

The existence of persistent NPEV infections in human glomerulonephritis has been well demonstrated in this study. It raises the possibility of an abnormal immune response, possibly genetic, possibly specific to the infectious agent, in these nephritic patients.

The signification of persistent NPEV infections remains speculative. Chronic viruria, the consequence of a probable chronic viraemia, represents a situation of chronic antigenic stimulation, with the possibility of an immune complex mechanism resulting in chronic glomerulonephritis. The viral antigens may be either initiating or triggering pre-existing nephritis. The deposition of such viral antigens in the kidney has already been reported for Coxsackie types [5].

We hypothesise that persistent NPEV infections represent the cause rather than the consequence of human GN. The incidence of this suspected aetiological factor could be as high as 25%. This finding is of major importance for the future.
Acknowledgments

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References

11. Burch GE. Amer Heart J 1974; 87: 139

Open Discussion

DONINI (Bologna) I want to ask if you measured viruria during treatment with immunosuppression or not. Was the patient with glomerulonephritis on medical treatment or not?

BERTHOUX No, the patients have been screened by the time they get into hospital to have the renal biopsy so at the first control the patient has had no treatment. For some with persistent viruria there were patients on treatment because we have to wait three months. For the patient with the nephrotic syndrome we have to give steroids and you cannot wait three months. So some were on steroids, but when we know that these patients have viruria we stop the immunosuppressive treatment and we check again.

DONINI Have you looked for non polio enterovirus in the healthy population?

BERTHOUX Not ourselves. We have just 20 controls in the healthy population because it is our staff members. But we have not done a large screening. Many

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studies have been done and it is very common in children. It is quite rare in adults. In children the incidence is about 30%. We have another control group which is not in this study. We have checked people with neurological disease. In these patients we find such virus in the cerebrospinal fluid but not in the urine, so viruria is quite specific for renal disease.