Gut, 1981, **22**, 698–701

Cytomegalovirus inclusions in the gastroduodenal mucosa of patients after renal transplantation

G FRANZIN,* A MUOLO, AND THEA GRIMINELLI

From the Gastroenterology Division of the Istituti Ospitalieri of Verona, and the Department of Surgical Nephrology of the University of Padova, Verona, Italy

SUMMARY Biopsies collected from gastroduodenal mucosa during endoscopic examination of 20 patients having undergone renal transplantation and subsequent immunosuppressive therapy showed cytomegalovirus (CMV) inclusion bodies in nine cases. CMV antibody titres were tested in all patients before and after the transplant procedure. Not all patients exhibited viraemia-related symptoms at the time of endoscopy. No correlation was found between the presence of CMV-type cells within the gastroduodenal mucosa, endoscopic and histological findings, the duration of the transplant, and the dosage of immunosuppressive drugs. The duodenum seems to be the elective site of CMV. The involvement of gastric mucosa seems to represent a worsening of the illness. Eight of nine patients with positive biopsies for CMV inclusion had negative pretransplant antibody titres to CMV. All nine patients were seropositive after transplantation and showed seroconversion. Five of 11 recipients with negative biopsies for CMV inclusion bodies, were seronegative before transplantation. Seroconversion occurred in five patients after the transplant; the other six had no rise in antibody titres. The lack of pre-transplant CMV antibody titre and its subsequent increase after transplantation identifies a greater risk of developing post-transplant CMV infection.

Cytomegalovirus (CMV) is an ubiquitous agent which has been associated with various clinical manifestations such as immunodeficiency syndrome, impaired host defences, gastric ulcer, uraemia, and immunosuppression after organ transplantation.[1–5] CMV infection occurs in up to 90% of patients who have undergone renal transplantation and who are maintained on immunosuppressive therapy.[4–8]

Some of these infections represent reactivation of latent virus, while some may be primary infections transmitted from donor to recipient.[7–9] It has been suggested that reactivation infection is usually asymptomatic, whereas primary infection may be associated with various clinical manifestations such as fever, haematological abnormalities, transplantation pneumonia, and hepatitis.[4 6 8–10]

The diagnosis of CMV is difficult and is established through positive viral cultures, a fourfold rise in CMV antibody titre, and/or histological evidence of CMV inclusion bodies in biopsy tissues.[5 6 8–10]

*Address for requests for reprints: G Franzin, Servizio di Endoscopia, Istituti Ospitalieri di Verona, USL 25 della Regione Veneto—37100, Verona, Italy.

Received for publication 26 February 1981

CMV has been isolated from blood, urine, oral secretions, bone marrow, grafted kidney, lung, pancreas, and the brain. It has been sampled by biopsy and at necropsy.[5 6 8 10 11] Antemortem detection of CMV infection of the gastroduodenal tract in renal transplanted patients is rare.[12]

We studied 20 patients who underwent renal transplantation, and present the histological evidence of CMV inclusion bodies found in nine of those patients. The biopsies presenting CMV were taken, during endoscopic examination, from the duodenal bulb, the gastric antrum and fundus. The findings reveal a strong correlation between the histological presence of CMV and the antibody titre after transplantation.

Methods

At our institution, patients undergoing transplant procedures are routinely studied endoscopically and bioptically[13] and tested for CMV infection by periodic antibody titre determinations. In the present study a group of 20 patients who had undergone renal transplantation was retrospectively evaluated. The

study group consisted of 17 males and three females. The mean age was 35 years and ranged from 20 to 50 years of age. All patients were asymptomatic for manifest CMV infection at the time of endoscopy. As a rule, immunosuppressive protocol included azathioprine and steroids; however, three patients were also given antilymphocyte globulin.

Upper gastrointestinal endoscopy was performed on 19 of the patients from one to 41 months after transplantation and was carried out by means of a fibre-endoscope—Olympus GIF D-3. The remaining patient underwent two endoscopic investigations at 20 months and 74 months after the transplantation.

Four to six biopsies were taken from the duodenal, the antral, and the fundic mucosa in all but one of the patients, who had undergone a Billroth II partial gastrectomy and, henceforth, whose specimens were taken from the stoma and the gastric stump. The specimens were properly oriented and fixed in 10% calcium formalin. Multiple serial sections were stained with H and E, Alcian blue (pH 2·5)—PAS, and Gordon and Sweet's reticulin.

Sera for CMV antibody titres were obtained before and at the time of transplantation, at 10, 30, 60, 90 and 180 days after surgery and at the time of endoscopy. Titres less than 1:8 were regarded as negative. The pre-transplant presence of complement-fixing CMV antibodies was considered indicative of previous contact with the virus. A fourfold or greater change in antibody titre (seroconversion) was deemed significant.

The 20 patients were divided into two groups based upon the presence (group A) and the absence (group B) of CMV inclusion bodies in bioptic specimens.

Each was separately evaluated for pre- and post-transplant changes of CMV antibody titres (Tables 1–4).

Results

Examination of the biopsies of the nine patients in group A revealed greatly enlarged cells with a prominent nuclear inclusion and, to a lesser extent, cytoplasmic inclusions, which were found in specimens taken from the duodenal bulb in eight of the patients and from the stoma in another.

The nuclear inclusions were usually separated from the nuclear membrane by a wide, prominent halo (Figure). Such a configuration is accepted as diagnostic of CMV infection.[14]

In one instance CMV inclusions were found also in the antral mucosa. In another case CMV inclusions were found both in the antral and in the fundic mucosa during the bioptic control performed 74 months after transplantation.

It is worth noting that several CMV-type cells[2] were

Table 1 *Pre- and post-transplant CMV antibody titres in nine recipients with CMV inclusion bodies in gastroduodenal mucosa*

Case no.	*Sex*	*Age (yr)*	*Pre-transplant CF–CMV titre*	*Months*	*Post-tranplant CF–CMV titre*	
1	M	48	<8 (negative)	20–74	64	64
2	F	23	16	6	64	
3	M	35	<8	3	16	
4	M	33	<8	17	64	
5	M	45	<8	17	32	
6	M	36	<8	3	64	
7	M	32	<8	3	64	
8	M	45	<8	1	32	
9	M	30	<8	4	64	

CF–CMV: complement fixing antibody to CMV.

Table 2 *Pre- and post-transplant CMV antibody titres in 11 recipients without CMV inclusion bodies in gastroduodenal mucosa*

Case no.	*Sex*	*Age (yr)*	*Pre-transplant CF–CMV titre*	*Months*	*Post-transplant CF–CMV*
1	M	20	<8 (negative)	41	64
2	F	35	8	9	<8
3	F	38	32	9	<8
4	M	28	<8	8	32
5	M	46	<8	7	<8
6	M	26	16	6	64
7	M	33	<8	11	64
8	M	20	<8	3	32
9	M	38	32	3	8
10	M	23	16	3	8
11	M	41	64	3	64

CF–CMV: complement fixing antibody to CMV.

Table 3 *Correlations between CMV inclusion bodies and pre-transplant antibody titre to CMV*

	Pre-transplant antibody titre:		
	Positive	*Negative*	*Total*
CMV inclusions			
Present (Group A)	1	8	9
Absent (Group B)	6	5	11
Total	7	13	20

A→B (χ^2=4·10; P<0·05).

Table 4 *Correlation between CMV inclusion bodies and post-transplant rise of antibody titre to CMV*

	Post-transplant antibody titre:		
	Raised	*Not raised*	*Total*
CMV inclusions			
Present (Group A)	9	0	9
Absent (Group B)	5	6	11
Total	14	6	20

A→B (χ^2=7·01; P<0·01).

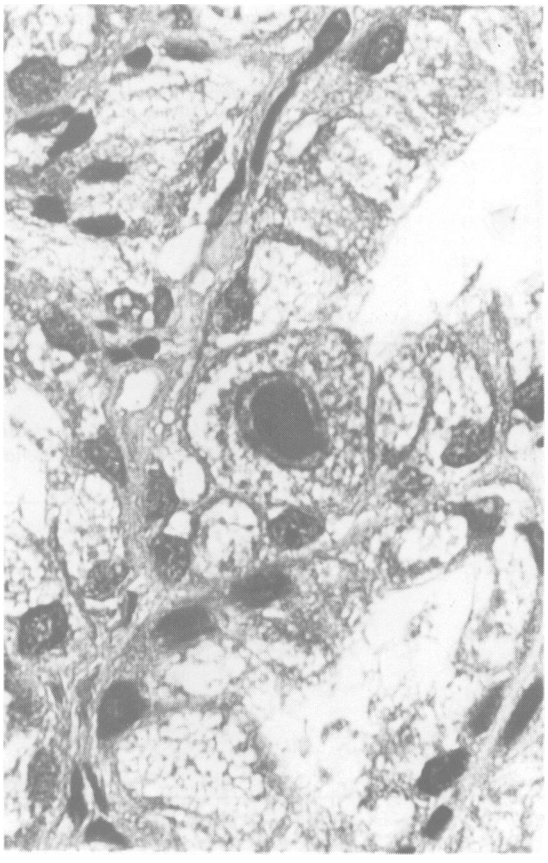

Figure *A cytomegalovirus-type cell in a Brunner's gland. H and E, × 1000.*

observed in all specimens and in all sections in the following instances: (1) in those cases in which both the duodenal and the antral mucosa presented CMV; (2) in the case involving the partial gastrectomy; (3) in case no. 1 (Table 1) at the 74 month control, in which the duodenal, the antral, and the fundic mucosa were affected by CMV.

In those cases in which the duodenal mucosa alone was involved, only one or two CMV-type cells were observed among the multiple serial sections.

CMV-type cells were located in the Brunner's gland area in the duodenum; in the pyloric glands, and in the chorion of the antrum; in the chief cells and in the chorion of the fundic mucosa as well as in the stomal pseudopyloric glands in the case of the partial gastrectomy.

Mild duodenitis occurred in six patients of group A. In the others, the duodenal mucosa was otherwise normal. In the same group, four cases of superficial antral gastritis and one case of slightly atrophic antral gastritis were detected. Fundic superficial gastritis was found in two instance (Table 5).

Symptoms such as pyrosis and dyspepsia occurred in only two instances.

No correlation between CMV inclusions and digestive symptoms and the morphological aspect of gastroduodenal mucosa was found.

Immunosuppressive therapy given to both the groups of recipients was assessed to see whether the differences could account for the frequency rate of CMV inclusion bodies: no correlation was found. Eight of the patients in group A were seronegative before transplantation (Table 1). In group B, five were seronegative and six were seropositive before the transplant procedure. The data in the two groups differ significantly: $\chi^2=4{\cdot}10$, $P<0{\cdot}05$ (Table 3).

At the time of endoscopy, all patients of group A were seropositive and showed seroconversion (Table 1). In group B only five patients showed seroconversion, whereas six had no rise in antibody titres (Table 2). These results in the two groups also reveal a significant difference: $\chi^2=7{\cdot}01$; $P<0{\cdot}01$ (Table 4). In group A, the patient in whom both the antral and the duodenal mucosa presented CMV inclusions lost his grafted kidney from chronic rejection and leucopenia. The partial gastrectomy patient died of TBC meningitis five months after endoscopy. Chorieoretinitis occurred a few days before the second endoscopic examination in one patient (case no. 1, Table 1).

Discussion

Occasionally CMV inclusions have been found in the gastrointestinal tract at postmortem examination of recipients who have died of severe CMV infection.[10] In the present series the same discovery was observed *in vivo*—that is, in biopsies obtained during routine post-transplant endoscopic examination of patients who did not present specific symptoms at the time of the investigation. The duodenum, and specifically the Brunner's gland area, seems to be the elective site of CMV. The involvement of the gastric mucosa (cases 1, 7, and 8, Table 1) coincided with a wider spreading

Table 5 *State of fundic, antral, and duodenal mucosae in patients with and without CMV inclusion bodies*

CMV inclusion bodies		*Fundus*		*Antrum*		*Duodenum*	
		SG	*AG*	*SG*	*AG*	*SD*	*AD*
Patients with	9*	2	—	4	1	6	—
Patients without	11	2	—	3	—	4	—
Total	20	4	—	7	1	10	—

* One patient with partial gastrectomy. SG: superficial gastritis. AG: atrophic gastritis. SD: superficial duodenitis. AD: atrophic duodenitis.

of CMV and may represent an indication of the underlying severity of the illness, still asymptomatic.

No correlation was found between the presence of CMV-type cells in the gastroduodenal mucosa and digestive symptoms or the endoscopic and histological findings. Similarly, no correlation was evidenced between the duration of the transplant, the daily dosage of azathioprine and steroids, and ALG administration.

It is noteworthy that eight of nine recipients with CMV inclusions had negative pre-transplant CMV antibody titres (Tables 1 and 3), whereas only five of the 11 recipients without CMV inclusions were seronegative before transplant procedures. Moreover, six patients in whom no post-transplant rise of titres was detected (Table 4) had no CMV inclusion bodies. On the other hand, most of the patients with post-transplant rise of titre (Tables 1 and 4) also had positive biopsies for CMV inclusions. Thus, the absence of pre-transplant CMV antibodies, and the post-transplant rise of antibody titres identifies a greater risk of developing post-transplant CMV infection.

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