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IgM IMMUNE COMPLEXES, LEUCOCYTOTOXINS AND RHEUMATOID FACTORS DURING ACTIVE CYTOMEGALOVIRUS INFECTIONS IN RENAL GRAFT RECIPIENTS TREATED WITH AZATHIOPRINE, CYCLOSPORIN A OR ANTI-THYMOCYTE GLOBULIN

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Summary

Circulating IgM immune complexes (CIC) were detected in all 11 renal transplant recipients with primary cytomegalovirus (CMV) infections and in 12 of 20 patients with secondary CMV. The incidence, kinetics and composition of the IgM CIC did not differ between patients immunosuppressed with Cyclosporin A (CyA) and those with azathioprine. Uncomplicated reversible or irreversible rejections did not cause increases in IgM CIC and treatment of rejection episodes with prednisone, methylprednisone, or rabbit antithymocyte globulin (ATG) did not prevent increases in IgM-PEG values due to coincident CMV infection. Leucocytotoxic CIC were associated with CMV related graft loss.

Introduction

CMV infections have been associated with a wide range of clinical features, including fever, pneumonia, renal dysfunction, hepatic dysfunction, lymphadenopathy, leucopenia, thrombocytopenia, rashes, arthralgia, and retinitis. Some of these findings may be due to direct cytopathic effects of CMV in the afflicted organs, but others may be related to indirect effects on the neonatal or immunocompromised patient's immune system. In 1977 Stagno and co-workers [1] detected IgG containing CIC by the Raji cell assay in sera from 39 of 86 neonates with CMV. In addition, granular deposits of IgG, IgM and C_3 were present in the glomeruli. Later, Richardson and co-workers [2] described a lesion in renal transplants, which they termed CMV glomerulopathy, that was characterized by swelling of endothelium and deposits of fibrin, IgM and C_3. These investigators detected CIC in sera from only two of eight patients with the Raji cell assay. However, the Fc receptor on the Raji cell binds IgG but not IgM, and IgG was deposited in only two of eight biopsies. We found prominent glomerular deposits of IgM and only minor amounts of IgG in renal

transplant biopsies from recipients with active CMV disease [3]. A polyethylene glycol (PEG) precipitation assay detected IgM CIC in the sera from all 10 patients with primary CMV and two of four patients with secondary CMV. In contrast, a Clq-binding assay which is more sensitive for soluble IgG than IgM CIC was positive in only three of 14 patients. Further studies [4–6] demonstrated that the PEG precipitates contained IgM rheumatoid factor and IgM leucocytotoxins. All of the patients in our earlier studies had been treated with azathioprine and low dose steroids. The present study was designed to extend these observations to a larger group of patients, many of whom were treated with CyA or ATG.

Materials and methods

Serial serum samples from 57 adults that were obtained in the first three months after they had recieved renal transplants at Leiden between October, 1983 and February, 1985 were studied prospectively with the IgM-PEG and radio-immune rheumatoid factor assays. These assays were performed as described in detail previously [3] with the exception that ^{125}I radio-labelled mouse monoclonal anti-human IgM antibodies were used to quantify the IgM. Two monoclonals, HB 57 (American Type Culture Collection, Rockville, MD) and 179–1.1 (TNO, Rijswijk) were found to be equally effective in the assays.

PEG precipitates and supernatants of patients' sera were prepared as previously described [4] for testing in a complement dependent cytotoxicity assay performed at 22°C on peripheral blood leucocytes from a panel of 10 donors. CMV antibody titres were measured by a standard immune adherence haemagglutination technique (IAHT) and indirect immunofluorescence assay on CMV infected human fibroblasts (IFA-IgM and IFA-IgG). The diagnosis of active CMV was based on a four-fold or greater increase in CMV antibody titre or on a positive CMV culture from urine, blood or saliva.

Results

In patients who had not received rabbit ATG, ^{125}I radio-labelled antibodies to human IgM from rabbit or mouse gave similar values in the IgM-PEG assay. Consistently discordant results were obtained after ATG treatment. When the rabbit, but not the two mouse, reagents were used, extremely high IgM-PEG values were recorded beginning 14 to 25 days after ATG treatment was initiated and persisting for up to six months (Figure 1A). These high values were probably due to the anti-rabbit antibodies that patients are known to produce to the rabbit ATG [7].

When the IgM-PEG assay was performed with mouse monoclonals an elevated IgM-PEG value was observed only once within two weeks of 47 rejection episodes treated with prednisone, methylprednisone or ATG when there was no evidence of concomitant CMV infection (Table I). This patient had serological and culture evidence of a herpes simplex infection which was first evident three weeks prior to the initiation of ATG therapy. Decreases in the IgM-PEG values accompanied treated rejections in four patients, three of whom were treated with ATG and

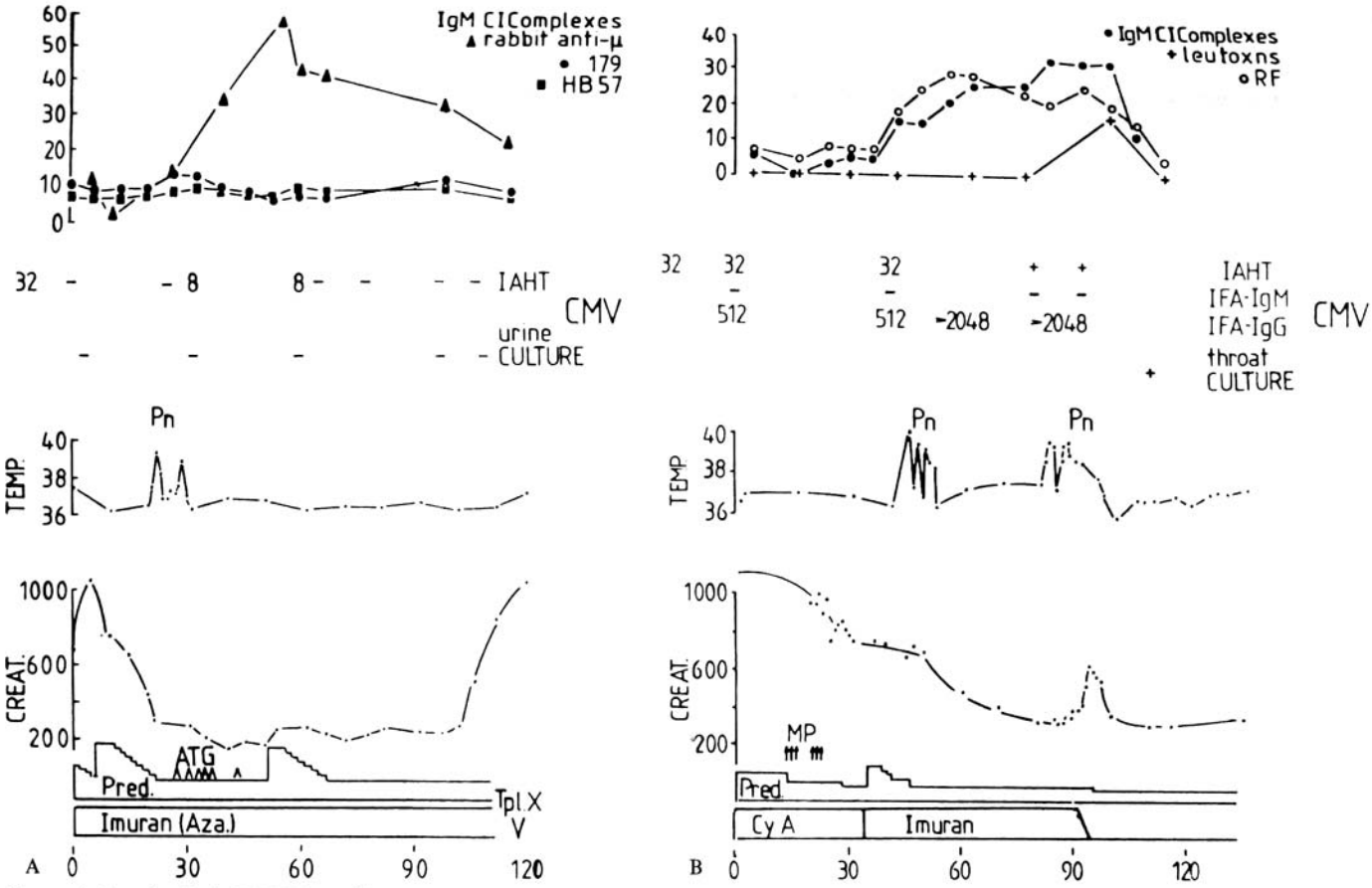

Figure 1. Longitudinal IgM-PEG studies on two renal transplant patients. Patient A was treated with prednisone and ATG for rejection and had a bacterial pneumonia (Pn). IAHT CMV antibody titres did not increase above the pre-transplantation value of 32 and urine cultures were negative. The IgM-PEG assay was consistently negative with mouse monoclonal reagents 179 and HB57 but increased with the rabbit reagent after treatment with ATG. Patient B was treated twice with methylprednisone (MP) for rejection with no increase in IgM-PEG values. Subsequently the IgM-PEG, rheumatoid factor (RF) and leucotoxins increased during a secondary CMV infection

TABLE I. Association of increased IgM-PEG values with active CMV infections but not with rejection episodes

Source of grafts	Maintenance immuno-suppression	Incidence of increased[1] IgM-PEG values within two weeks of Rejection episodes[2] treated with				Active CMV infections	
		Prednisone	Methylpred	ATG	Nephrec-tomy	Primary	Secondary
Family	Azathioprine + prednisone	0/0	0/0	0/4	0/0	2/2	0/2
Cadaver	Azathioprine + prednisone	0/6	0/0	1/19	0/3	1/1	6/10
Cadaver	Cyclosporin A → azathioprine[3]	0/1	0/5	0/1	0/0	1/1	3/5
Cadaver	Cyclosporin A	0/0	0/5	0/2	0/2	7/7	3/3
		0/7	0/10	1/26	1/5	11/11	12/20

1 Greater than 15 per cent increase over the patients' base line values

2 Not associated with active CMV infections

3 Converted from Cyclosporin A to azathioprine one to three months after transplantation

one of whom was treated with methylprednisone. Nephrectomy was also accompanied by a reduction in base line IgM-PEG levels in three of five cases.

In all 11 patients with primary CMV infections and in more than half (12 of 20) of the patients with secondary CMV infections the IgM-PEG values increased more than 15 per cent above the patients previous base line levels. In primary infections this increase usually coincided with the increase in IgM antibodies to CMV and in secondary infections it sometimes occurred weeks in advance of the increase in IgM or IgG antibodies to CMV (Figure 1B). In both the CyA- and azathioprine-treated patients the maximum IgM-PEG values ranged between 20–45 per cent above base line levels and they remained elevated for 3–12 weeks. Treatment with ATG, methylprednisone, prednisone or nephrectomy just before or during a CMV infection did not prevent the increase in IgM-PEG values.

The rheumatoid factor values paralleled the IgM-PEG values in eight of 11 patients with primary CMV infections. In one patient with a primary infection the rheumatoid factor remained within the normal range while the IgM-PEG values were elevated, and in two other patients the rheumatoid factor values were elevated for two months prior to the increase in IgM-PEG values and in CMV antibody titres. Fewer patients with secondary CMV infections had elevated rheumatoid factor values. In four patients with secondary infections, the increased rheumatoid factor values paralleled the increase in IgM-PEG values (Figure 1B), but in eight other patients the rheumatoid factor values remained in the normal range while the IgM-PEG values were increased. Seven of eight

patients who did not have increased IgM-PEG values during their secondary CMV infections also did not have elevated rheumatoid factor values.

Primary CMV infections were associated with circulating leucocytotoxins in eight of 11 patients. The leucocytotoxins were partially or completely precipitated by 3.5 per cent PEG in five of these eight patients. Eleven of 16 patients with secondary CMV infections had circulating leucocytotoxins (4 patients were not tested), and in eight of these patients the leucocytotoxins were PEG precipitable. Six of seven patients who rejected their kidney transplants within a month of the onset of their CMV infections produced PEG precipitable leucocytotoxins during their infections.

Discussion

The present study extends our previous reports that active CMV infections, particularly primary infections, in renal transplant recipients are associated with IgM CIC that are detectable by PEG precipitation [3–6]. The frequency, maximum value, and kinetics of IgM CIC were similar in patients treated with CyA to those in patients treated with azathioprine and low dose steroids. Harkiss and Weirzbicki [8] have recently reported that IgM CIC detected by a solid phase conglutinin binding ELISA correlated with viral infections in 28 heart transplant recipients who were treated with CyA and/or ATG. Reversible or irreversible rejection episodes were not associated with increased IgM-PEG values regardless of whether they were treated with prednisone, methylprednisone or ATG. In fact, methylprednisone, ATG and nephrectomy were sometimes associated with transient decreases in the base line IgM-PEG values. For this reason, the IgM-PEG assay may help distinguish between renal dysfunction associated with CMV infection versus that due to rejection.

In addition, investigations into the composition of the IgM CIC may provide some insights into the mechanism of some of the clinical findings associated with CMV infections. In the present group of patients PEG precipitable circulating leucocytotoxins correlated with loss of graft function.

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