Renal graft dysfunction during infection with cytomegalovirus: association with IgM lymphocytotoxins and HLA-DR3 and DR7

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Abstract

Of 121 consecutive adult recipients of cadaver renal transplants who were treated with low dose steroids and azathioprine, 23 developed active cytomegalovirus infections. These 23 patients were divided into three groups on the basis of their symptoms related to the infection: five patients had no renal, respiratory, or haematological abnormalities; seven had renal dysfunction; and nine had renal dysfunction plus respiratory or haematological abnormalities. Two patients were regarded as a separate group because their infections occurred two to four weeks after graft nephrectomy.

All but three of the patients produced IgM or IgG lymphocytotoxins during their infections. In the patients with mild infections and in control patients without infections, however, these lymphocytotoxins were predominantly IgG antibodies that were not precipitated by 3.5% macrogol (polyethylene glycol). In contrast, 12 of the 16 patients with renal dysfunction during their infections had broadly reactive IgM lymphocytotoxins. These IgM lymphocytotoxins lysed T as well as B lymphocytes at 22°C and were precipitated by 3.5% macrogol, suggesting that they were circulating as immune complexes. Rheumatoid factors were found in sera from nine patients with cytomegalovirus infections, seven of whom developed leukopenia or pneumonia, or both, in addition to renal dysfunction.

Some of these immune responses associated with cytomegalovirus infection in transplant recipients may be genetically controlled since 10 of 11 patients positive for HLA-DR3 or DR7 produced IgM lymphocytotoxins.

Introduction

Cytomegalovirus infections are a major cause of morbidity in transplant recipients. Fever, leukopenia, thrombocytopenia, pneumonia, liver dysfunction, and renal dysfunction have been associated with such infections.¹⁻⁵ The pathogenesis of these various manifestations has not been fully clarified. During cytomegalovirus infection, however, immune complexes have been detected in the circulation and granular deposits of immunoglobulins shown in the glomeruli of patients with and without renal transplants,⁶⁻⁹ suggesting that renal dysfunction may be caused by immune complexes. Yet many patients with cytomegalovirus infections and circulating immune complexes

do not develop renal dysfunction, and in all but one case cytomegalovirus antigens have not been shown in the glomerular deposits.⁷ Many of the antibodies generated during cytomegalovirus infections are directed to autoantigens such as DNA, smooth muscle, IgG (rheumatoid factor), lymphocytes, and erythrocytes.⁸⁻¹¹

We undertook the present investigation to determine whether certain autoantibodies induced by cytomegalovirus may circulate as immune complexes and cause renal damage.

Patients and methods

PATIENTS

We studied all 23 adults who developed well documented primary or secondary cytomegalovirus infections in the first four months after cadaver renal transplantation at this hospital between January 1980 and January 1983. Eight patients with primary infections and eight with secondary infections experienced renal dysfunction during their infections. An additional 15 patients without active cytomegalovirus infections who experienced renal graft dysfunction during the same period were studied as controls for antibody responses related solely to rejection of the transplant. All 121 adults who received cadaver renal transplants at this hospital during the study period were treated with low dose steroids and azathioprine¹² and followed up by one team of physicians.

METHODS

Serum samples were obtained weekly from each patient after transplantation. In the case of patients with active cytomegalovirus infections samples were selected from the time when titres of antibodies to cytomegalovirus increased and renal dysfunction or other symptoms were recorded. Additional samples obtained before and after the active infections were tested for longitudinal studies. In the case of control patients, who experienced acute rejection but did not have cytomegalovirus infections, samples were selected from those obtained within a week after removal of the transplant to maximise the chances of finding lymphocytotoxins in the circulation.

Macrogol (polyethylene glycol) 6000 precipitates were prepared by incubating 0.5 ml serum with 0.5 ml phosphate buffered saline (0.07 mol sodium chloride and 0.001 mol phosphate buffer) containing 35 mg macrogol at 0°C for one hour. These samples were centrifuged at 2000 g for 15 minutes at 4°C. The supernatant was saved and the precipitate dissolved in 0.5 ml phosphate buffered saline.

Assays—The following standard determinations were performed on the macrogol precipitates and supernatants: a Folin determination for total protein content; an immunodiffusion assay for IgM immunoglobulin concentration; latex agglutination for rheumatoid factor; and the National Institutes of Health complement dependent cytotoxicity assay performed at 22°C on peripheral blood lymphocytes from a panel of 10 donors for lymphocytotoxins. The classes of the lymphocyte reactive antibodies and rheumatoid factors were determined by indirect immunofluorescence with heavy chain specific antibodies.¹³ All HLA typing of graft recipients had been routinely performed by Eurotransplant in Leiden before transplantation. Titres of antibodies to cytomegalovirus were determined by a standard immune adherence haemagglutination technique.¹⁴ Titres of less than 8 were considered to be negative. A diagnosis of active cytomegalovirus infection was based on a fourfold or greater increase in the titre of antibodies to cytomegalovirus or on culture of cytomegalovirus from urine, blood, or sputum.

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Results

The 23 patients who developed active cytomegalovirus infections after cadaver renal transplantation were divided into four groups on the basis of renal, respiratory, and haematological findings. Five patients had no detectable renal, respiratory, or haematological abnormalities (group 1); seven patients had renal dysfunction as evidenced by otherwise unexplainable increases in serum creatinine concentration (>50 mmol/l (>566 mg/100 ml)) or proteinuria (1.3-8.0 g/day) (group 2); and nine patients had leukopenia ($<2 \times 10^9$ cells/l), thrombocytopenia ($<100 \times 10^9$ cells/l), or radiological evidence of respiratory infections in addition to renal dysfunction (group 3). Two patients were considered separately (group 4) because they developed cytomegalovirus infections two and four weeks after irreversible graft rejections.

Six of the 12 patients in groups 1 and 2 and all of the patients in groups 3 and 4 were febrile. Five of the patients in groups 1 and 2 and two in group 3 had raised transaminase activities during their infections. Titres of antibodies to cytomegalovirus were inversely correlated with the severity of symptoms: patients with mild symptoms had higher titres (geometric mean titre >512 in group 1) than patients with more severe symptoms (geometric mean titre 256 in group 2 and 128 in groups 3 and 4). This may reflect in part the debilitating effects of the infections in patients in group 3, since four of these patients had considerable leukopenia, eight had respiratory infections, and three died of causes related to the cytomegalovirus infection. No significant differences were observed between the four groups of patients in the distribution of primary and secondary infections (12:11), age (38 (SD 11) years), or sex (13 men:10 women).

Although the amount of material similar to IgM immune complexes that was precipitated by 3.5% macrogol was raised in all four groups of patients with active cytomegalovirus infections (35 (6)% *v* 15.4 (4)% in transplant recipients without such infections⁸), the reactivity of the IgM antibodies contained in the precipitate varied. Macrogol precipitated lymphocytotoxins in only one of the five patients without renal dysfunction (group 1) but in 12 of the 16 patients with renal dysfunction (table I; $p < 0.05$ by χ^2 analysis). Seven of the nine patients with pneumonia or leukopenia, or both, in addition to renal dysfunction (group 3) had rheumatoid factor that was precipitated by macrogol (mean titre 640), whereas only two of the 12 patients in groups 1 and 2 had circulating rheumatoid factors, with titres of 240 and 320. Longitudinal studies showed that the appearance of rheumatoid factors and lymphocytotoxins precipitated by macrogol coincided with the clinical findings of renal dysfunction, whereas the non-precipitable lymphocytotoxins did not. The rheumatoid factors were predominantly IgM with only minor amounts of IgG and IgA. The antibodies that were precipitated by macrogol were shown to be IgM when the lymphocytotoxin test was performed on ultracentrifuged fractions of the patients' sera and when lymphocytes were stained with the patients' sera and specific anti-IgM or IgG labelled with fluorescein. In contrast, the non-precipitable lymphocytotoxins were 7S IgG antibodies. Both types of lymphocytotoxins usually lysed cells from 50-100% of the donor panel. The antibodies precipitated by macrogol lysed T as well as B lymphocytes, and their reactivity did not correlate with the HLA antigens of the cell donors.

Sera from 15 patients who received renal transplants at Leiden during the same period and had renal dysfunction due to irreversible acute rejection in the absence of active cytomegalovirus infections were tested in the same assays. These patients were similar to the patients with renal dysfunction associated with infection in age (38 (12) years), sex (10 men:5 women), and titres of antibodies to cytomegalovirus before transplantation (seven without detectable antibodies and eight with antibodies). Rejection was not accompanied by production of rheumatoid factor in any of these 15 patients. Ciciarelli *et al*¹⁵ also reported that renal graft rejection is not associated with production of rheumatoid factor. Only two of these control patients developed lymphocytotoxins precipitable by macrogol, which reacted weakly with 10-20% of the lymphocyte panel; eight of these 15 patients had IgG lymphocytotoxins not precipitable by macrogol at the time of graft rejection (table I).

As the tendency to form certain antibodies may be linked to immune response genes (HLA-DR) the HLA type of each group of patients was analysed. The prevalence of HLA-DR types in the patients with cytomegalovirus infections did not differ significantly from that in the total population of 121 adults who received cadaver renal transplants at Leiden during the study period. Ten of the 11 patients positive for HLA-DR3 or DR7 who developed active cytomegalovirus infections, however, produced IgM lymphocytotoxins that were precipitated by macrogol (table II). In addition, the

two patients who produced weak IgM lymphocytotoxins that were precipitable by macrogol in the absence of active infections were also positive for DR3 or DR7. The incidence as well as the intensity of the lymphocytotoxin response was increased by cytomegalovirus infection in the patients positive for DR3 and DR7 because seven patients in the control group without infections who were positive for DR3 or DR7 did not produce detectable IgM lymphocytotoxins that were precipitable by macrogol (table II).

TABLE I—Laboratory findings in renal transplant recipients* with active cytomegalovirus infections (figures are numbers of patients)

	Group 1 (n=5)	Group 2 (n=7)	Group 3 (n=9)	Group 4 (n=2)	Controls (n=15)
Primary; secondary infection	3:2	3:4	5:4	1:1	0:0
Lymphocytotoxins in:					
Whole serum	3	7	9	2	10
Macrogol supernatant (IgG)	2	2	7	1	8
Macrogol precipitate (IgM)	1	6	6	2	2
Rheumatoid factor (IgM)	1	1	7	0	0

*Group 1 = patients without renal, respiratory, or haematological abnormalities during their infection. Group 2 = patients with renal dysfunction. Group 3 = patients with renal dysfunction and respiratory or haematological abnormalities. Group 4 = patients who developed cytomegalovirus infections after graft rejection. Controls = patients with graft rejection without active cytomegalovirus infections.

TABLE II—Association of IgM lymphocytotoxins precipitable by macrogol with HLA-DR3 or DR7 in renal transplant recipients

HLA-DR3 or DR7	Lymphocytotoxins precipitable by macrogol		Significance
	Present	Absent	
<i>Patients with cytomegalovirus infections</i>			
Present	10	1	} $p < 0.05$
Absent	5	7	
<i>Patients without cytomegalovirus infections</i>			
Present	2	7	} NS
Absent	0	6	

NS = Not significant.

Discussion

Active cytomegalovirus infections were documented in 23 of 121 adult recipients of cadaver renal transplants. This low incidence of infection is probably related to the low dosage of steroids used for immunosuppression in our patients. Others have reported that the incidence of such infections decreases with decreased immunosuppressive treatment.^{16 17} None the less, 16 of our patients developed renal dysfunction and nine developed leukopenia, thrombocytopenia, or respiratory infections in addition to renal dysfunction.

As both the susceptibility to and the symptoms from murine cytomegalovirus infection are linked to the major histocompatibility locus in mice^{18 19} we searched for such a linkage in our patients. No genetic predisposition to active cytomegalovirus infections was discerned in our patients. Some of the immune responses to the infection, however, appeared to be linked to certain HLA-DR genes. During active cytomegalovirus infections 10 of the 11 patients who were positive for DR3 or DR7 formed broadly reactive IgM lymphocytotoxins, and eight of these patients developed renal dysfunction which coincided with the appearance of these antibodies and was associated with heavy granular deposits of IgM in the glomeruli.^{5 8} In one patient this IgM response persisted for over eight months after graft nephrectomy and the end of immunosuppressive treatment, suggesting that neither foreign histocompatibility antigens nor immunosuppression were required to maintain this response. Another patient, who was positive for DR3 and developed an active cytomegalovirus infection one month

after removal of the transplant, also produced a strong IgM lymphocytotoxin response in the absence of foreign histocompatibility antigens and immunosuppressive treatment. Finally, one patient positive for DR3 and one positive for DR7, who rejected their kidneys in the absence of demonstrable cytomegalovirus infections, developed weak IgM lymphocytotoxin responses, while 13 other patients undergoing acute graft rejections did not (table II). These observations emphasise the contribution of genetic factors to the IgM lymphocytotoxin response in our patients. Other investigators have reported that normal subjects and patients with autoimmune diseases who are positive for DR3 produce increased amounts of certain antibodies and clear immune complexes from their circulation at decreased rates.²⁰⁻²²

The IgM lymphocytotoxins in our patients may have been circulating in the form of immune complexes as they were precipitated by 3.5% macrogol and this procedure preferentially precipitates material that is similar to immune complexes from serum.⁸ In eight patients the precipitate contained only 37 (8)% of the total IgM in the sera but all of the lymphocytotoxic activity. These lymphocytotoxins do not appear to be directed to HLA antigens as there was no correlation between the HLA type of the lymphocytes in the panel and the patterns of lymphocytotoxicity observed. They also were not exclusively "cold B-lymphocytotoxins"²³⁻²⁴ as they reacted strongly with T lymphocytes at 22°C. Chalopin and Rife¹¹ have also noted that virally induced IgM antibodies with T lymphocyte reactivity are associated with renal graft dysfunction.

The nine patients in our study who experienced leukopenia or respiratory infections, or both, in addition to renal graft dysfunction frequently had not only circulating IgM lymphocytotoxins but also IgM rheumatoid factors and IgG lymphocytotoxins. The symptoms of the cytomegalovirus infections did not coincide in time with the appearance of the IgG lymphocytotoxins but they did with the appearance of the rheumatoid factors. In one patient rheumatoid factor and symptoms were present for only two weeks. In the eight other patients rheumatoid factors were present for from three to eight weeks. Although producing the most rheumatoid factors and lymphocytotoxins, this group of patients with severe symptoms related to the cytomegalovirus infection produced the lowest titres of antibodies to cytomegalovirus. The possibility that the production or presence of lymphocytotoxins or rheumatoid factors may inhibit the production or activity of antibodies to cytomegalovirus is currently under investigation.

Our data suggest that although all transplant recipients may have an increased risk of cytomegalovirus infections, certain immune responses to this infection may be linked to HLA-DR genes and these responses may influence the symptoms of the infection. These observations may explain in part the "protean clinical manifestations"²⁵ of cytomegalovirus infections.

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ONE HUNDRED YEARS AGO A CORRESPONDENT writes: "Having worn a Spanish sash for some time, many years ago, whilst walking in the Pyrenees, I am decidedly of opinion that the weight of the trousers is supported much more easily, and pleasantly, by a sash than by braces; these last are narrow, about 2 inches wide, and though custom enables us to wear them without conscious inconvenience, I think any one using them for the first time would find them very unpleasant. The sash worn by the middle and lower class in Aragon is of wool 8 or 9 inches broad, and (if my recollection is correct) about 4½ feet long; when of such width and length it does not need to be drawn tight, but only closely wrapped round the waist and the end tucked in. I should certainly wear one constantly but that I do not wish to have an eccentric appearance. Medical men, I believe, attach great value to the wearing of sashes or bands round the stomach, especially in hot countries. A narrow silken sash which must be drawn tight is, I should suppose, far less pleasant to wear. (British Medical Journal 1883; **i**:1051.)