Cytomegalovirus infections in heart and heart and lung transplant recipients

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SUMMARY Of the first 166 heart and 15 heart and lung transplant recipients at Papworth Hospital, Cambridge, who survived for more than one month after transplantation, 162 were investigated for cytomegalovirus (CMV) infection by serological methods. Altogether, 73 (45%) developed CMV infection after transplantation: 30 (18·5%) had acquired primary infection and 43 (26·5%) reactivation or reinfection. Six patients died of primary infection, probably acquired from the donor organ. Recipients negative for CMV antibody who received an organ from an antibody positive donor had the most severe disease. Heart and lung transplant recipients experienced more severe primary CMV infection than those in whom the heart alone was transplanted.

The most sensitive and rapid serological method was a μ-capture enzyme linked immunosorbent assay (ELISA) for detecting CMV specific IgM, the amount of which was often of prognostic value and influenced the management of patients.

Infections are a major cause of morbidity and mortality in the first few months after cardiac transplantation because immunosuppressive treatment is most intense during this time and renders the patient more susceptible to microbial infection.1–3 Cytomegalovirus (CMV) is the most important of these infections and is transmitted to heart transplant recipients by the donated organ, blood, or blood products.4–6 Primary CMV infection is the most severe form of the disease, particularly when the infection is acquired from the donated organ.7 CMV reactivation or reinfection, although rarely fatal, may be associated with high morbidity, particularly when patients are concurrently infected with other organisms, such as Pneumocystis carinii.8 Other workers, notably Dummer et al9 have described the CMV infections arising in heart transplant recipients in the United States of America. In this paper we give a detailed account of CMV infections in the first 166 heart and 15 heart and lung transplant recipients at Papworth Hospital.

Material and methods

Of the first 166 heart and 15 heart and lung transplant recipients in the Papworth Hospital series, who received a transplant between January 1979 and August 1986, only the 162 who survived for more than one month were included in this study. Of these, 148 were male and 14 were female, with ages ranging from 6 to 56 (mean 40) years.

Serum samples were available from donors whose age range was 8–45 (mean 25) years. CMV antibody status was determined by complement fixation test and and a competitive enzyme linked immunosorbent assay (ELISA).

The first 29 patients received conventional immunosuppression with azathioprine and prednisolone including a prophylactic course of intravenous equine antithymocyte globulin (ATG) (Upjohn Ltd, Kalamazoo, USA) lasting 28 days. The next 60 patients received cyclosporin A and low dose steroids, and the next 60 patients were randomised to receive either this regimen or cyclosporin A and azathioprine. Thereafter, patients received cyclosporin A, prednisolone, and azathioprine. The initial maintenance doses were: cyclosporin A (10–12 mg/kg/day to maintain whole blood trough concentration at 1200–2000 mg/ml, reducing to 6 mg/kg/day by three months); azathioprine (2 mg/kg/day, adjusted to white cell count); and prednisolone (1 mg/kg/day reducing to a maintenance dose of 0·25 mg/kg/day after two weeks). All patients treated with cyclosporin A received a short (three to five day) course of ATG in the early postoperative period.
**Complement fixation test** was performed in U well microtitre trays as described by Bradstreet and Taylor, with three haemolytic doses of complement (HD50). All serum samples were tested over a range of dilutions from 1 in 8 to 1 in 8192.

**Competitive ELISA** tests were performed in Falcon flexible microtitre enzyme immunoassay (EIA) plates (Becton Dickinson) as described by Wreghitt et al. Briefly, 100 µl of CMV complement fixation test antigen (Public Health Laboratory Service (PHLS) Division of Microbiological Reagents and Quality Control, DMRQC) at optimal dilution (previously determined in a checkerboard assay), in carbonate/bicarbonate buffer (pH 9-6), was placed in each well of the plate and incubated overnight in a moist chamber at 4°C. The plates were then washed three times with 0.85% sodium chloride containing 0.08% Tween 20. A total of 200 µl of 0.1% (w/v) bovine plasma albumin was added and the plate left at room temperature for one to four hours after which the wells were aspirated to dryness. Neat serum (100 µl) was then added to duplicate wells in the assay plate after which it was incubated at room temperature overnight in a moist chamber.

Included in each assay was a positive control serum with a complement fixation titre of 64, and a negative control serum which did not contain CMV antibody in any assay. The plate was washed three times as before and 100 µl anti-CMV horse radish peroxidase conjugate (mouse-monoclonal) at a dilution of 1 in 2500 in PBS (pH 7.6), containing 0.08% Tween 20 (PBST) was added to each well. The plates were then incubated in a moist chamber at room temperature for three to four hours and then washed three times as before. After this, 100 µl enzyme substrate (orthophenylenediamine 1 mg/ml and hydrogen peroxide 0.4 µl/ml in citric acid/phosphate buffer, pH 5.0) was added to each well. After incubating the plate in the dark at room temperature for 20 to 30 minutes 25 µl of 3 mol/l sulphuric acid was added to each well to stop the reaction. Absorbance was then measured at a wavelength of 492 nm. Results were expressed as percentage inhibition derived by the use of the following formula:

\[
\text{% inhibition} = \frac{\text{Absorbance with positive serum} - \text{Absorbance with test serum}}{\text{Absorbance with negative control serum} - \text{Absorbance with test serum}} \times 100\%
\]

The cut off point was arbitrarily defined as 50% inhibition. Samples giving more than 50% inhibition were regarded as positive, and those giving less than 50% inhibition were regarded as negative for CMV antibody.

The μ-capture ELISA was performed, as described by Wreghitt et al. Briefly, the wells of Falcon flexible EIA plates (Becton Dickinson) were coated overnight at 4°C with 100 µl Dako rabbit anti-human IgM antibody (diluted 1 in 2000 in carbonate/bicarbonate buffer, pH 9.6). Plates were washed three times with 0.85% sodium chloride containing 0.8% Tween 20. A total of 100 µl human test serum (diluted 1 in 100 in PBST) was added and the plates incubated at 37°C for three hours. Serum dilutions were not placed in the top row of each plate. Reference control sera containing 100, 33, 10, 3.3, 1.0, 0.33, 0.1, and 0 arbitrary units per ml of CMV specific IgM, made by diluting positive control serum in negative control serum, were included in each assay.

The working dilutions of antigen and conjugate were determined by checkerboard titrations. The plates were washed as before and 100 µl CMV antigen (PHLS, DMRQC diluted 1 in 10 in PBST with 10% fetal calf serum) was added to every well that had contained serum. The plates were incubated at 4°C overnight and then washed three times as previously, before 100 µl peroxidase conjugated anti-CMV monoclonal antibody was added to all wells, except those in the top row. The plates were incubated at 37°C for three hours, washed three times, before 100 µl substrate solution (o-phenylenediamine 1 mg/ml and hydrogen peroxide 0.4 µl/ml in citric acid/phosphate buffer, pH 5) was added to all wells. After incubating plates at room temperature for 30 minutes the reaction was stopped by adding 25 µl of 3 mol/l sulphuric acid to each well. Absorbance was read at 492 nm in a Titertek Multiskan (Flow Laboratories). The top row of wells, coated with anti-human IgM and containing substrate and stop solution, provided blank readings. The amount of CMV specific IgM in serum was determined by reference to a standard curve plotted from the results obtained with the positive control serum.

Severity of symptoms associated with CMV infection was assessed according to the scoring system described by Smiley et al.

**Results**

Although each patient was investigated serologically for evidence of CMV infection at the time at which it occurred, this study represents a thorough retrospective review using several serological techniques. Primary CMV infection was indicated in patients whose serum did not contain any detectable CMV antibody in any assay (particularly competitive ELISA) before transplantation and whose serum samples taken at least a month after transplantation showed a significant rise in CMV antibody (fou-fold rise by complement fixation test, > 50% inhibi-
tion in competitive ELISA). CMV reactivation or reinfection was indicated in those patients whose serum contained detectable CMV antibody in any assay before transplantation and whose serum samples taken at least a month after transplantation showed a significant rise in CMV antibody (≥ fourfold rise by complement fixation test).

Further evidence of primary CMV infection or CMV reactivation or reinfection was sought by determining the amounts of CMV specific IgM in serum samples showing a rise in complement fixation test antibody. In general, higher concentrations of CMV specific IgM were found in patients with primary CMV infection than in those with CMV reactivation or reinfection (fig 1). CMV specific IgM could also be detected in serum samples taken several days (and sometimes several weeks) before a rise in complement fixation antibody was detected (fig 2).

Typical CMV antibody profiles of patients with primary CMV (fig 3) and CMV reactivation or reinfection (fig 4) show that CMV specific IgM is present at a higher concentration, and is detectable for longer in primary CMV infection compared with CMV reactivation or reinfection.

Thirty patients (18.5%) experienced primary CMV infection and 43 (26.5%) were found to have CMV reactivation or reinfection at some time after transplantation. The number of patients who developed CMV infection and the severity of disease was related to the CMV antibody status of the recipient and donor. CMV antibody negative recipients were more likely to experience primary CMV infection if they received a heart or heart and lungs from a CMV antibody positive donor (83%) than if they received a heart or heart and lungs from a CMV antibody negative donor (27%) (table 1). The six patients who died of CMV infection all had primary disease and were in the mismatched group—that is, CMV antibody positive donor, CMV antibody negative recipient. Similarly, CMV antibody positive recipients were more likely to experience CMV reactivation or reinfection if they received a heart or heart and lungs from a CMV antibody positive (62%) rather than a CMV antibody negative (42%) donor (table 1).
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Table 1 Summary of infections in 129 heart and 15 heart and lung transplant recipients

<table>
<thead>
<tr>
<th>CMV status of:</th>
<th>No (%) of patients with:</th>
<th>CMV reactivation/reinfection</th>
<th>No (%) died of CMV</th>
<th>Clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>Recipient</td>
<td>Total</td>
<td>Primary CMV</td>
<td>-</td>
</tr>
<tr>
<td>Heart transplants:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ -</td>
<td>18</td>
<td>15 (83)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- +</td>
<td>33</td>
<td>-</td>
<td>20 (61)</td>
<td>-</td>
</tr>
<tr>
<td>- -</td>
<td>44</td>
<td>-</td>
<td>19 (43)</td>
<td>-</td>
</tr>
<tr>
<td>Heart and lung transplants:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ -</td>
<td>5</td>
<td>4 (80)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- +</td>
<td>3</td>
<td>1 (20)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- +</td>
<td>2</td>
<td>-</td>
<td>3 (100)</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig 3 CMV complement fixation (●—●) and specific IgM (○—○) antibody responses in patient with primary infection.

Fig 4 CMV complement fixation (●—●) and specific IgM (○—○) antibody responses in patient with reactivation/reinfection.
It was difficult to perform a detailed statistical analysis of all four donor/recipient groups individually because of the uneven distribution of the numbers of patients in the groups. Analysis of variance (F ratio) using a three factor (between organs, between recipients, and between donors) model showed that the donor and recipient CMV antibody status had a significant effect on the clinical score associated with CMV disease (between donors F = 18·2, df = 1·68, p < 0·01; between recipients F = 12·4, df = 1·68, p < 0·01). There was no interaction between recipient and donors (F = < 1, df = 1·68, p > 0·05).

The route of infection had a profound effect on the severity of disease (table 1). Of the 30 patients who experienced primary CMV infection, those who were likely to have acquired the infection from the donor organ(s) (CMV antibody positive donor, CMV antibody negative recipient) had a much more severe form of the disease (clinical score 8·5) than those who acquired the infection from blood or blood products (CMV antibody negative donor, CMV antibody-negative recipient, clinical score 2·8).

Similarly, of the 43 patients who experienced CMV reactivation or reinfection, the 23 recipients who received an organ from a CMV antibody positive donor had a more severe form of the disease (clinical score 3·2) than those who received an organ from a CMV antibody negative donor (clinical score 1·8). Interestingly, the reactivation of or reinfection with CMV in the CMV antibody positive recipients who received a heart from a CMV antibody positive donor was more severe (clinical score 3·2) than was primary CMV infection in the CMV antibody negative patients who received a heart from a CMV antibody negative donor (clinical score 2·8) who were most likely to have acquired the infection from blood or blood products, but this difference is not significant.

Heart and lung transplant recipients who were CMV antibody negative before transplantation and who received organs from a CMV antibody positive donor were as likely (80%) to develop primary CMV infection after transplantation as heart recipients (84%) but far more likely to have severe life threatening or fatal CMV infection (clinical score 12·3 compared with 8·1). Only three of the 16 heart transplant recipients who received an organ from a CMV antibody positive donor and who acquired primary CMV infection, died, compared with three of the four heart and lung transplant recipients in this group; the fourth had a very prolonged and severe CMV infection. The small number of patients in the heart and lung group made statistical comparison with the heart group impossible.

The impact of CMV infection after transplantation depends both on the CMV antibody status of recipients and donors as well as on the immunosuppressive regimen. CMV infections in patients receiving various combinations of immunosuppressive agents are shown in table 2. Patients with CMV infection who were receiving combinations of azathioprine and prednisolone experienced more severe disease than those receiving cyclosporin A combined with azathioprine or prednisolone.

As CMV seropositivity increased with age (table 3) more recipients than donors were CMV antibody positive (58% compared with 42%). Although nine patients experienced Pneumocystis carinii infection at some time after transplantation (range 75–100 days), this was not concurrent with their CMV infection. One patient who experienced Aspergillus infection after transplantation subsequently had primary CMV infection.

### Discussion

This study represents a detailed analysis of the CMV infections in the first 147 heart and 15 heart and lung transplant recipients at Papworth Hospital, Cambridge from 1979 to 1986. A total of 73 (45%) experienced CMV infection after transplantation, usually in the first few months.

Primary CMV infection was noted in 18·5% patients, while CMV reactivation/reinfection was experienced by 26·5% recipients. This incidence is much lower than that reported in most series of
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In probably the most detailed study of 49 heart transplant recipients, Dummer et al. found evidence of CMV infection in 89% of patients; 73% of the CMV antibody negative recipients had acquired primary infection and 100% of CMV antibody positive patients experienced reactivation or reinfection with CMV after transplantation. These patients all received cyclosporin A immunosuppression treatment, as did most of our patients. Other studies of heart, kidney, and liver transplant recipients have shown a greater percentage of patients experiencing CMV infection after transplantation than was found in this study. In a similar study of CMV infections in heart, kidney, and liver transplant recipients in Cambridge (TG Wrehitt, unpublished observations) a similar rate of infection in each group of patients was found.

It is difficult to explain why other studies have found more CMV infections in transplant recipients. We used sensitive antibody assays such as competitive ELISA, which we have found to be five times more sensitive than the complement fixation test and twice as sensitive as indirect ELISA. Furthermore, μ-capture ELISA for detecting CMV specific IgM is a sensitive test. Although all our patients were closely followed up serologically, culture of CMV was generally attempted only in those patients with serologically confirmed CMV infection or with symptoms. We are confident that no primary CMV infections were missed using this strategy, but the number of cases of CMV reactivation or reinfection detected in this study may be an underestimate, as patients may have been excreting CMV in the absence of more than a four-fold rise in complement fixation antibody titre or the presence of specific IgM. They would, however, have a very low grade infection in the absence of an antibody response. Furthermore, regular urine samples were cultured from many patients who did not produce a CMV antibody response and CMV was never cultured from the urine of a patient who failed to produce an antibody response.

The intensity of immunosuppressive treatment affects the seriousness of CMV infection. It has been reported that ATG may precipitate severe symptoms. There is not any evidence, however, to suggest that any of the various kinds of immunosuppressive treatment affects the rate of CMV infection. ATG was given to all our transplant recipients, but they also received cyclosporin A, azathioprine, and prednisolone in various combinations at different times in the series. We found that primary CMV infections were more severe when azathioprine was included in the regimen than when it was not (table 2), although such an effect was not noted in CMV reactivation or reinfection. In other studies of heart transplant recipients no difference was found between the severity of CMV infections in patients receiving cyclosporin A or azathioprine. Preiksaitis et al. however, showed that heart transplant recipients receiving high doses of ATG, azathioprine, and prednisolone experienced more severe CMV infections than those given low doses.

We found that heart and lung transplant recipients were more likely to experience severe or fatal CMV infections than those receiving a heart transplant alone. Of the four heart and lung transplant patients who developed primary CMV infection, three died and the fourth had a very severe illness. This compares with three deaths in the 16 heart transplant recipients with primary CMV infection. Other workers have noted similar findings. As the immunosuppressive regimen in our two groups of transplant recipients was similar, we presume that the more severe infection in the heart and lung transplant patients relates to the greatly increased dose of CMV which may be acquired from the two organs. All our heart and lung recipients with fatal CMV had pneumonitis.

The most important clinical factor affecting the rate and severity of CMV disease is the route of infection. Several studies have shown that the most severe form of primary CMV disease arises when infection is acquired from the donated organ. Primary CMV infection is principally acquired through two routes—the donated organ or blood and blood products. In our series 27% of CMV antibody negative recipients who received a heart from a CMV antibody negative donor acquired primary CMV infection, presumably through blood and blood products given perioperatively. Dummer et al found that 64% of such patients acquired primary CMV infection, while Ho et al found that 30% of kidney transplant patients acquired infection by this route. The extent to which CMV is transmitted by blood or blood products depends on three factors: the amount of blood given, the CMV antibody positivity rate of the donor population, and the age of the transfused blood or blood products. Much less blood is given to heart and kidney transplant recipients perioperatively than to liver transplant recipients, yet in a retrospective study we were unable to show any blood borne CMV infections in kidney and liver transplant recipients in Cambridge. Our heart transplant recipients receive relatively fresh blood, which may be important as Glenn et al showed that 30% of open heart surgery patients acquired CMV from blood less than 48 hours old, yet none acquired infection from blood more than 48 hours old. This, however, cannot be the only factor as many liver transplant recipients with clotting problems at operation receive very fresh blood. Judging by the antibody positivity rate (49%) given by Preiksaitis et al. for American blood donors, this would not be very different from United Kingdom blood donors (or
organ donors) (table 3).

Primary CMV infection acquired from the donor heart or heart and lung is more severe than that acquired from blood or blood products (table 1). It must be presumed that some of the CMV antibody negative patients, who received a heart from a CMV antibody positive donor, acquired primary CMV infection from blood or blood products. Therefore, in our series we can probably say that around 60% of this group of patients acquired their disease from the donor organ. This agrees with the findings of others.14,17

As all six heart or heart and lung transplant recipients in our series who died from overwhelming CMV infection were CMV antibody negative and they had acquired a heart from a CMV antibody positive donor, it is most important to minimise this risk by (i) testing blood from prospective donors and recipients by some means such as a latex agglutination test (CMV scan, Becton Dickinson), which is quick, reliable, and sensitive20; and (ii) avoiding transplant of a heart (or more particularly heart and lungs) from a CMV antibody positive donor to an antibody negative recipient.

We have shown that 62% of CMV antibody positive recipients experienced CMV disease when given a heart from an antibody positive donor compared with 42% who received a heart from a seronegative donor. Not only were these patients more likely to experience CMV disease if they received an organ from a seropositive donor, but also the disease was likely to be more severe (table 1)—even more severe than primary CMV infection acquired through blood or blood products. Some of the CMV infection in seropositive recipients receiving a heart from a seropositive donor were probably reinfections rather than reactiavtions.

Some workers have shown that life threatening or fatal Aspergillus, P carinii, or Candida infections are more often experienced by transplant recipients who have concurrent primary CMV infection.14,21,22 Rubin et al found that 36% of heart transplant recipients with primary CMV disease had concurrent P carinii pneumonia, as did 5% of patients with CMV reactivation or reinfection.14 Only one of nine patients in our series with P carinii pneumonia had previously had primary CMV infection; four had experienced CMV reactivation or reinfection, and four had not had CMV infection. In all of the patients with CMV and P carinii infection, CMV disease was experienced several weeks before P carinii infection was diagnosed. One patient experienced Aspergillus infection after transplantation and subsequently had primary CMV disease.

Early diagnosis of CMV infection is most important as effective drugs are now available to treat this life threatening disease. Some workers have advocated the use of rapid methods for antigen detection using anti-CMV monoclonal antibodies.23 In our patients we relied on antibody detection, and in particular, the estimation of CMV specific IgM. Not only have we found that CMV specific IgM is always detectable before complement fixation antibody in primary CMV disease (figs 2 and 3) but that the amount of CMV specific IgM present in serum may have important prognostic value. This is particularly so if large amounts are present, as they are in primary CMV infection, even in the early stages. Other workers have shown that more CMV specific IgM is produced after primary CMV infection than after CMV reactivation or reinfection15,24,25 and, like us, they have shown that CMV specific IgM may be detectable for years after primary CMV infection (fig 3). In our series we found that 85% of patients with CMV reactivation or reinfection had detectable CMV specific IgM—more than that reported in kidney transplant recipients.23

Now that the risks of acquiring CMV disease after heart transplantation have been evaluated it is important to avoid transplanting organs from seropositive donors into seronegative recipients and to use seronegative blood if this is available. With the advent of antiviral drugs effective against CMV it is also important to have available effective methods for rapid diagnosis of CMV infection so that treatment may be started early.

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