Lack of association between cytomegalovirus infection of heart and rejection-like inflammation

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SUMMARY Serial myocardial biopsy specimens, taken up to the time of serological evidence of primary cytomegalovirus (CMV) infection in 22 heart transplant patients, were examined and compared with those taken over similar times after transplantation in 21 patients who did not develop CMV infection. None of these 43 patients had serological evidence of CMV infection before their heart transplantation. There was no evidence of an increased cellular infiltrate in the myocardium at the time of the active CMV infection, even though the donor heart is the likeliest source of infection, nor was there any change in myocyte, interstitial cell, or vascular endothelial cell nuclei to identify active CMV infection.

Cytomegalovirus (CMV) infection often occurs after heart transplantation and primary infection is usually associated with high morbidity, especially when the infection is acquired from the donor organ.¹ Histopathological descriptions of cardiac CMV infection are rare.²⁻⁴ Even in lethal, severe primary CMV infection it has been shown by nucleic acid hybridisation that myocardial cells carrying viral DNA appear quite normal on conventional histological examination and may therefore be able to act as a latent source of infection.³

Distinguishing between CMV infection and rejection in transplanted kidneys may be difficult. Richardson *et al* described a glomerular lesion in CMV infection in the transplanted kidney which they regarded as distinct from rejection.⁵ The same group⁶ and others⁷ have found monoclonal antibodies to circulating T cell subsets of use in differentiating between CMV infection and rejection in the kidney. In heart transplant recipients changes in circulating T cell subset ratios have been found to be more specific for indicating CMV infection than rejection.⁸ In an earlier report one of us⁹ was concerned that some of the biopsy specimens reported as moderate rejection might be viral infection with myocyte necrosis and a lymphocytic infiltration. With increasing numbers of heart transplant patients it was decided to review the Papworth experience of CMV infection and its possible effects on the heart.

In the normal management of patients after transplantation endomyocardial biopsy specimens and

serum samples are taken at regular intervals.^{1 10} These routine examinations have given us the opportunity to study the histology of CMV infection in the human heart. In a substantial proportion of heart transplant patients who have not experienced CMV before the operation and who receive a heart from a donor with evidence of previous CMV infection the CMV in the donor heart may reactivate and produce a primary infection in the recipient.¹

Material and methods

Forty three patients who received heart transplants and who had no detectable CMV antibody before transplantation by complement fixation test¹¹ or competitive enzyme linked immunosorbent assay (ELISA)¹² were studied. Twenty two patients experienced primary CMV infection and showed CMV seroconversion about 57 days after transplantation and produced significant amounts (above 0.33 arbitrary units per 0.1 ml serum)¹ of CMV specific IgM (table 1).¹³ Half of these patients received hearts from CMV-positive donors. All the endomyocardial biopsy specimens, which had been taken up to the time of the CMV antibody titre change (when any specific inflammatory changes should have occurred), were reviewed. Twenty one control cases were selected who did not develop primary CMV infection over the same period after transplantation; only one of these control patients received a heart from a CMV-positive donor. All the patients in this study were receiving immunosuppressive treatment with cyclosporin A.¹⁴ In all the

Table 1 Patient details

	Primary CMV	Control group
Number	22	21
Male/female	20/2	19/2
Age in years		
Range	34-52	15-54
Mean	43.5	34.9
Duration of study		
Range (days)	27-119	28-108
Mean	57.3	57.8
Donor CMV state		
Positive	11	1
Negative	8	19
Not known	3	1
Recipient disease		
Ischaemic	9	8
Dilated cardiomyopathy	8	9
Hypertrophic cardiomyopathy	1	1
Endomyocardial fibrosis	0	1
Myocarditis	2	1
Rheumatic	2	1

CMV = cytomegalovirus infection.

biopsy specimens, the rejection reaction was graded according to Pomerance and Stovin.¹⁰ Besides rejection, note was made of any multinucleation of myocytes, or of interstitial cells, of any large nuclei with increased chromatin and blurred outline, and of any haloed "owl-eye" nuclei.

Results

The mean time from operation to the development of primary CMV infection was 57.3 days (range 27-119 days). Three of the controls and nine of the cases of primary infection developed Epstein-Barr virus (EBV) infection, diagnosed by an increase in EBV viral capsid antigen IgG antibody titre. In all but one case this occurred well after CMV infection; this one case was in the primary CMV infection group. One other case in the primary group developed a herpes simplex virus infection, detected by a rise in complement fixing titre, and this also occurred after the CMV infection.

There was no significant difference (Student's *t* test) in the distribution of grades of rejection between the endomyocardial biopsy specimens in the CMV infection group and those of the control group (table 2), nor were there any differences in the primary infection group between those receiving hearts from the CMV-positive donors and those receiving hearts from CMV-negative donors.

The subsequent clinical course of the patients in the CMV infection group differed from the controls in that one of the five deaths in the primary infection group was due to disseminated CMV infection complicated by *Candida* pneumonia and peritonitis occurring 22 days after the serological detection of the CMV

Table 2 Biopsy sets

	No (%)	
	Primary CMV	Control group
<i>All cases:</i>		
Rejection grade ¹⁰		
No rejection	23 (20.0)	25 (20.7)
Minimal	51 (44.4)	32 (26.4)
Mild	35 (30.4)	56 (46.3)
Moderate	6 (5.2)	8 (6.6)
Severe	0	0
<i>Nuclear changes</i>		
Multinuclear interstitial	4	3
Multinuclear myocyte	3	1
Large, blurred interstitial	9	6
Large, blurred myocyte	15	12
<i>Primary cases and donor state:</i>		
	Donor CMV positive	Donor CMV negative
Rejection grade		
No rejection	14 (25.0)	7 (16.3)
Minimal	23 (41.1)	19 (44.2)
Mild	17 (30.4)	13 (30.2)
Moderate	2 (3.5)	4 (9.3)

infection. Two of the primary CMV infection group died of coronary artery disease associated with transplant and both of these had had EBV infections. The other two deaths in the primary infection group were due to lymphoma and rejection. One of the two control patients died from bacterial infection. The remaining patient in the control group underwent retransplantation for probable recurrence of his rheumatic myocarditis.

None of the biopsy specimens showed typical "owl-eye" nuclei of CMV infection. There was no difference between the two groups in the incidence of multinucleate myocytes (three primary, one control), multinucleate interstitial cells (4:3), hyperchromatic blurred nuclei in myocytes (15:12), hyperchromatic blurred nuclei in interstitial cells (10:8), interstitial oedema (9:6), or vasculitis (2:2). In both groups these changes were found on average a month (nine to 119, mean 29.6 days) after transplantation. The hyperchromatic blurred myocyte nuclear change in the primary infection cases receiving CMV-positive donor hearts occurred on average 18.4 days (9-40 days) after transplantation; this nuclear change was seen on average at 31.8 days (11-63 days) in those receiving hearts from CMV-negative donors and at 42.7 days (10-76 days) in the control group. Although this time of 18.4 days is just significantly shorter ($p < 0.5$) than the other two groups, the nuclear change is not sufficiently distinctive to enable a diagnosis of CMV infection to be made.

Discussion

The results indicate that there is no evidence of a

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cellular myocardial infiltrate or of non-specific nuclear changes associated with primary infection with CMV, even though there is evidence¹ that the donor heart is the most common source of the infecting virus. This study suggests that a non-specific nuclear change may occur earlier in the biopsy specimens, from CMV-negative patients receiving hearts than from CMV-positive donors, but this change is not associated specifically with CMV infection. In the infant case of CMV myocardial infection described by Iwasaki *et al.*⁴ lymphocytic and macrophage reaction was sparse, and no mention as made of any cellular reaction being present in the two cases reported by Myerson *et al.*³ In mice it has been shown that the heart can transmit CMV infection and in this model immunosuppression exacerbated the infection in both primary and latently infected animals.¹⁵ Further studies indicated that transmission of the CMV infection was unlikely to be through any of the donor leucocytes carried over in the heart.¹⁶

Earlier speculation⁹ that moderate rejection might be confused with a cellular response to CMV infection was due to CMV reactivation following increased immunosuppression for moderate rejection. This earlier observation was made on patients receiving conventional immunosuppressive treatment¹⁷; all of the patients in this study were receiving cyclosporin A. Although CMV does not seem to produce a cellular infiltration in the myocardium that is likely to be confused with the infiltrate of rejection, CMV infected cells with characteristic "owl-eye" nuclei may be found in myocardial biopsy specimens and at necropsy before serological evidence of infection. No such infected cells were seen in any of the specimens reviewed for this study. Even though the nuclear changes observed were more common in the primary infected group than in the controls these differences were not significant. The possibility that the nuclear changes seen both in the cases of primary infection and in the controls might be due to some virus other than CMV cannot be discounted, although our evidence indicates that EBV and herpes simplex virus are unlikely candidates.

The incidence of lethal coronary artery disease in the cases of primary infection is of interest in view of the recent suggestions that CMV infection may be related to coronary atherosclerosis.¹⁸

In conclusion, there is no evidence to suggest that primary CMV infection is associated with a cellular myocardial infiltrate that could be mistaken for rejection in the Papworth heart transplant patients. Furthermore, there is no evidence that CMV infection is often associated with specific nuclear changes in interstitial or myocardial cells. Lastly, there is no evidence in this series to suggest that CMV infection acts as a clinically important immunosuppressant.

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