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Donor directed cytotoxicity of cardiac graft infiltrating cells during cytomegalovirus infection

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Abstract. We investigated whether cytomegalovirus (CMV) infection had an effect on donor directed cytotoxicity of cardiac graft infiltrating cells. The group we studied comprised 89 heart transplant recipients. Thirtyeight showed signs of CMV infection, and in 27 of them cytolytic activity of biopsy-derived cultures could be tested during the infection. Fity-one patients had never had CMV infection, and they were used as the control group. Eight patients had a primary, and 19 a secondary infection. We found that during CMV infection, both primary and secondary, a significantly higher proportion of the biopsy-derived cultures showed cytotoxicity against donor antigens ($P < 0.01$ when compared to the control group). In secondary infections, this was only due to an increase in donor class I directed cytotoxicity, while in primary infections a significant increase of class II directed cytotoxicity was also found ($P < 0.005$ when compared to secondary infection).

Key words: Cytomegalovirus – Alloreactivity – Graft infiltrating cells – Heart transplantation

Cytomegalovirus (CMV) infection is the most common problem due to infectious disease in immunosuppressed allograft recipients, and is a major cause of morbidity and mortality. The virus can induce viral disease, superinfections with other micro-organisms and immunomodulation. Furthermore, there may be a relationship between CMV infection and graft rejection. In the present study, we investigated the effect of CMV infection on donor directed cytotoxicity of graft infiltrating cells, and this was compared with a control group without infection.

Materials and methods

Patients. We studied CML reactivity of biopsy-derived lymphocytes from 89 heart transplant recipients. The immunosuppressive regimen consisted of cyclosporin A (CsA) and low dose prednisone.

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The CMV serostatus of the transplant recipients was screened for anti-CMV IgG by an ELISA. All CMV seronegative recipients received anti-CMV Immunoglobulins (Cytotect, Biotest Pharma GmbH, Dreieich, FRG), irrespective of the CMV serostatus of the allograft donor [4]. Samples of urine, throat wash, and blood were collected for virus isolation or a CMV early antigen test every 14 days for 3 months [6]. Specific anti-CMV immunofluorescence studies were done on all cultures. Routine monitoring included serology using an indirect immunofluorescence assay for IgM antibodies and an ELISA for IgG antibodies. A patient was considered to have CMV infection when a rise of CMV IgM antibodies and/or a positive CMV early antigen test was found.

All 89 patients were followed from the day of transplantation. Thirty-eight of them had CMV infection, and in 27 out of the 38 patients cytolytic activity of graft infiltrating cells could be tested during the infection. Of the 27 patients tested, 10 had clinical symptoms. Fifty-one patients had never and CMV infection, and they were used as the control group. In the patients without infection, we analyzed the biopsy-derived cells over a comparable period to the patients with infection, which was the first 228 days after transplantation (the median follow up in the patients with infection).

Culture method. Lymphocyte cultures were established from endomyocardial biopsies (EMB) as described by us in an earlier study [5]. In brief, each biopsy was cultured in a 96 well roundbottom tissue culture plate (Costar 3799, Cambridge, Mass.) with 200 µl culture medium per well, in the presence of 10^5 irradiated (40 Gy) autologous peripheral blood mononuclear leukocytes (PBMC) as feeders. Culture medium consisted of RPMI-1640-Dutch modification (Gibco, Paisley, Scotland) supplemented with 10% v/v lectin-free lymphocult-T-LF (Biotest GmbH, Dreieich, FRG) as an exogenous source of interleukin 2 (IL-2), 10% pooled human serum, 4 mM L-glutamine, 100 IU/ml penicillin and 100 µgg/ml streptomycin.

Cell-mediated cytotoxicity assays. A 4-h ^{51}Cr release assay was used to measure the cytotoxic capacity of the cultures against donor cells and a panel of unrelated target cells (EBV transformed B-cell lines or PHA blasts) sharing one or more HLA antigens with the donor. Serial double dilutions with E:T ratios varying from 1.25:1 up to 80:1 were used.

Results

Eighty-four EMB-derived cultures from 27 patients could be tested during CMV infection (1–7 per patient, median 2). From the individual patients without infection, one to ten biopsies (median four) yielded sufficient

Table 1. Donor directed cytotoxicity of EMB-derived cultures before, during and after infection with CMV. This is compared with the control group without infection. All patient groups were analyzed over a comparable period after transplantation

	CML specificity		
	donor n^a (%)	class I n^a (%)	DR n^a (%)
< 41 days[b]			
Before infection	62 (79)	57 (73)	43 (55)
Control group	101 (71)	92 (65)	54 (41)
≤ 228 days[c]			
During infection	74* (88)	71** (85)	37 (47)
Control group	173 (74)	157 (67)	106 (48)
229–365 days			
After infection	11 (65)	11 (65)	6 (35)
Control group	17 (65)	15 (58)	9 (38)

[a] n = number of reactive cultures
[b] median follow up before the first clinical evidence of infection (a rise of CMV-IgM and/or a positive CMV-EA test)
[c] median follow up in the patients with infection
* $P = 0.01$ when compared to the control group (X^2 test)
** $P < 0.005$ when compared to the control group (X^2 test)

Table 2. Donor directed cytotoxicity of EMB-derived cultures during primary and secondary CMV infection

	CML specificity		
≤ 228 days	donor n (%)	class I n (%)	DR n (%)
Primary infection	27 (90)	26 (87)	21* (70)
Secondary infection	47 (87)	45 (83)	16 (33)

* $P < 0.005$ when compared to cultures from secondary infected patients, and $P < 0.05$ when compared to the control group (X^2 test)

cells for cell mediated lympholysis. During CMV infection, a significantly higher percentage of donor reactive cultures was found compared to the control group ($P = 0.01$, Table 1). This proved to be due to an increase in HLA class I directed cytotoxicity ($P < 0.005$). Before signs of infection could be demonstrated, the percentage of donor (class I) reactive cultures was comparable to that in the control group over a similar period after transplantation. The highest values were measured during the infection (Table 1). After the period of the highest CMV infection incidence, CML reactivity returned to the level of the control group (Table 1). To ascertain that the increased cytolytic reactivity during CMV infection was not caused by a few patients who had provided the majority of the biopsies, we also calculated the percentage reactive cultures for each individual patient. Figure 1 shows that the increased number of HLA class I directed cytolytic biopsies during infection were derived from 24 of the 27 patients tested, and that in each of these 24 patients, the majority of the biopsies were reactive against class I antigens. In our group of 27 patients, 8 had a primary and 19 a secondary infection of whom respectively 6 (75%) and 7 (37%) had clinical symptoms. Both during primary and secondary infection, a significantly higher proportion of the EMB-derived lymphocyte cultures showed cytotoxicity against donor class I antigens compared to the control group, but in primary infected patients the percentage of class II reactive cultures was also increased (Table 2).

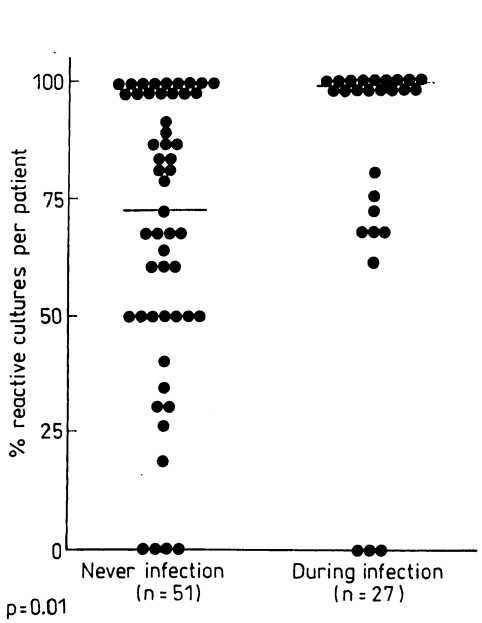

Fig. 1. The percentage of cultures reactive against HLA class I mismatches for each individual patient (each *not* represents one patient). During CMV infection significantly more patients yielded a high number of reactive cultures compared to the control group who never had CMV infection ($P = 0.01$, Wilcoxon test)

Discussion

Many reports describe a relationship between CMV infection and an increased incidence of acute rejection. This relationship with rejection suggests that during infection, donor directed cytotoxic reactivity is increased. Indeed, in the present study we found an increased donor reactivity of graft infiltrating cells during infection. This proved to be due mainly to an increased cytolytic activity directed against HLA class I mismatches. Before the first clinical signs of infection could be demonstrated, we found that the cytolytic activity was comparable to the control group. This was in contrast to data described by Grundy et al. [2], who found a depression of in vitro CML responses against alloantigens early after inoculation of the virus in susceptible mice. This was followed by a phase of enhanced alloreactivity. This may have been due to other characteristics of the mouse-CMV. Our finding of increased class I directed cytotoxicity during CMV infection was consistent with the findings of others [1, 3], who have shown that the virus could directly enhance MHC class I and ICAM-1 expression on graft tissue. Grundy has shown that the virus not only increases MHC class I expression, but it also has a direct enhancing effect on the alloreactive CTL response. Von Willebrand et al. [7] have described an increased expression of HLA class II antigens on tubulus cells and endothelial cells directly after the onset of clinical symptoms, and they suggested that this was an indirect (lymphokines) consequence of the virus infection. We found that in patients with primary infection, of whom the majority had clinical symptoms, not only class I, but also class II directed cytotoxicity of graft infiltrating cells was increased. In conclusion, during

CMV infection we found an increased incidence of cytotoxic graft infiltrating cells directed against HLA class I mismatches. We found no evidence for an early immunosuppressive phase. In primary infections, which were often symptomatic, both class I and class II directed cytotoxicity were increased.

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