Accelerated rejection of murine cardiac allografts by murine cytomegalovirus-infected recipients. Lack of haplotype specificity.

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Research Article

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Accelerated Rejection of Murine Cardiac Allografts by Murine Cytomegalovirus-infected Recipients

Lack of Haplotype Specificity

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Abstract

Clinical studies have revealed a correlation between cytomegalovirus (CMV) infection and acute allograft rejection. Likewise, for a murine model we observed that C3H (H-2^k) recipients infected with murine CMV (MCMV) rejected BALB/c (H-2^d) cardiac allografts earlier than uninfected recipients (6.9±0.1 d compared with 8.1±0.6 d; $P < 0.001$). It has been hypothesized that MCMV epitopes crossreact with alloantigens and in this manner induce rejection. However, we also demonstrated that MCMV caused accelerated rejection in the reverse combination (C3H heart engrafted to BALB/c recipient; 7.2±0.3 and 9.4±0.4 d for infected and control animals, respectively; $P < 0.001$) and accelerated rejection of grafts of a third, unrelated haplotype (C57Bl/6; H-2^b; 8.0±0.7 d compared with 10.1±0.6 for infected and control C3H recipients, respectively; $P < 0.03$). Ultraviolet (UV) inactivation of MCMV before administration to the graft recipient abrogated the ability to induce rapid rejection. Activated T lymphocytes were present in grafts from infected recipients 2 d before control recipients ($P < 0.02$) and, at the time of graft rejection, were almost exclusively CD8+ for both infected and control recipients. Thus, MCMV accelerated rejection appears not to result from crossreaction between MCMV epitopes and MHC products. The failure of UV-inactivated virus to accelerate rejection and the high proportion of CD8+ T cells recovered from all rejected grafts suggest that the class I pathway of antigen presentation may be important in the induction of early rejection. (*J. Clin. Invest.* 1993. 91:2602–2608.) Key words: T cell • transplantation • immune recognition • alloantigen • H-2

Introduction

Cytomegalovirus (CMV)[1] is of primary clinical significance as a source of morbidity and mortality in immunocompromised patients. Among those at risk are patients with heritable or acquired immunodeficiency syndromes and transplant recipients. The latter group of patients bears an added degree of risk in that CMV infection (either primary or reinfection) can be transmitted through the transplanted organ. For transplant recipients, the factors that influence the incidence and severity of CMV infection are the pretransplant level of CMV immunity, the source of infection, and the degree of immunosuppression (1). In addition to the direct complications attributable to CMV infection, the virus has been found to have modulating effects on the immune system of the infected individual (2). In some instances, CMV infection can result in generalized immunosuppression that predisposes the patient to secondary infection with other opportunistic pathogens (1, 3). In contrast to these immunosuppressive effects of CMV infection, other evidence suggests that CMV infection can in fact induce or accelerate the rejection of an allograft (4).

Clinical studies have revealed a correlation between CMV infection and acute rejection of allografts. A multicenter study of kidney recipients found a 25% increased incidence of graft loss in those patients with serologic evidence of CMV infection as compared with uninfected counterparts (5). Similarly, a significantly elevated rejection rate of cardiac allografts with a dramatic decrease in patient survival has been reported in conjunction with active CMV infections (6).

Studies in murine models have duplicated the immunomodulatory effects of CMV infection on the human immune system. Infection of animals with murine CMV (MCMV) has been shown to induce an immunosuppressed state shortly after infection, which is later followed by a heightened response to alloantigens as measured in mixed lymphocyte cultures (7–9). The survival of BALB/c cardiac allografts in C3H recipients has recently been shown to be significantly decreased if the recipients are infected before transplantation with MCMV (10). Thus, it appears that the virus-immune interactions that have been described in the clinical setting have counterparts in a murine animal model system.

The purposes of this study were to (*a*) confirm the MCMV-induced acceleration of graft rejection in the BALB/c to C3H cardiac allograft model, (*b*) determine whether accelerated rejection is associated with a particular haplotype specificity by examining rejection of grafts bearing multiple haplotypes, and (*c*) determine whether there is a difference in the kinetics of appearance within the graft of activated T lymphocytes or a difference in the phenotypes of these graft-infiltrating lymphocytes (GIL) between infected and uninfected graft recipients.

Methods

Mice. C3H/HeNCr1BR (H-2^k), BALB/c AnNCr1BR (H-2^d), and C57Bl/6NCr1BR (H-2^b) strains of mice were used in different donor–recipient combinations as dictated by the design of the experiment. The animals used in these experiments were all male mice 2–4 mo of age

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1. *Abbreviations used in this paper:* CMV, cytomegalovirus; GIL, graft-infiltrating lymphocytes; MCMV, murine cytomegalovirus; pfu, plaque-forming units.

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obtained from Charles River Laboratories (Wilmington, MA). Animals were screened for murine pathogens (*Mycoplasma pulmonis*, Sendai virus, murine hepatitis virus) by murine immunocomb solid phase immunoassay (Orgenics Ltd., Yavne, Israel).

Cardiac transplantation. Heterotopic heart transplantation was performed as previously described (11). Briefly, a heart from the donor strain animal was removed and sutured into the abdomen of the recipient by joining the aorta to the abdominal aorta of the recipient and the pulmonary artery to the inferior vena cava. Ligatures were then released, allowing reperfusion and reestablishment of normal sinus rhythm. Contraction of the transplanted heart was monitored daily by palpation and graded on a scale of 0–4. Graft rejection was scored as the time at which the first substantial impulse decline was observed. Determination of rejection by palpation has been shown to coincide with electrocardiographic findings and histopathologic evidence of rejection (11).

Viral infection of graft recipients. The Smith strain of MCMV (provided by Dr. Earl Kern, University of Alabama, Birmingham, AL), which was maintained by passage in BALB/c mice and prepared as a 10% (wt/vol) salivary gland homogenate using MEM with 10% FCS, was used for these studies. The stock virus was free of other murine pathogens as determined by solid phase immunoassay and was stored at −70°C with 10% DMSO as a stabilizer. The virus stock had a titer of 10^8 plaque forming units (pfu) per ml as determined by plaque assay on mouse embryo fibroblasts; animals were infected by subcutaneous challenge. As a control, salivary gland homogenate from uninfected animals was prepared following the same dilution scheme as used for salivary glands removed from infected animals and was administered to graft recipients in a manner identical to challenge with the live virus. For ultraviolet (UV) inactivation experiments, virus stock (10^7 pfu/ml) was divided into two aliquots; one aliquot was exposed to UV irradiation from a 30-W germicidal UV lamp at a distance of 8 cm with constant agitation for 30 min. The second aliquot was held at room temperature as a control. Both UV-inactivated and control aliquots were tested for pathogenicity in BALB/c mice at a challenge dose of 10^6 pfu administered intravenously in a volume of 0.1 ml.

Determination of IL-2 responsiveness of graft-infiltrating lymphocytes. Explanted heart grafts were gently minced and placed in culture in RPMI tissue culture medium containing 10% FCS, 0.05 mmol 2-mercaptoethanol, 24 mmol sodium bicarbonate, 10 mmol HEPES buffer, 1 mmol sodium pyruvate, antibiotics, and 160 U/ml of purified human IL-2. The cultures were observed daily with an inverted microscope and the degree of growth was assessed as described (12). Briefly, the scores represent the highest degree of growth seen for triplicate cultures; positive growth was considered to be moderate to numerous dividing cells and cell clusters.

Phenotype determination by flow cytometry. Positive cultures were examined for the expression of lymphocyte phenotypic markers. Growing cultures were incubated with rat anti-L3T4 (CD4) and anti-Lyt 2 (CD8) (Becton Dickinson, Mountain View, CA) monoclonal antibodies for 30 min at 4°C, washed, and stained with fluorescein-labeled, goat-anti-rat(Fab')2 second antibody (Cappel Laboratories, Malvern, PA) for an additional 30 min at 4°C. Stained cells were analyzed for fluorescence with a Coulter Epics Profile flow cytometer with a 488 nm argon laser (Coulter Corp., Hialeah, FL). Cells were sorted by the log of their fluorescence intensity and histograms of the cell populations generated.

Analyses. Comparison of graft survival times between experimental and control groups of animals used the nonparametric Mann-Whitney U test. The comparison of lymphocyte graft infiltration between infected and control recipients (as measured by IL-2 culture growth) was done by chi-square analysis.

Results

Accelerated allograft rejection after CMV infection. C3H (n = 14) mice were infected 12 d before transplantation with 10^4 pfu of MCMV administered subcutaneously. These animals along with uninfected controls (n = 9) received heterotopic cardiac allografts from uninfected BALB/c donors and the graft survival monitored (11). As described in Methods, the virus pool was prepared from a salivary gland homogenate from infected BALB/c mice. Therefore, as an additional control, salivary gland homogenate from uninfected animals (n = 6) was included in the experiment. These results are presented in Fig. 1. The uninfected control animals had a mean (±SE) survival time of 8.1±0.6 d. Those animals receiving the salivary gland homogenate from uninfected animals had a mean graft survival time of 8.0±0.4 d. These mean survival times do not differ.

In contrast, C3H recipients infected with CMV before transplantation had a mean graft survival of 6.9±0.1 d; this represents a significantly decreased survival time compared with either uninfected graft recipients ($P < 0.001$, Mann-Whitney) or control animals receiving salivary gland homogenate prepared from normal animals ($P < 0.001$, Mann-Whitney). These results confirm the preliminary evidence that MCMV infection is associated with accelerated graft rejection in this model.

Effects of a second infective dose of MCMV or salivary gland homogenate on allograft survival. The effect of a second challenge with MCMV before engraftment has previously been examined (10). However, for these experiments parallel challenge with salivary gland homogenate was not tested. Therefore, C3H test animals were given salivary gland homogenate from healthy animals at 26 d and again at 5 d (days −26 and −5; n = 7) or 12 d (day −12; n = 6) before engraftment. On day 0, these animals each received a heterotopic cardiac allograft from a BALB/c donor and graft survival was monitored. The graft survival for these groups (and also the previously described graft survival curves for animals given a primary and

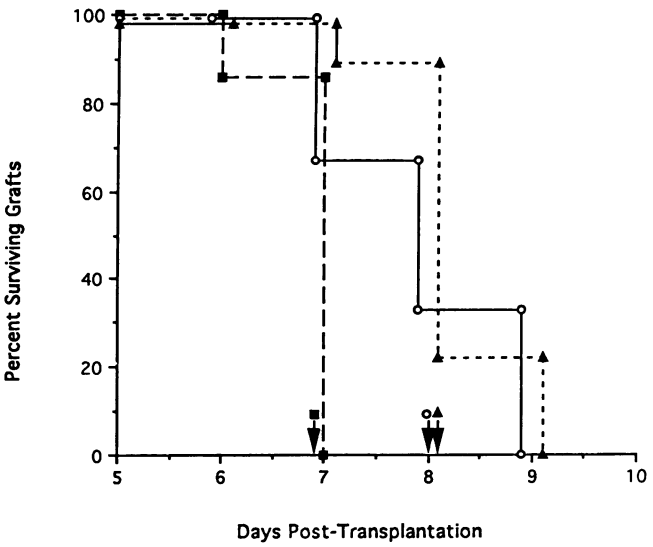

Figure 1. Survival of BALB/c cardiac allografts for MCMV-infected (■), untreated control C3H graft recipients (▲), and C3H recipients receiving salivary gland homogenate from uninfected animals (○). The mean day of rejection (indicated by arrows) of hearts engrafted to infected recipients was significantly shorter (6.9±0.1 d) than in untreated animals (8.1±0.6 d; $P < 0.001$, Mann-Whitney) or in animals receiving the salivary gland homogenate from uninfected animals (8.0±0.4 d; $P < 0.001$).

secondary infection with MCMV) are presented in Fig. 2. Groups of animals receiving one or two challenges with salivary gland homogenate did not differ with respect to graft survival (mean graft survival±SE: 8.0±0.37 and 7.9±0.3 d, respectively). Further, neither group differed significantly from untreated control recipients (8.1±0.2 d). As previously seen, however, secondary infection further accelerated the rejection process (mean day of rejection, 5.6±0.4 d). Thus, although an appropriately timed second infection increases the rate of graft rejection, this effect is not seen when recipients are given salivary gland homogenate from healthy animals.

Graft rejection in the C3H to BALB/c (reverse combination) transplant model for MCMV-infected and control animals. We examined the reverse combination of MCMV-infected BALB/c recipients engrafted with a heart from a C3H donor. This experiment addressed the question of whether the observed foreshortening of graft survival results from sensitization of C3H recipients with MCMV epitopes that crossreact with BALB/c alloantigens. This experiment also serves as an additional control against the potential contribution of the BALB/c salivary homogenate in accelerated graft rejection. The results of these experiments are given in Fig. 3. Untreated BALB/c animals (n = 5) had a mean graft survival time of 9.4±0.4 d. Animals infected at day −10 with 10^4 pfu (n = 6) rejected C3H grafts at a mean of 8.6±0.5 d (not significant compared with untreated control animals). BALB/c recipients infected with 10^2 pfu (n = 6) at day −10 had a graft survival time of 7.2±0.3 d ($P < 0.001$ compared with uninfected animals, Mann-Whitney; $P < 0.01$ compared with animals given 10^4 pfu, Mann-Whitney).

These observations indicate that MCMV-accelerated rejection is not haplotype specific in that accelerated rejection of grafts of both the H-2^d and H-2^k haplotypes can be induced by viral infection. Additionally, these results further confirm that accelerated rejection of BALB/c hearts in C3H recipients is not due to contamination of the virus pool with BALB/c alloantigens. It is of interest that the inoculum of virus found to induce accelerated rejection of BALB/c grafts by C3H recipients (10^4 pfu) is ineffective in the C3H to BALB/c combination. In this latter combination, a much lower infective dose (10^2 pfu) results in rapid rejection, suggesting that genetic factors influence the response to MCMV infection.

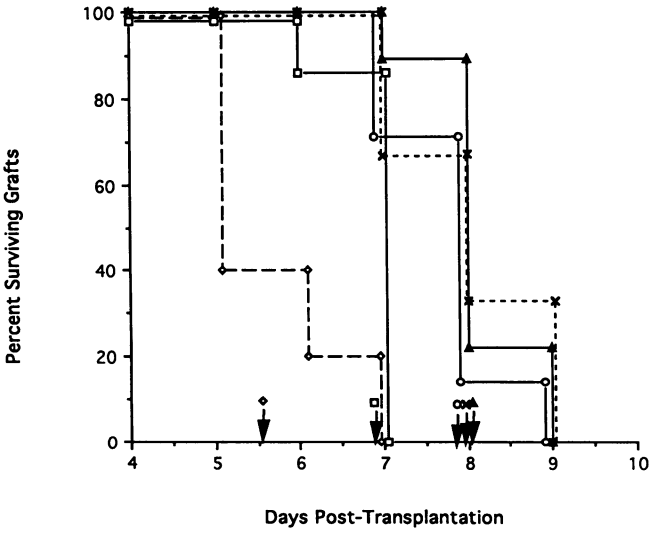

Figure 2. The effect of secondary MCMV infection on the survival of BALB/c hearts engrafted to infected, control, or C3H recipients. Animals receiving one (×) or two (○) challenges with salivary gland homogenate did not differ with respect to mean graft survival (indicated by arrows) (means±SE: 8.0±0.37 and 7.9±0.3 d, respectively). These also did not differ from untreated control recipients (▲; 8.1±0.2 d). However, a single MCMV infection induced early rejection (□), and a secondary infection (◇) further accelerated the rejection process (mean day of rejection 5.6±0.4 d; $P < 0.001$ compared with the three control groups, Mann-Whitney).

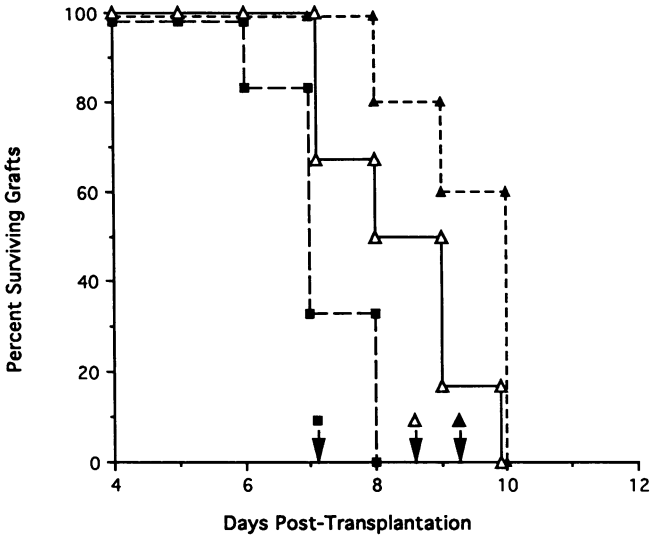

Figure 3. Survival of C3H cardiac allografts for infected and control BALB/c recipients. Untreated BALB/c animals (▲) had a mean graft survival time of 9.4±0.4 d. BALB/c recipients infected with 10^4 pfu (△) rejected C3H grafts at a mean of 8.6±0.5 d (not significant compared with untreated control animals). Animals infected with 10^2 pfu (■) had a graft survival time of 7.2±0.3 d ($P < 0.001$ compared with uninfected animals, Mann-Whitney; $P < 0.01$ compared with animals given 10^4 pfu, Mann-Whitney).

Graft rejection by C3H mice of a donor graft unrelated to BALB/c. Having demonstrated that accelerated rejection can occur in response to alloantigens of either H-2^k (C3H) or H-2^d (BALB/c) haplotypes, we wished to examine one additional strain combination that introduced an untested H-2 haplotype in order to substantiate and broaden these original observations. For this experiment, C57Bl/6 (H-2^b) hearts (n = 6) were transplanted to either MCMV-infected (10^4 pfu subcutaneously) or uninfected control C3H recipients. The graft survival time (Fig. 4) for uninfected animals (n = 10) was observed to be 10.1±0.6 d. Infected animals (n = 6) rejected grafts at 8.0±0.7 d ($P < 0.03$, Mann-Whitney). This observation once again supports the hypothesis that MCMV-mediated acceleration of allograft rejection results from a mechanism other than presentation of shared epitopes expressed on both MCMV and H-2 encoded alloantigens.

The effect of viral inactivation on graft rejection. The importance of viral replication on MCMV-induced early graft rejection was examined. Virus stock was adjusted to 10^7 pfu/ml and divided into two aliquots: one was UV irradiated (Methods) and the other was held at room temperature to be used as a control.

To assess the efficacy of the UV irradiation on viral inactivation, BALB/c mice were challenged with 0.1 ml (10^6 pfu)

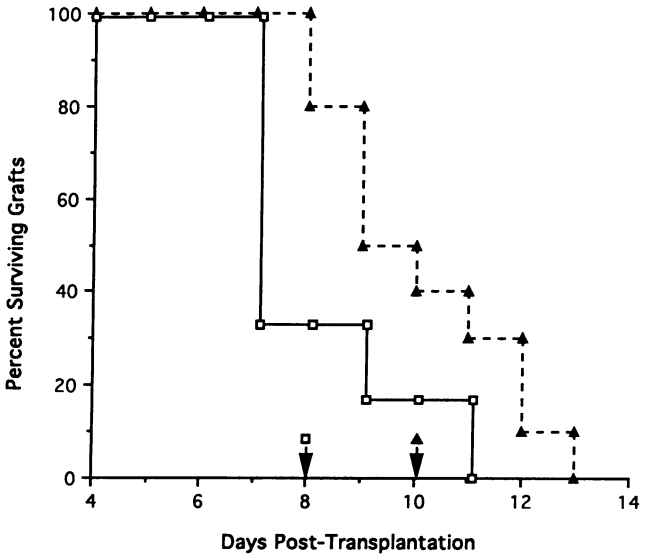

Figure 4. Survival of C57Bl/6 hearts engrafted to infected (□) and control (▲) C3H recipients. The graft survival time for uninfected animals was 10.1±0.6 d compared with 8.0±0.7 d for infected animals ($P < 0.03$, Mann-Whitney).

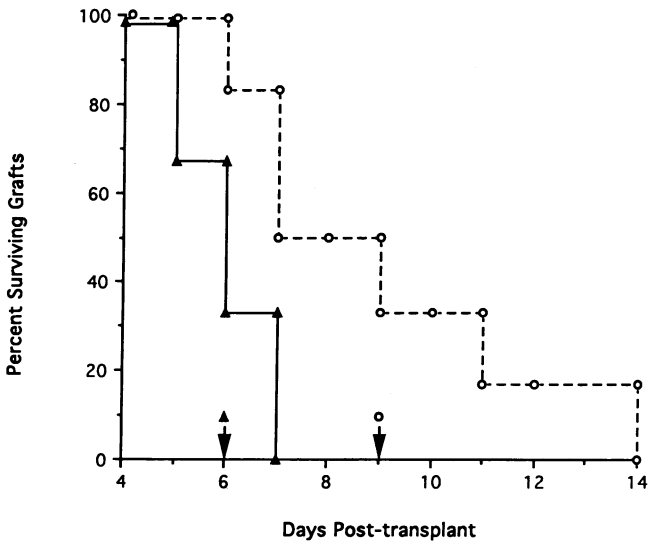

Figure 5. Survival of C57Bl/6 hearts engrafted to C3H recipients challenged with infectious (▲) or UV-inactivated virus (○). The graft survival time for animals given infectious virus was 6.0±0.4 d compared with 9.0±1.2 d for animals given UV-inactivated virus ($P < 0.04$, Mann-Whitney).

each of UV-irradiated ($n = 3$) and control ($n = 3$) viral suspensions and monitored for survival. The three animals given control virus died on days 4, 4, and 5 after infection, respectively. The animals given UV-inactivated virus did not develop overt signs of illness and survived indefinitely. Thus, pretreatment of MCMV with UV irradiation was judged to reduce the titer of infectious virus to a level below that detectable by this method. However, the presence of residual infectious virus in the UV-treated virus pool cannot be excluded.

To test the effect of UV irradiation on the ability of MCMV to induce early rejection, C3H recipients were given 10^4 pfu of either infectious ($n = 6$) or inactivated virus ($n = 6$) 12 d before engraftment with a C57Bl/6 cardiac allograft. The survival of these grafts for recipients receiving inactivated and infectious virus are presented in Fig. 5. Grafts transplanted to animals given infectious virus were rejected a mean of 6.0±0.4 d after engraftment, whereas animals receiving inactivated virus rejected the C57Bl/6 grafts a mean of 9.0±1.2 d after transplantation ($P < 0.04$, Mann-Whitney). There was no difference in the survival of grafts for untreated graft recipients and recipients given UV-inactivated virus (10.1 vs. 9.0 d, respectively, $P = 0.34$). These observations clearly illustrate the importance of viral replication in the induction of the observed acceleration of graft rejection.

Isolation of activated lymphocytes from cardiac allografts. To observe the pattern of graft infiltration by activated lymphocytes, BALB/c allografts were removed from C3H recipients (uninfected or MCMV infected) at various times after transplantation. The explanted grafts were minced and the tissue was placed in culture with IL-2. These cultures were observed microscopically and growth was scored as previously described (12). Results are given in Table I. Lymphocyte growth was evident in two of three cultures of hearts removed 3 d after transplantation and in two of three cultures of hearts removed 4 d after transplantation from MCMV-infected animals. Cultures of hearts removed from control animals at these times did not exhibit lymphocyte growth. Thus, growth was evident in graft cultures from four of six hearts from MCMV-infected recipients before day 4 after transplantation as compared with none of six grafts from controls ($P < 0.02$, χ^2).

Phenotypic analysis of the lymphocytes present in the IL-2-supplemented cultures is presented in Fig. 6. Percentages (mean±SE; $n = 3$ animals/time point) of CD4+ and CD8+ lymphocytes were determined by flow cytometry using anti-L3T4 and anti-Lyt 2 rat monoclonal antibodies as previously described (2). Qualitatively and quantitatively the phenotypes identified in the cultures of grafts harvested serially after transplantation are similar for both infected and control recipients. In both groups of animals there was an early appearance of CD4 (L3T4) positive lymphocytes that decreased with time. The phenotype of T cells isolated from the grafts surrounding the time of rejection were almost exclusively CD8 (Lyt 2) positive. One observed difference was that for MCMV-infected recipients, both CD4+ and CD8+ lymphocytes appeared earlier in culture (~ 2 d) as compared with control animals. Thus, as judged by the pattern of T lymphocyte infiltration after transplantation, the rejection mechanisms in the two models appear

Table I. Growth of GIL from BALB/c Hearts Engrafted to MCMV-infected and Uninfected C3H Recipients and Removed Serially after Transplantation

Day of graft removal	Control			Infected		
	Animal			Animal		
	1	2	3	1	2	3
3	−	−	−	+	−	+
4	−	−	−	+	+	−
5	+	+	+	+	+	+
7	+	+	+	+	+	+

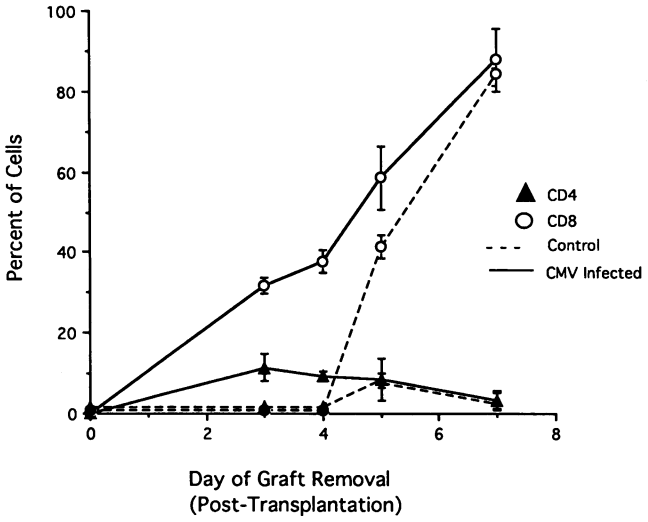

Figure 6. Phenotypes of infiltrating lymphocytes from BALB/c grafts removed serially posttransplantation from MCMV infected (——) and untreated (- - -) C3H recipients and grown in culture with IL-2. The percentage of each culture (±SE) bearing the CD4 (△) and CD8 (○) phenotypic markers are presented.

to be similar, the difference being that the process begins ~ 1–2 d earlier in graft recipients infected with MCMV. In both infected and uninfected graft recipients, the principal cellular mediator of rejection appears to be the CD8+ T cell.

Discussion

Human studies suggest that CMV infection in transplant recipients may correlate with an increased incidence of graft rejection. Recently, a murine model has been described by Shao et al. (10) which shows a similar relationship between cardiac allograft rejection and experimental infection with murine CMV. In that report, preliminary evidence of accelerated graft rejection was presented which indicated that BALB/c cardiac grafts were more rapidly rejected by C3H mice when the recipient was infected with MCMV before engraftment. One question left unanswered was the potential sensitization of C3H recipients by BALB/c alloantigens contaminating the virus pool that was prepared from MCMV-infected BALB/c salivary glands. In the study reported here we confirmed their initial observation and further demonstrated that BALB/c salivary gland homogenate (prepared in a manner identical to the method used for preparation of virus pools) did not induce rejection of BALB/c grafts more rapidly than untreated controls. This was in contrast to MCMV-infected recipients that rejected BALB/c grafts 1.2 d earlier (single infection) or 2.2 d earlier (double infection) than untreated control recipients.

Although the rapid rejection of heart grafts by MCMV-infected recipients has been clearly demonstrated, the mechanism through which this phenomenon is mediated is as yet unknown. One possible mechanism that has been proposed to explain the relationship between CMV infection and graft rejection in humans relates to a conserved region (common to several alleles) of the HLA-DR β chain that shares sequence homology with a 5 amino acid peptide from the immediate-early-2 (IE-2) antigen of CMV. Furthermore, immunologic crossreactivity between products of this conserved region and the CMV IE-2 has been demonstrated (13). Thus, it has been hypothesized that the increased risk for rejection is due to an anti-graft response initiated by the CMV epitope, IE-2. Interestingly, the region showing sequence homology with CMV is also conserved between the human HLA-DR β chain and the class II H-2 antigens for mice. It can therefore be argued that in the murine model, rapid rejection might be due to immune reactivity directed at the H-2 class II antigen induced by infection with CMV. In addressing this hypothesis, we found that both rapid rejection of BALB/c hearts by infected C3H recipients and rapid rejection of C3H hearts by infected BALB/c recipients (reverse combination) occurred. It is believed that self-reactive clones of T lymphocytes are eliminated during thymic development. Thymic and peripheral deletion of T cell receptors recognizing host-expressing antigens has been demonstrated (14–16). Thus, for BALB/c mice autoreactive (H-2^d reactive) T cells have been eliminated and similarly, H-2^k reactive T cells are deleted in the H-2^k C3H animals. Therefore, cellular rejection of a BALB/c heart by a C3H animal is necessarily mediated by a population of lymphocytes quite different in T cell receptor expression than those mediating rejection of a C3H heart by a BALB/c recipient. Thus, accelerated rejection in the forward (BALB/c to C3H) and the reverse (C3H to BALB/c) strain combinations must be mediated by populations of T lymphocytes bearing quite different repertoires of T cell receptors. Thus, the hypothesis that rapid rejection is due to sensitization by a specific crossreactive epitope seems unlikely, although other possibilities may exist.

It is conceivable that MCMV bears multiple epitopes, one crossreactive with the H-2^k haplotype and one crossreactive with the H-2^d haplotype. Under these conditions, sensitization by MCMV could occur in both strains of recipients and induce accelerated rejection in both combinations. Therefore, we tested a third, unrelated haplotype. C57Bl/6 hearts (H-2^b) grafted to MCMV-infected C3H recipients were also rejected more rapidly than those grafted to uninfected C3H recipients. The observation that multiple strain combinations are susceptible to accelerated graft rejection induced by MCMV reduces the probability that MCMV epitopes and alloantigens are crossreactive. At this time, however, the involvement of minor lymphocyte stimulating antigens or as yet unidentified alloantigens in MCMV accelerated rejection is not known and remains to be examined.

It is of interest that for the C3H to BALB/c graft–recipient combination, the infecting dose of virus that results in accelerated rejection is 100-fold lower than that for the BALB/c to C3H combination. A biphasic response to MCMV has been described in which there is an initial state of immunosuppression followed by a heightened response to alloantigens (9). Unlike other strains tested, BALB/c animals have been found to remain in an immunosuppressed state for a prolonged period after a single infective dose of MCMV (17). This immunosuppressed state is less prolonged in BALB/c animals given a single, low-dose challenge, or can be reversed by a second MCMV challenge (9, 17). Thus, BALB/c animals appear to be uniquely sensitive to immunosuppression resulting from MCMV infection. These previous observations (which are consistent with our findings reported here) suggest that genetic factors govern the host response to MCMV infection and the subsequent increased risk for graft rejection. In view of these findings, an investigation of the relationship between CMV, MHC antigen expression, and increased risk for graft rejection in the clinical setting might prove fruitful.

Alloactivated T lymphocytes express, among other activation markers, the high-affinity IL-2 receptor and will undergo clonal proliferation after the binding of IL-2 to this receptor (18). This two-signal requirement is an important control mechanism for regulating the immune response and also provides an investigational tool through which activated lymphocytes can be identified by their proliferative response to IL-2 (12, 19). We have previously used this technique (12) to study the kinetics of graft infiltration by activated T cells and the phenotypes of these GIL. In this study, we likewise used this method to compare the appearance and phenotype of lymphocytes infiltrating BALB/c grafts in healthy and infected C3H recipients. IL-2 responsive lymphocytes were present 2 d earlier in grafts removed from infected recipients, but the phenotypic pattern of infiltration (i.e., early appearance of CD4+ lymphocytes gradually replaced by CD8+ lymphocytes with approaching rejection) was essentially the same for both infected and uninfected recipients. This phenotypic pattern was identical to that previously seen for the C57Bl/6 to C3H combination (12). Thus, with respect to the principal T lymphocyte subsets, MCMV infection does not appear to alter the phenotypic characteristics of the rejection process, only the time of graft infiltration.

An observation of significance was the failure of virus subjected to UV irradiation to effect accelerated rejection. This finding provides some insights into the mechanism(s) through which MCMV modifies the alloresponse of the graft recipients. Although inactivated viral particles are generally capable of inducing interferons, the ability of the UV-inactivated virus (or residual infectious virus) to induce interferon production and the relative importance of interferon with respect to the regulation of the immune response in this system is not known. Gamma interferon is a potent upregulator of MHC expression on most cell types (which could potentially lead to more rapid allorecognition or provide additional targets for alloreactive cytotoxic T lymphocytes) and thus could contribute to the observations reported here. Likewise, α interferon (along with γ interferon) has been shown to shift the helper T lymphocytes to the Th1 pattern of cytokine production and inhibit the production of Th2 associated cytokines (20). The contribution of these effects to the observed results will require additional investigation.

Perhaps more importantly, inactivation of MCMV before infection could potentially have marked effects on the presentation of viral peptides to the immune system of the infected animal. Two main pathways have been identified through which foreign antigens are presented to the immune system of the host. First, exogenously derived peptides enter an endosomal processing pathway and are ultimately expressed on the cell surface complexed with MHC class II molecules (21). The peptide–MHC class II complex is then recognized by the T cell receptor of CD4+ helper T lymphocytes. Endogenously derived peptides are generated in the cytosol from intracellularly synthesized proteins (e.g., cytosolic proteins, viral proteins, tumor antigens) and are transported to the endoplasmic reticulum where they complex with MHC class I products (22–24). These complexes are transported to the cell surface where they interact (via the T cell receptor) with CD8+-expressing T cells.

Whereas UV inactivation of MCMV presumably would not affect the exogenous class II presentation pathway, UV inactivation does interfere with transcription (25) and would thus reduce the number of newly synthesized viral proteins available for presentation via the class I pathway. The end result would be a comparative reduction in the number of activated CD8+ T cells for animals given inactivated virus. Although an association between virus-activated CD8+ T cells and graft rejection has not been demonstrated, it is of interest that for both the BALB/c to C3H and C57Bl/6 to C3H donor–recipient combinations we have demonstrated that the infiltrating T cell phenotype is exclusively CD8+ at the time of graft loss. This illustrates the importance of this subset as an effector cell in the rejection of these particular grafts. Thus, there may be a connection between MCMV activation of recipient CD8+ T cells and accelerated rejection.

In this study, we have confirmed an earlier report of accelerated rejection of murine cardiac allografts induced by MCMV and have shown that accelerated rejection is not due to sensitization of the recipient to antigens present in the salivary gland homogenate used to prepare the virus pools. Our observation that rapid rejection of grafts occurs in three unrelated haplotypes provides evidence that the observed acceleration of the rejection process is not due to crossreactivity between MCMV epitopes and a specific graft alloantigen. Failure of UV-inactivated virus to induce the observed effect may suggest the involvement of cytokine networks or MCMV peptide presentation in mediating early graft loss. Further, although grafts from infected recipients are infiltrated 2 d earlier than grafts in uninfected controls, the phenotypic sequence of graft infiltration by T lymphocyte subsets is similar for infected and uninfected recipients and indicates the importance of CD8+ T cells in graft rejection. Taken together, these findings suggest that early rejection results from a mechanism that foreshortens the alloantigen recognition/T lymphocyte activation process. This mechanism could potentially act on the immune system of the recipient or effect recognition through upregulation of MHC antigens (i.e., gamma interferon mediated) on the graft. Although it leaves some additional questions to be answered, this murine model appears to parallel the clinical situation of graft rejection episodes associated with CMV infection and suggests unique immunomodulating properties of this virus which underscore the significance of this agent as a complicating factor in transplantation.

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