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Evidence for a Bidirectional Relationship between Cytomegalovirus Replication and acute Graft-versus-Host Disease

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Cytomegalovirus (CMV) infection and graft-versus-host disease (GVHD) are important complications after allogeneic hematopoietic stem cell transplantation (HSCT) with a clear link. Multiple studies show that GVHD and its treatment put patients at risk for CMV replication. Data on CMV replication as a cause of GVHD, in contrast, are controversial. We analyzed the reciprocal association of CMV replication with acute GVHD (aGVHD) in 515 patients treated with allogeneic HSCT between 1993 and 2008. Cumulative incidences at day 100 were 17% for CMV replication, 68% for aGVHD grade I-IV, and 48% for GVHD grade II-IV. Multivariate time-dependent analyses revealed that the presence of GVHD increased the risk of CMV replication in a dose-dependent manner: hazard ratio (HR) for CMV replication for patients with aGVHD grade I was 1.35 (95% confidence interval [CI] 0.82-2.21); HR for patients with aGVHD grade II-IV was 1.61 (95% CI 1.11-2.36, *P*-value for trend = .01). During phases of CMV replication, patients were at increased risk of developing aGVHD (HR 2.18, 95% CI 1.30-3.65, $P < .01$). These data confirm that GVHD and its therapy can induce CMV replication. They further demonstrate the reciprocal novel finding that patients are at significantly increased risk of developing aGVHD during CMV replication.

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INTRODUCTION

During the past 50 years hematopoietic stem cell transplantation (HSCT) has evolved from experimental therapy to standard of care for many severe congenital or acquired diseases of the bone marrow [1]. Despite progress in immunosuppressive and antiviral therapy, acute graft-versus-host disease (aGVHD) and cytomegalovirus (CMV) infection remain important complications after allogeneic HSCT [2].

GVHD and CMV replication are pathogenetically associated: multiple studies show that GVHD and its treatment put patients at risk for CMV replication [3-5]. In contrast, the role of CMV replication as a cause of GVHD is controversial. Review articles on the topic frequently suggest that CMV replication might induce GVHD [6]; however, data that directly link CMV replication and GVHD development are lacking. One recent small study found no effect of CMV replication on subsequent development of aGVHD [7].

We were therefore interested to examine in a single-center study the bidirectional association of CMV replication and aGVHD in 515 patients treated with T cell-replete allogeneic HSCT between 1993 and 2008.

PATIENTS AND METHODS

Data Collection

This retrospective cohort study is based on standardized, prospectively collected clinical data from the database of the Division of Hematology at the University Hospital of Basel, Switzerland, supplemented by chart review of clinical records. Data on CMV assays were collected from the database of the Institute

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for Medical Microbiology of the University Basel. All patients gave written informed consent to their treatment and to analysis of transplant outcome data.

Patient Population

Between January 1st 1993 and December 31st 2008, 530 patients received 619 allogeneic HSCT. For reasons of cohort homogeneity, 10 cord blood transplants were excluded from the study, as were 73 T cell-depleted grafts, and 21 transplant procedures with incomplete data on CMV replication. Data are presented on the remaining 515 transplants performed in 479 patients.

Median age at transplant was 42 years (range: 16-70 years). Treatment indications were predominantly hematologic malignancies (acute myeloid leukemia (AML) 31%, acute lymphoblastic leukemia (ALL) 15%, chronic myelogenous leukemia (CML) 15%, myelodysplastic syndrome (MDS) and myeloproliferative neoplasia (MPN) 13%, and lymphoma 21%). Donors were HLA-identical sibling (68%), other family members (1%), or volunteer unrelated donors (28% HLA-matched and 3% HLA-mismatched). The conditioning regimen was primarily cyclophosphamide/total-body irradiation (TBI) ± etoposide (49%), cyclophosphamide/busulfan (17%), or fludarabine/TBI (17%). GVHD prophylaxis consisted of cyclosporine A (CSA) and methotrexate (71%) or CSA and mycophenolate mofetil (MMF) (21%) in the majority of cases. Antithymocyte globulin (ATG) was administered in 7% of transplants. The serologic CMV risk constellation was donor negative/recipient negative (D−/R−) in 33% of the patients, D+/R− in 12%, D+/R+ in 25%, D−/R+ in 19%, and missing in 11% of transplants. Further patient and transplant characteristics are summarized in the Table 1.

CMV and GVHD Diagnosis and Treatment

We applied a standardized CMV surveillance policy over almost 2 decades. CMV replication was tested weekly using pp65 antigenemia assay or real-time polymerase chain reaction (PCR). PCR was introduced routinely in November 2003, and was thereafter performed in parallel to the antigenemia assay. Patients with detectable CMV replication were monitored twice weekly. CMV replication was defined as ≥1 positive cell per 10^5 peripheral blood mononuclear cells (PBMCs) detected by CMV pp65 antigenemia assay, or ≥1000 CMV copies/mL whole EDTA-blood detected by real-time PCR. Cytomegalovirus replication was preemptively treated with (val-)gancyclovir or foscarnet until PCR or antigenemia were negative in 2 consecutive assays. Severity and organ involvement of aGVHD was assessed daily according to established criteria [8,9]. Whenever possible, diagnosis of GVHD was confirmed by skin or gut biopsy. Isolated grade I GVHD of the skin was treated with topical steroids. Any GVHD of grade II or more was treated with systemic corticosteroids (methylprednisolone 2 mg/kg/day).

Statistical Analysis

Cumulative incidences of CMV replication and of aGVHD were calculated, treating death from any cause as a competing outcome.

To analyze the complex relationship between CMV replication and aGVHD, 4 states were defined: "No CMV replication, no GVHD," "CMV replication," "aGVHD," and "Dead." All patients entered the analysis in the "No CMV replication, no GVHD" state at day of transplant. Patients transitioned to a state of CMV replication on the day CMV replication was detected for the first time, and left this state on the day of the first negative assay. Patients with established GVHD remained in this state until the end of the analysis (unless they developed CMV replication or died). Possible transitions between the states are summarized in Figure 1. Transition probabilities between the states were compared by calculating hazard ratios (HR) using time-dependent Cox models adjusted for covariates (patient age, disease, disease stage, donor type, stem cell source, conditioning regimen, degree of HLA match, and type of pharmacologic GVHD prophylaxis). To analyze the impact of GVHD on CMV replication we compared transition probabilities (1) (CMV replication in patient without GVHD) and (2) (CMV replication in patient with GVHD). To analyze the impact of CMV replication on the occurrence of GVHD, we compared transition probabilities (3) (GVHD occurring in patient without CMV replication) and (4) (GVHD occurring in patient with CMV replication).

Patient and transplant characteristics were compared using Pearson's chi-square or Mann-Whitney U-test, as appropriate. Two-sided P-values $< .05$ were considered significant. The analysis was restricted to the time from transplant until day 100.

RESULTS

Incidence of CMV Replication and aGVHD

CMV replication was found in 86 of 515 transplants giving rise to a cumulative incidence at day 100 of 17% (95% confidence interval [CI] 14%-20%). Median interval from transplant to CMV replication was 33 days (range: 1-95), and median duration of CMV replication was 8.5 days (range: 2-62). Nineteen patients (4%) showed multiple episodes of CMV replication within the first 100 days posttransplant. Donor (D) and recipient (R) serostatus significantly

Table 1. Transplant Characteristics

Total transplants, n (%)	515 (100)	Donor, n (%)	
Sex, n (%)		Identical Sibling	351 (68.2)
Male/female	299/216 (58.1/41.9)	Other family member	5 (1.0)
Patient age		Volunteer unrelated donor, HLA-matched	142 (27.6%)
Median (range)	42.0 (16-70)	Volunteer unrelated donor, HLA-mismatched	17 (3.2%)
Age distribution, n (%)		Conditioning regimen	
<20 years	28 (5.4)	Cyclophosphamide + TBI ± Etoposide	251 (48.7)
20-40 years	204 (39.6)	Cyclophosphamide + Busulfan	88 (17.1)
>40 years	283 (55.0)	Fludarabin + TBI	85 (16.5)
Underlying disease, n (%)		Other	91 (17.7)
Acute leukemia	239 (46.4)	GVHD prophylaxis, n (%)	
Chronic myelogenous leukemia	79 (15.3)	Cyclosporine A ± Methotrexate	400 (77.7)
Lymphoproliferative disease	109 (21.2)	Cyclosporine A + Mycophenolate Mofetil	110 (21.3)
MDS/MPN	68 (13.2)	Other	5 (1.0)
Other	20 (3.9)	CMV constellation	
Disease stage, n (%)		Donor−/Recipient−	172 (33.4)
Early/advanced disease	202/313 (39.2/60.8)	Donor+/Recipient−	61 (11.8)
Stem cell source, n (%)		Donor+/Recipient+	128 (24.9)
Bone marrow	110 (21.4)	Donor−/Recipient+	99 (19.2)
Peripheral blood	405 (78.6)	Unknown	55 (10.7)

MDS indicates myelodysplastic syndrome; MPN, myeloproliferative neoplasia; TBI, total-body irradiation; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

influenced the day 100 cumulative incidence of CMV replication: D−/R− 6%, D+/R− 10%, D+/R+ 25%, and D−/R+ 37% ($P < .01$). The introduction of PCR for the detection of CMV led to a nonsignificant increase in the detection of CMV replication compared to patients monitored by antigenemia only (cumulative incidence of CMV replication at day 100 of 14.7% for patients tested with antigenemia assay only versus 19.8% for patients tested with antigenemia and PCR assays, $P = .13$).

The cumulative incidence for aGVHD grade I-IV was 68% (95% CI 64%-72%) with a median onset at day 14 (range: days 5-94); grade II-IV aGVHD occurred in 48% of transplants (95% CI 45%-53%).

Combining both endpoints, 149 patients (29%) experienced neither aGVHD nor CMV replication, 280 (54%) aGVHD only, 19 (4%) CMV replication only, and 67 (13%) both CMV replication and aGVHD. Of the 67 patients with both aGVHD and CMV replication, 46 (69%) developed aGVHD prior to CMV replication, 17 (25%) developed aGVHD during CMV replication, and 4 (6%) developed aGVHD after resolution of CMV replication.

Effect of aGVHD on CMV Replication

Among the 86 patients with CMV replication, cytomegalovirus replication initiated after onset of GVHD in 46 (53%). Cox modeling revealed that the presence of aGVHD grade I-IV significantly increased the risk of CMV replication (HR 1.55, 95% CI 1.07-2.23, $P = .02$). As GVHD itself and GVHD treatment are both immunosuppressive and may therefore contribute to the increased risk of CMV reactivation, we aimed to separate the direct influence of GVHD from that of GVHD treatment. Compared to patients without GVHD, we found a hazard ratio of 1.35 (95% CI 0.82-2.21) for patients with GVHD grade I (ie, GVHD that was not treated with systemic steroids). In comparison, patients with more severe GVHD (grade II-IV, treated with systemic steroids) were at a more increased risk for CMV replication (HR 1.61, 95% CI 1.11-2.36, overall P for trend [no GVHD/GVHD grade I/GVHD grade II-IV] = .01, Table 2).

Median duration of CMV replication was shortest in patients without GVHD (7 days). In comparison, clearance of CMV was delayed both in patients with GVHD at start of CMV replication (9 days) and in those developing GVHD during CMV replication (10 days), an effect compatible with slower virus clearance in patients immunosuppressed by GVHD and its

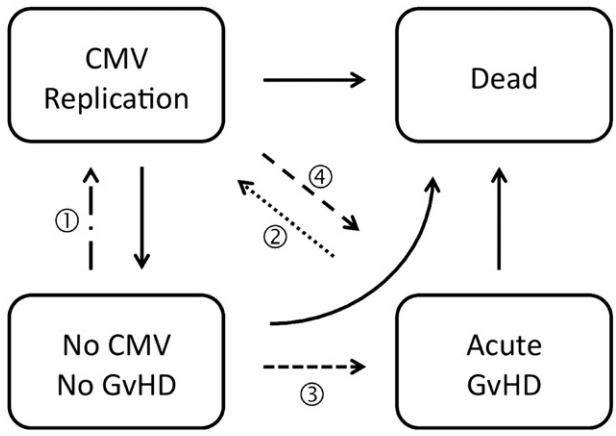

Figure 1. Summary representation of transitions analyzed in time-dependent Cox models. All patients start in the "No CMV/No GVHD" state at day of transplant, and transition to another state on the day they develop CMV replication, or GVHD, or die. Arrows indicate possible transitions: (1) CMV replication in patient without GVHD; (2) CMV replication in patient with GVHD; (3) GVHD occurring in patient without CMV replication; (4) GVHD occurring in patient with CMV replication.

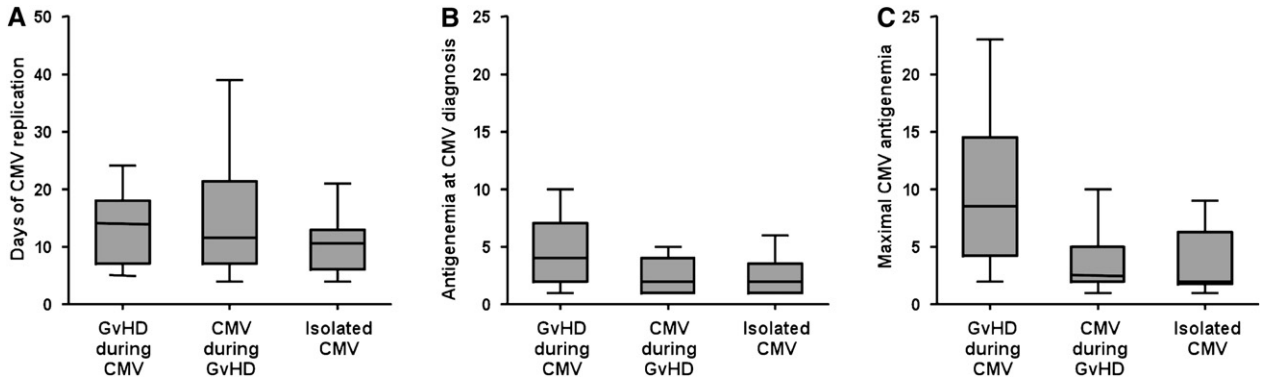

Figure 2. Impact of aGVHD on duration and severity of CMV reactivation. Duration of CMV reactivation (A), first antigenemia during CMV replication episode (pp65-positive cells/500,000 cells, B), and maximal CMV antigenemia (pp65-positive cells/500,000 cells, C) in episodes of CMV replication during which GVHD occurred (left bar), episodes of CMV replication that initiated during established GVHD (middle bar), and isolated episodes of CMV occurring in patients without GVHD (right bar). Lines represent medians, boxes interquartile ranges, and whiskers represent ranges.

treatment. Differences for duration of CMV replication between the 3 cohorts did not reach statistical significance, however ($P = .64$, Figure 2A).

The severity of CMV replication during established GVHD (median pp65 positive cells at CMV diagnosis = 2/500,000 cells, median peak antigenemia 3/500,000 cells) was similar to that of isolated CMV replication in patients occurring without GVHD (2/500,000 and 2/500,000 cells, respectively) ($P = .85$ and $P = .68$ respectively, Figure 2B and C). Interestingly, CMV episodes during which GVHD initiated were more severe in comparison to isolated CMV replication without GVHD (4/500,000 cells and 8.5/500,000 cells, $P = .08$ and $P = .03$, respectively, Figure 2B and C). A similar trend was also seen in the analysis of patients presenting with CMV disease: 6 cases of CMV pneumonitis and 2 cases of CMV colitis occurred up to day 100; CMV replication initiated during GVHD in 7 of these 8 cases (HR for patients during GVHD 8.58, $P = .06$).

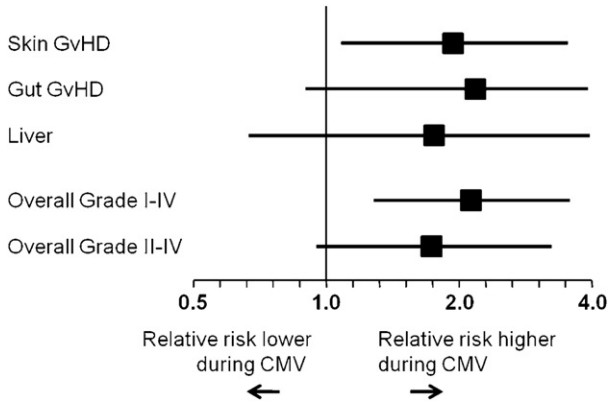

Figure 3. Impact of CMV replication on aGVHD incidence. Forest plots representing the impact of CMV replication on GVHD incidence. Each row represents a separate Cox model. Boxes represent the hazard ratios derived from the Cox model, whiskers the 95% confidence intervals. Hazard ratios were adjusted for patient age, disease, disease stage, donor type, stem cell source, conditioning regimen, degree of HLA match, and type of pharmacological GVHD prophylaxis.

Effect of CMV Replication on aGVHD

In multivariate analysis, patients were at increased risk of developing aGVHD during episodes of CMV replication (HR for development of any grade aGVHD: 2.18, 95% CI 1.30-3.65, $P < .01$). Hazard ratios for the development of grade II-IV aGVHD and for involvement of skin, gut, and liver were comparable, and are shown with 95% confidence intervals in Figure 3.

Severity of aGVHD grade was not significantly influenced by concomitant CMV replication: Median grade of aGVHD was II in patients developing GVHD during CMV replication, as well as in patients with GVHD occurring before or after CMV replication ($P = .62$, Figure 4). Distribution and grade of organ aGVHD were equally not significantly influenced by concomitant CMV replication (Figure 4).

Finally, we analyzed the relationship between CMV pretransplant serology and CMV replication. The relative risk for GVHD development remained virtually unchanged if donor/recipient CMV serology were included in the model in addition to CMV replication (HR for CMV replication: 2.16, 95% CI 1.28-3.65, $P < .01$). In contrast, CMV serologic constellation was not a significant predictor of GVHD after correction for individual CMV replication (HR versus D−/R− 1.00: D+/R− 0.90 [0.62-1.28], D−/R+ 0.94 [0.71-1.26], D+/R+ 1.18 [0.87-1.59]). These data suggest that CMV replication rather than CMV serostatus is the true risk factor for GVHD development.

DISCUSSION

This retrospective single-center study examines and describes the complex relationship between

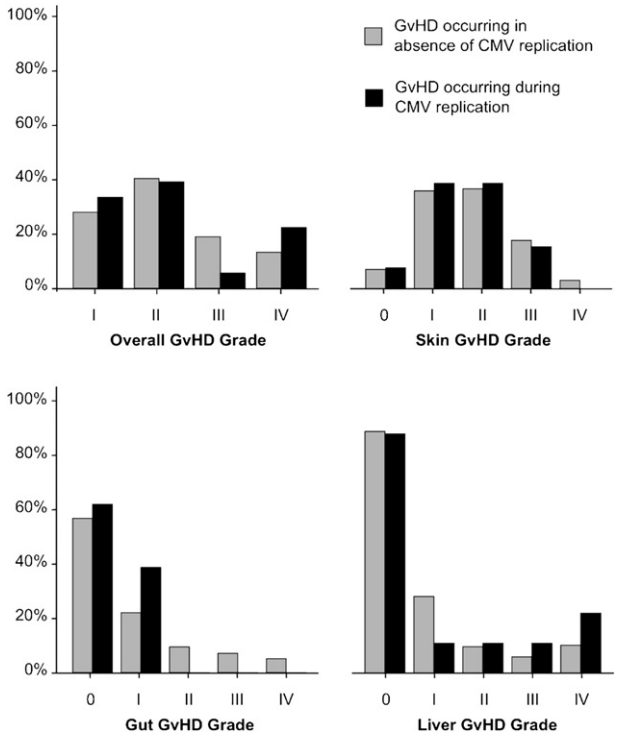

Figure 4. Severity and organ distribution of GVHD episodes occurring during CMV replication (black bars) and in the absence of CMV replication (gray bars).

CMV replication and aGVHD in patients undergoing allogeneic HSCT.

The role of aGVHD as a risk factor for CMV disease is well known. Miller et al. [3], in 1986, described aGVHD as an important risk factor for CMV replication. These early data also suggested a direct immunosuppressive effect of aGVHD independent from that of GVHD treatment (ie, systemic steroids), which increases the risk of CMV replication. Multiple other studies have since confirmed that patients with aGVHD are at an increased risk of CMV disease [4,5].

The data in our current cohort are in line with these studies, and confirm the association between aGVHD and CMV replication. For patients with aGVHD grade II-IV (treated with systemic corticosteroids), we found a risk increase of 61% compared to patients without GVHD. In comparison, the risk increase of 35% documented in patients with aGVHD I (not treated with systemic steroids) accounted for more than half of the effect seen in aGVHD grade II-IV patients, indicating that the immunosuppressive effect of GVHD itself is at least as important as that of GVHD treatment in the pathogenesis of CMV replication.

On the other hand, CMV may also play a role in the development of GVHD. The increased risk of GVHD development in patients with CMV seropositivity pretransplant [10-18] and reduced rates of chronic GVHD (cGVHD) after preemptive CMV treatment [19] are both indicative of a pathogenetic association between CMV replication and aGVHD. CMV-infected endothelial cells have been shown to produce inflammatory cytokines such as interleukin 6, which plays a crucial role in the initial phase of the GVHD [20]. The inflammatory response in patients after allogeneic HSCT with CMV replication could thereby contribute to the initiation of aGVHD [10]. However, data that directly link CMV replication with aGVHD development are so far lacking [21,22].

Our large study provides this missing link and clearly demonstrates that patients with active CMV replication are at increased risk of developing aGVHD in the transplant setting reported, which contains a majority of patients receiving peripheral blood grafts after myeloablative conditioning and leads to a high incidence of aGVHD with an early onset. Our analysis sheds light on the complex and reciprocal relationship between CMV replication and mortality after HSCT: since the introduction of a preemptive treatment, CMV disease has become rare, and CMV replication is no longer a substantial direct cause of death in patients after HSCT [23]. However, CMV remains a clear risk factor for treatment-related mortality after allogeneic HSCT [24]. This is elegantly explained by the excess of aGVHD associated with CMV replication demonstrated in this study.

Although some studies have shown positive results [25-27], most centers do not use antiviral prophylaxis to prevent CMV replication, because of hematologic (ie gancyclovir) and renal (ie foscarnet) toxicity, and because preemptive treatment has proven to be very successful in preventing CMV disease. In view of the results our study, which showed that patients with CMV replication are at increased risk for aGVHD,

Table 2. Multivariate Analysis for CMV Reactivation at Day 100

	Hazard Ratio (95% CI)	*P*-Value
Acute GVHD		
No acute GVHD	1.00	
Acute GVHD grade I	1.35 (0.82-2.21)	.01*
Acute GVHD grad II-IV	1.61 (1.11-2.36)	
Graft		
Bone marrow	1.00	
Peripheral stem cells	0.70 (0.49-1.00)	.05
Conditioning		
Cyclophosphamide + TBI ± Etoposide	1.00	
Cyclophosphamide + Busulfan	0.88 (0.56-1.41)	.60
Fludarabin + TBI	0.30 (0.12-0.78)	.01
Other	1.13 (0.61-2.10)	.69

CMV indicates cytomegalovirus; GVHD, graft-versus-host disease; TBI, total-body irradiation.
Cox model adjusted for acute GVHD, patient age, disease, disease stage, donor type, stem cell source, conditioning regimen, degree of HLA match, and type of pharmacological GVHD prophylaxis.
*P-value for trend.

and consequently also for transplant-related mortality, and of the introduction of new drugs, such as maribavir, this policy has to be discussed. Maribavir is a new antiviral drug under investigation for prophylaxis for CMV [28]. A randomized, placebo-controlled dose-ranging phase 2 study has been published with promising results, showing lower risk of CMV replication and limited toxicity (principally gastrointestinal side effects) [29]. The use of CMV prophylaxis routinely with new agents with lower toxicity, especially in patients at high risk of CMV replication, might reduce the incidence of CMV replications, reducing so the risk for aGVHD.

In conclusion, this study confirms aGVHD as a risk factor for CMV replication, and documents that patients with active CMV replication have a significantly higher risk of developing aGVHD compared to patients without CMV replication. The data suggest that prospective studies of prophylactic CMV treatment to reduce aGVHD incidence might be warranted.

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