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Intensification of GVHD prophylaxis interferes with the effects of pretransplant herpes virus serology on the occurrence of grades II – IV acute graft-versus-host disease

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Summary. The effects of pretransplant herpes virus serology on the occurrence of grades II – IV acute graft-versus-host disease (GVHD) were studied in 262 recipients and their HLA-identical family donors. In 131 recipients on standard GVHD prophylaxis (either methotrexate or cyclosporin A) significant effects were observed for donor HSV serology (seropositivity associated with increased risk for GVHD) and donor EBV serology (seronegativity associated with increased risk). However, these effects were nonsignificant in the other 131 recipients on intensified GVHD prophylaxis (i.e., methotrexate combined with cyclosporin A, in vivo anti-T-cell monoclonal antibodies, or various procedures to reduce the T-cell numbers in the transplants).

Key words: Bone marrow transplantation – Graft-versus-host-disease – Herpes viruses

Introduction

The incidence and severity of acute graft-versus-host disease (GVHD) can be reduced by removing the bacterial microflora from the gastrointestinal tracts of animals [1] and human beings [11]. The potentiating effect of the gastrointestinal microflora on graft-versus-host reactivity may occur via the activation of lymphocytes or their precursors during the immediate post-transplant period. Similar effects may be mediated by viruses and parasites carried by the recipients at the time of transplantation [6]. Herpes viruses are important candidates since they establish life-long carrier status after initial infection and frequently reactivate during immunosuppression. In addition, herpes virus carrier status leads to the development of virus-specific humoral and cellular immunity, so that the presence of virus-specific antibodies may be taken as a marker for carrier status and presence of virus-specific memory cells.

Several studies of the effects of pretransplant herpes virus serology in allogeneic bone marrow transplant (BMT) recipients and their donors on the development of acute GVHD have been performed (Table 1). Two single-center studies [2, 7] were followed by a retrospective multicenter study of data collected by the European Group for Bone Marrow Transplantation [3]. In all studies, the risk of grades II – IV GVHD increased proportionally with the number of herpes viruses carried. However, results were discordant at the level of individual viruses (Table 1).

In 1986, the two BMT teams in Leiden intensified their GVHD prophylactic protocols to reduce GVHD-related morbidity and mortality. Around the same time, the BMT team in Utrecht embarked on a protocol in which T-lymphocyte-depleted bone marrow grafts were reconstituted with a fixed low number of T cells prior to administration [10]. The present analysis of 262 patients who underwent transplantation by these three teams was performed to assess the effect of intensification of GVHD prophylaxis on the interaction between herpes virus carrier status and grades II – IV acute GVHD.

Patients and methods

Between 1978 and 1991, 262 patients received transplants with bone marrow grafts from their HLA-identical siblings in Leiden (Dept. of Hematology, $n = 126$; Dept. of Pediatrics, $n = 76$) and in Utrecht ($n = 60$). The diagnostic indications for BMT were severe aplastic anemia ($n = 28$), acute nonlymphoblastic leukemia ($n = 120$), acute lymphoblastic leukemia ($n = 50$), chronic myelogenous leukemia ($n = 35$), non-Hodgkin's lymphoma ($n = 9$), Kahler's disease ($n = 5$), other hematological malignancies ($n = 8$) or other indications ($n = 7$). The median age of the patients was 35 years (range 10 months to 48 years). Standard GVHD prophylaxis (either methotrexate or cyclosporin A) was given to 131 patients. Intensified GVHD prophylaxis was given to the other 131 patients (methotrexate + cyclosporin A, $n = 21$; in vivo anti-T-cell monoclonal anti-

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Table 1. Pretransplant herpes virus serology and acute GVHD; summary of three clinical studies

Lower risk	Higher risk	Leiden 1987	Huddinge 1988	EGBMT 1990
Donor				
0–2 Viruses	3–4 Viruses	$p < 0.05$	$p < 0.05$	$p < 0.05$
HSV negative	HSV positive	$p < 0.05$	NS	NS
EBV positive	EBV negative	$p < 0.05$	NS	NS
Recipient				
0–2 Viruses	3–4 Viruses	$p < 0.05$	$p < 0.05$	$p < 0.05$
CMV negative	CMV positive	NS	$p < 0.05$	NS
EBV negative	EBV positive	$p < 0.05$	NS	NS

bodies, $n = 23$; in vitro T-cell depletion using anti-T-cell monoclonal antibodies, $n = 28$; and fixed low T-cell numbers in the grafts, $n = 60$). All transplants resulted in full engraftment. Four patients (three on standard and one on intensified GVHD prophylaxis) were excluded from subsequent analyses because they died without clinical signs of GVHD while being at risk for the disease (i.e., prior to day 100 post BMT).

IgG antibodies against herpes simplex virus (HSV), varicella zoster virus (VZV), and Epstein-Barr virus (EBV) capsid antigen were detected using indirect immunofluorescence assays, IgG antibodies against cytomegalovirus (CMV) late antigen by an enzyme-linked immunoassay.

Results

Grades II–IV acute GVHD occurred in 53 of the 128 patients on standard GVHD prophylaxis (41%) and in only 28% of the 130 patients on intensified GVHD prophylaxis (22%). First, a multivariate analysis of the interaction between donor and recipient pretransplant herpes virus serology and acute GVHD was performed on all 262 patients. Competing nonvirological factors in the analysis were chosen on the basis of their significant role in previous analyses (Table 2, upper panel) [5, 7]. With respect to herpes virus serology, significant but opposite effects were observed for donor HSV serology (higher risk vs. lower risk, seropositive vs. seronegative; $p = 0.03$) and donor EBV serology (higher risk vs. lower risk, seronegative vs. seropositive; $p = 0.01$) (Table 2, lower panel). Separate analysis of patients on standard and intensified GVHD prophylaxis revealed that the significant effects of donor HSV and EBV serology were confined to the patients on standard GVHD prohylaxis; the effects of herpes virus serology in the patients on intensified GVHD prophylaxis were nonsignificant (Table 3). Cross-tabulations for the individual viruses are shown in Table 4 (HSV, upper panel; EBV, lower panel) and illustrate the disappearance of the effect of donor HSV and EBV serology on acute GVHD. In both patient groups, grades II–IV acute GVHD are rare to absent in the (young) seronegative recipients of marrow from seronegative donors.

Table 2. Multivariate analysis on 262 patients

A. Competing factors in the analysis

Pre-BMT serology	Odds ratio	p value
Intensified prophylaxis for GVHD given	0.20	0.0001
Total GI decontamination	0.24	0.01
Increasing age, recipient	1.0	NS
Increasing age, donor	1.0	NS
Sex mismatch	1.5	NS

B. Pretransplant herpes virus serology

Pre-BMT serology	Odds ratio	p value
Donor		
HSV positive	2.8	0.03
CMV positive	1.6	NS
VZV positive	2.1	NS
EBV positive	0.3	0.01
Recipient		
HSV positive	1.0	NS
CMV positive	1.5	NS
VZV positive	1.4	NS
EBV positive	2.6	NS

Discussion

We have shown that various protocols of intensified GVHD prophylaxis interfere with the effects of pretransplant herpes virus serology on the occurrence of grades II–IV acute GVHD. With respect to HSV, we have inferred that HSV-specific memory T lymphocytes in the grafts are responsible for the effect [7]. This contention is supported by the observation of Boström et al. [4] that strong donor mononuclear cell reactivity to HSV is associated with an increased frequency of grades II–III GVHD. Thus, the activation of HSV-specific memory T lymphocytes by the HSV-encoded or cross-reactive antigens in the recipients may contribute to GVHD, possibly via the release of cytokines that induce systemic T-cell activation.

The first encounter of T lymphocytes from EBV-seronegative donors with the virus or with EBV-transformed cells leads to their polyclonal activation, as observed in infectious mononucleosis [9]. Indeed, an increased rate of EBV production in the oropharynx has been observed in the immediate post-BMT period [8], similar to the situation in immunosuppressed renal transplant recipients [12]. Again, the depletion or the functional suppression of T lymphocytes would prevent their activation and cytokine production.

Table 3. Effect of intensification of GVHD prophylaxis

Pre-BMT serology	Standard		Intensified	
	O.R.	*p* value	O.R.	*p* value
Donor				
HSV positive	5.4	0.007	1.2	NS
CMV positive	3.4	0.03	1.1	NS
VZV positive	2.2	NS	2.1	NS
EBV positive	0.08	0.002	0.8	NS
Recipient				
HSV positive	2.6	NS	0.7	NS
CMV positive	1.1	NS	1.9	NS
VZV positive	2.0	NS	1.1	NS
EBV positive	6.0	0.05	1.1	NS

Table 4. Effects of intensification of GVHD prophylaxis

A. Pretransplant HSV serology and acute GVHD

Pre-BMT serology		Standard		Intensified	
Recipient	Donor	Gr. 0–I	Gr. II–IV	Gr. 0–I	Gr. II–IV
Negative	Negative	11	1 (8%)	12	0 (0%)
Negative	Positive	10	2 (17%)	7	4 (36%)
Positive	Negative	19	5 (21%)	10	6 (38%)
Positive	Positive	35	45 (56%)	73	18 (20%)

B. Pretransplant EBV serology and acute GVHD

Pre-BMT serology		Standard		Intensified	
Recipient	Donor	Gr. 0–I	Gr. II–IV	Gr. 0–I	Gr. II–IV
Negative	Negative	11	2 (15%)	4	0 (0%)
Negative	Positive	9	1 (10%)	6	2 (25%)
Positive	Negative	4	10 (71%)	6	2 (25%)
Positive	Positive	51	40 (44%)	86	24 (22%)

T lymphocytes constitute the final common pathway through which GVHD is effectuated. This study shows that the depletion of T lymphocytes from the marrow grafts or the prevention of their activation post BMT abolishes the significance of pretransplant herpes virus serology as a risk factor for grades II–IV acute GVHD.

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