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Longitudinal analysis of anti-host directed cytotoxic T cell reactivities after HLA-identical bone marrow transplantation (BMT)

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INTRODUCTION

Putative minor Histocompatibility antigens (minor HAs) may severely complicate the outcome of BMT between HLA-identical siblings, by in vivo triggering of effector T lymphocytes involved in Graft versus Host Disease (GvHD) or in graft rejection (1). With regard to GvHD, the impact of HLA-restricted host-directed cytotoxic T lymphocytes (CTLs), isolated from recipients' PBL at one time point after grafting has been demonstrated earlier (2,3). To gain insight into the longitudinal development of such anti-host CTL reactivities, we performed in vitro studies following 12 patients up until 25 months after grafting.

PATIENTS AND T CELL LINES

One patient with severe aplastic anemia and 11 patients with acute leukemia in first or second remission received non-T cell depleted bone marrow from their HLA-A-, B-, Cw-, DR identical, MLR non-reactive sibling donors. As prophylaxis for acute GvHD methotrexate (n = 9) and cyclosporin A (n = 3) were given. Based on the presence or absence of clinical acute and/or chronic GvHD patients were divided into three groups; 3 patients (1,2,3) had no GvHD, 5 patients (4,5,6,7,8) developed acute GvHD (grade I-II) with onset between day 12 and 54 post-BMT, and 4 patients (9,10,11,12) developed acute GvHD (gradeII-IV) with onset between day 11 and 28 post-BMT followed by chronic GvHD.
Host-specific T cell lines were induced from patients' PBL isolated periodically after BMT and as a control from unsensitized donors' PBL through in vitro stimulation with patients' pre-BMT PBL. All patients' T cell lines were tested for anti-host cytotoxic activity against patients' pre-BMT PHA induced T lymphoblasts in a standard chromium release assay. To further analyse the CTL specificity of one patient's T cell lines panelstudies were performed.

RESULTS AND CONCLUSIONS

Table 1 illustrates that, whereas none of the donor-derived T cell lines showed any host-directed cytotoxicity, significant anti-host lysis (>20%) developed in 10 patients; in 2/3 patients without GvHD, in 4/5 patients with acute GvHD and in 4/4 patients with acute followed by chronic GvHD.
Basically, the longitudinal analysis revealed at least two distinct patterns of anti-host cytotoxic reactivities; 1) non-phasic (n = 5, patients 1,5,8,9,10) and 2) phasic, either abruptly arising (n = 3, patients 7,11,12) and/or dispearing (n = 3, patients 3,4,7). Notably, the sudden appearance of anti-host CTLs in patients 7,11 and 12 coincided with the discontinuation of cyclosporin A treatment.
Strong early anti-host cytotoxicity (0-1,5 months after BMT) was found in two patients who developed acute and subsequently chronic GvHD (patients 9, 10), in one of them persisting for at least 25 months (patient 9). When specificities of "early" (i.e. 25 days post-BMT) and "late" (i.e. 773 days post-BMT) anti-host CTLs of this latter patient were compared (table 2), both T cell populations showed minor H antigen-reactivity, restricted via HLA-B7. The analysis further demonstrated that the "early" anti-host CTLs had a broader panelreactivity (23/24 HLA-B7+ targets) than the "late" anti-host CTLs (12/24 HLA-B7+ targets).

Table 1.
Post-BMT occurrence of anti-host CTLs in 12 patients.

patient	GvHD		patient after BMT (months)						donor
	acute	chronic	0-1,5	1,5-3	3-6	6-9	9-12	12-25	
1	no	no	+[a]			+++			ND
2	no	no	--	--		--	--		--
3	no	no	+		+++	++	(+)	--	--
4	yes	no			++	+	++	--	--
5	yes	no	++	+++					ND
6	yes	no			(+)	(+)	--	--	ND
7	yes	no	--	--		+++		--	--
8	yes	no		++		+	(+)	+	--
9	yes	yes	+++	+++	+++	++	+++	+++	ND
10	yes	yes	+++	+++	++	++	+		--
11	yes	yes	--	++	+++	+++			--
12	yes	yes	--	(+)	++				--

[a] percentage of specific lysis against patients' pre-BMT T lymphoblasts. - = <10%, (+) = 10-20%, + = 20-40%, ++ = 40-60%, +++ = 60-100%, ND = not determined.

Table 2.
Specificity analysis of a patient's anti-host CTLs, obtained 25 days and 773 days after BMT, on a panel of unrelated donorcells.

	CTL 25d panel reactivity		CTL 773d panel reactivity	
	+	-	+	-
$B7^+$ n=24	23	1	12	12
$B7^-$ n=13	0	13	0	13

In conclusion, although strongest anti-host T cell reactivities were found in two patients who developed chronic GvHD, the presence of anti-host CTLs per se was not correlated with the occurrence of GvHD in these 12 patients. Further longitudinal analysis of one patient's anti-host CTLs suggested that early multiple minor H antigen reactivity might be partially lost in time. Finally, preliminary evidence that cyclosporin A might interfere with the activation of anti-host CTLs remains to be further investigated.

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