

Acute Graft Versus Host Disease due to T Lymphocytes Recognizing a Single HLA-DPB1*0501 Mismatch

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Abstract

Analysis of a large number of unrelated bone marrow transplantations (BMT) has shown that HLA-DP incompatibility did not detectably influence the risk for acute graft-versus-host disease (aGVHD). Accordingly, it was proposed that HLA-DP determinants did not function as transplantation antigens in the same way as HLA-A, -B, or -DR. We have previously shown that HLA-DP (as well as HLA-A, -B, -DQ, or -DR)-specific T cells could be isolated from skin biopsies of patients who developed an aGVHD after semiallogeneic BMT. Nevertheless, whether a single HLA-DP mismatched allele could induce a detectable allo-specific reaction in vivo after BMT remained to be established. To directly address this issue we studied one patient who presented aGVHD after receiving purified CD34+ bone marrow (BM) cells from an unrelated donor with a single HLA-DP mismatch in the GVHD direction. To characterize the immunological events associated with GVHD, we analyzed the peripheral T cell repertoire, the T cell receptor V β diversity, and the specificity of T cells invading a skin biopsy at the onset of GVHD. Our results demonstrated that a large fraction of skin-infiltrating lymphocytes, which expressed diverse T cell receptors, were reactive against this single HLA-DPB1*0501 mismatch and consequently that a single HLA-DP mismatch between BM donor and recipient can activate a strong T cell response in vivo. (*J. Clin. Invest.* 1996. 98:100–107.) Key words: skin • T lymphocytes • HLA-DP • clone • specificity

Introduction

Allogeneic bone marrow transplantation has become a successful therapeutic approach for the treatment of several hematological malignancies as well as bone marrow failures such as aplastic anemia. However, its success remains limited by its chief complication: the acute graft-versus-host disease

(aGVHD)¹ (1, 2). Two main risk factors are linked to the occurrence of aGVHD: the T cell content of the graft and the HLA disparities between donor and recipient. With respect to HLA disparities, the increased risk of aGVHD linked to HLA-A, -B, -DR mismatching between donor and recipient is well established (3–8), in contrast HLA-DP incompatibility does not seem to be a risk factor for aGVHD (for review see references 9 and 10). Accordingly, it was proposed that HLA-DP determinants might not function as transplantation antigens in the same way as HLA-A, -B, or -DR (10). In the absence of direct evidence for HLA-DP recognition in such a graft situation, we took advantage of a clinical situation where an acute GVHD occurred in one of our patients who received an unrelated allogeneic bone marrow transplant (BMT) of CD34 purified precursor cells with a single HLA-DPB1*0501 mismatch to address two questions: (a) could a single HLA-DPB1 allele mismatch trigger a specific T cell response after BMT? and (b) if a specific T cell response occurs, is it mediated by T cells expressing restricted T cell receptor V- β (TCRBV) regions? In the course of this study additional observations were made concerning the relationship between T cells present in the skin and the patient's PBL at the onset of GVHD.

Methods

Case report

Patient. A 48-yr-old female with chronic myeloid leukemia diagnosed in June 1990 was treated first with hydroxyurea, IFN- α and subcutaneous low dose cytarabine. In July 1993 she presented an accelerated CML.

Bone marrow transplantation. A search in the French Bone Marrow transplant registry detected one possible donor (host and donor HLA typing are indicated in Table I). Results of mixed lymphocyte culture was 8% in the GVH direction and 17% in the HVG direction. The donor was a 20-yr-old male. Patient was CMV+; donor was CMV-; they were ABO identical.

Conditioning regimen. Total body irradiation: total dose 12 Gy on six fractions over 3 d with lung shielding at 8 Gy followed by cyclophosphamide 60 mg/kg/d on 2 consecutive d. Donor bone marrow was harvested under general anesthesia.

Characteristics of the graft. Because of GVHD risk factors (patient age, advanced disease, and unrelated donor), this patient received, after informed consent, selected bone marrow (BM) CD34+ cells with the aim of reducing GVHD risk through T cell reduction. 1,200 ml of whole marrow containing 5.31×10^8 mononuclear cells /

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1. **Abbreviations used in this paper:** aGVHD, acute graft-versus-host disease; BIL, biopsy-infiltrating cell line; BLCL, B lymphoblastoid cell line; BM, bone marrow; BMT, BM transplant; GVL, graft-versus-leukemia; TCRBV, T cell receptor V- β ; TK, thymidine kinase.

Table I. HLA-typing of Host, Donor, and BLCL Used for the Study

BLCL	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1	HLA-DPB1
Host (Ho)	2	7/44	1501/1101	0602/0301	0401/0501
Donor (Do)	2	7/44	1501/1101	0602/0301	0301/0401
DES (A)	2/28	7	0103/0413	0501/0302	1501/0501
YOT (B)	1/23	44/12	4	nd	0202/0501
EGM (C)	1/11	51/7	1501/0407	nd	0401/0501
BOV (E)	3	7/62	15	nd	W4/0501
DEM (F)	2.2	57.2	4/16	0502/0302	0401/1602
BH (G)	2.2	13.2	0701	0201	0401
QBL (I)	26.1	18.1	0301	0201	0202
E418 (J)	1	52	1502	0601	02012/0401
IBW9 (K)	33.1	65	0701	0201	0101
BSM (L)	2.2	62.3	0401	0302	02012
HOM2	3.2	27.5	1	0501	0401
DAB	2/11	18/W55	8/11	0402/0301	0401/1501

Letters between parentheses are used in Fig. 3 to refer to the corresponding BLCL. The two last cell lines HOM2 and DAB were used as controls in the initial screening of the clones presented in Table III. nd, not defined.

kg (27.6×10^9 total) were harvested in heparinized flask from the posterior iliac crest. A buffy coat was obtained by centrifugation using a Cobe 2991 blood cell processor (Cobe Laboratories Inc., Lakewood, CO). Nucleated cells (0.58×10^8 /kg) were stored unprocessed as a reserve. Mononuclear cells (14.2×10^9 total) including 1.70% CD34+ cells (4.6×10^6 /kg) were then incubated with a biotinylated anti-CD34 mAb (clone 12.8) for 25 min, washed in PBS; (Baxter, Munich, Germany) and processed onto a computer-driven avidin immunoaffinity column device (CEPRATE stem cell concentrator; Cell Pro Inc., Bothell, WA). Adsorbed CD34+ cells were removed by mechanical agitation from the column, washed in PBS, diluted in 10 ml PBS 4% HSA. The CD34+ fraction contained 3×10^6 mononuclear cells/kg (0.17×10^9 total), including 54%, i.e. 1.62×10^6 CD34+ cells/kg (recovery and enrichment for CD34+ cells were 35% and 32-fold, respectively). CFU-GM content using standard assay was 4.79×10^6 , total corresponding to 9.2×10^4 /kg. BFU-E content was 8.25×10^6 , total corresponding to 15.87×10^4 /kg. CD34+ cells content was 92.4×10^6 , total corresponding to 1.78×10^6 /kg. Graft was injected through a central venous line under 40 ml vol within 10 min on May 3, 1994.

Evolution. WBC was $> 1,000$ on day 13, PMN $> 1,000$ on day 14. G-CSF (Granocyte) was started on day 7 at a dose of 263 μ g/d i.v. No evidence of platelet or erythrocyte reconstitution was observed until death. No other GVHD prophylaxis except CD34+ selection was used. Only 3% CD3+ T cells contaminated the CD34+ preparation corresponding to a total number of T cell reinjected of 9.4×10^4 /kg. On day 10, the patient presented a general skin eruption. A skin biopsy was performed showing signs consistent with the diagnosis of acute GVHD: vacuolization of keratinocytes with dermo-infiltration with some lymphocytes. Immunophenotyping analysis showed a strong expression of DR antigen on keratinocytes and the presence of a mixed lymphocytic population composed of 30% CD4+ and 20% CD8+ T cells. Treatment with Cyclosporine A 3 mg/kg/d CIV + prednisone 4 mg/kg/d was started. Skin improved but liver function decreased rapidly with diarrhea and rising bilirubin up to > 600 mmol/liter at time of death. The patient died on day 39. The main cause of death was aspergillosis complicating GVHD. Liver post mortem examination showed signs compatible with GVHD. No evidence of liver infection with aspergillosis was present.

VNTR analysis

DNA were prepared from host and donor B lymphoblastoid cell line and digested by HaeIII. Digestion products were separated on a 1%

agarose gel, transferred onto hybond N+ membrane, and hybridized with a hyper polymorphic single locus probe (PH30; Gibco BRL, Life Technology, Gaithersburg, MD) labeled with λ CTP³².

Obtaining skin infiltrating T cells and clones

Skin specimens were washed at least five times in medium containing 10% pooled human sera, 1% L-glutamine (2 mM), and 50 μ g/ml gentamycin and then cultured in a 24-well plate (Nunclon, Copenhagen, Denmark) in the same medium supplemented with recombinant interleukin 2 (rIL-2, 150 BRMP U/ml). Cultures were kept at 37°C in a 5% CO₂ atmosphere. To generate a panel of clones, one responder T cell was seeded in every three culture wells in 96-microwell round bottom culture plate together with pooled allogeneic feeder cells (5×10^4 PBL and 5×10^3 B lymphoblastoid cell line (BLCL), 30 gray irradiated) in the presence of 1 μ g/ml leucoagglutinin-A (Pharmacia Fine Chemicals, Uppsala, Sweden), and rIL-2 (150 BRMP U/ml). These conditions, where 100% of T cells are stimulated, were chosen to avoid in vitro antigenic selection. This allows the best representation of T cells, which grew under rIL-2 alone during the initial culture period, i.e., the in vivo activated T cells infiltrating the biopsy (11, 12), since this procedure was shown to preserve the initial diversity of the amplified population (13). Before specificity assays, clones were cultured in IL-2 alone without stimulation for at least 3 wk. Because of the limited number of biological material available, T cells from total or CD34 purified BM cells as well as patient PBL were amplified for 10 d in vitro before T cell repertoire analysis using the polyclonal activation procedure described above.

Proliferation assay

Resting T cells ($2.5-5 \times 10^4$), taken > 3 wk after the last stimulation, were cocultured for 48-72 h with the indicated irradiated (30 Gy) B-lymphoblastoid cell line (BLCL) in 96-microwell flat-bottomed culture plates at a 1:1 responder-to-stimulator ratio. 6 h before harvesting, 1 μ Ci of [³H]thymidine was added to each well, and ³H uptake was then measured in a liquid scintillation counter. Results are expressed as the mean of triplicate or quadruplicate cultures. Host, donor, and the DAB BLCL, as well as the HLA-DPB1*0501 BLCL DES, YOT, EGM, and BOV which were derived from genotyped blood bank donors, were obtained by coculturing PBL with EBV containing supernatant from the virus-producing B95.8 cell line in the presence of 1 μ g/ml CSA. The BLCL DEM, BH, QBL, E418, IBW9, BSM, and HOM2 are from the 1987 HLA-workshop in New York.

Table II. Flow Cytometry Analysis of TCRBV Region Expression by the Different T Cell Populations Studied

	TCR beta V Subsets (TCRBV)																			T
	2S1	3S1	5S1	5S2	5S3	6S7	8S1	11S1	12S2	13S1	13S6	14S1	16S1	17S1	18S1	19S1	21S3	22S1		
Donor BM	6.3*	1.1	6.5	1.6	0.7	3.1	5.1	1.7	4.3	2.8	1.6	5.1	2.0	0.7	0.0	6.8	3.3	2.6	62.6	
CD34c	4.2	0.3	4.5	0.8	0.9	1.7	4.2	0.9	1.5	2.4	1.4	8.9	1.2	0.5	0.3	7.0	2.9	2.2	57.5	
PBLb	0.5	0.2	0.5	0.2	0.5	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.5	0.5	0.2	0.3	0.4	0.3	8.7	
PBLd	0.3	0.5	5.7	0.7	1.5	2.0	1.9	0.3	2.0	0.8	6.5	5.7	0.4	0.2	0.0	4.1	4.3	1.1	38.0	
BIL	0.3	0.0	0.3	0.4	0.0	9.6	2.7	0.2	1.5	0.4	nt	0.1	0.0	0.2	0.1	nt	0.1	11.3	27.3	

*Percentage of positive cells (green fluorescence) among CD3+ T cells (red fluorescence); T, total percentage of the CD3+ T cell population recognized; nt, not tested; PBLb, PBL harvested on day 10; PBLd, PBL harvested on day 39; BIL, Biopsy infiltrating lymphocytes (biopsy was performed on day 10); square values correspond to subsets from which HLA-DP0501-specific T cell clones were obtained.

The following mAbs were used for specificity studies: anti-HLA-DR (GSP41), -DQ (1A3 or Leu-10), -DP (B7.21).

Flow cytometry

The following TCRBV region-specific mAbs (Immunotech, Marseille, France) were used for flow cytometry: E2.2E7.2 (anti-BV2S1), LE89 (anti-BV3S1), IMMU157 (anti-BV5S1), 36213 (anti-BV5S2), OT145 (anti-BV6S7*1), 3G5D15 (anti-BV7S1), 56C5.2 (anti-BV8S1/S2), FIN9 (anti-BV9S1), C21 (anti-BV11S1), S511 (anti-BV12S1), IMMU1222 (anti-BV13S1), JU74 (anti-BV13S6), CAS1.1.13 (anti-BV14S1), TAMAYA1.2 (anti-BV16S1), E17.5F3 (anti-BV17S1), BA62.6 (anti-BV18S1), ELL1.4 (anti-BV20S1), IG125 (anti-BV21S3), IMMU546 (anti-BV22S1), and HUT78#1 (anti-BV23S1) (references compiled in the 1995 TCR Workshop, San Francisco). Cells were stained by two color immunofluorescence using phycoerythrin-conjugated CD3 (red fluorescence) and unconjugated TCRBV region-specific mAb, whose binding was revealed by FITC-conjugated goat anti-mouse IgG antiserum (green fluorescence). Labeled cells were analyzed on a FAC-Scan (Beckton Dickinson and Co., Mountain View, CA) using LYSIS II software.

Immunoscope analysis

TCR β chain-specific primers were as in reference 14 except for BV6 (5' CTC TGA AGA TCC AGC GCA CAS AGC) and BV21 (5' TCC AGC CTG CAA AGC TTG AGG ACT). RNA was extracted as previously described (14). Fluorescent DNA products were migrated on sequencing gels in an automated DNA sequencer (Applied Biosystems Inc., Foster City, CA), and raw data were analyzed by the immunoscope software package (15, 16).

Results

Obtaining skin-infiltrating T cells. The patient received 1.78×10^6 purified CD34+ cells/kg and T cell contamination was 0.94×10^5 /kg. 10 d after transplantation, a skin rash was observed, and GVHD was suspected. A 4-mm skin punch biopsy was performed and processed as described in Methods. After 3 d of culture, T cells started to exude the biopsy, and the biopsy infiltrating cell line (BIL) was shown to proliferate specifically against host BLCL but not against the irrelevant BLCL Boleth and BTB, suggesting that at least a significant proportion of these T cells were specific for host HLA antigens (data not shown).

Analysis of T cell repertoire diversity before and after transplantation. Because of the limited number of reinjected T cells and the presence of a single HLA-DP mismatch, T cell diversity analysis represented in this case a particularly important issue. Indeed, if a restricted T cell repertoire could be evi-

denced in such a graft setting, one could consider using specific immunotherapy as an alternative to global immunosuppression. $\alpha\beta$ T lymphocytes, the main effectors of the allogeneic reaction, possess antigenic receptors whose variable regions are made up of a combination of different TCR-V, -D, and -J elements (VDJ for β chains and VJ for α chains). Besides this combinatorial diversity, a junctional diversity is produced by the addition or removal of nucleotides at the junctions of the rearranged genes. Combinatorial (TCR-V gene usage) and junctional diversity of T cell receptors are now both amenable to analysis: the former by using TCR-V region specific mAbs and the latter by studying CDR3 length using recently developed techniques. An in depth TCR repertoire analysis was performed among the following populations: the donor BM CD3+ T cells before CD34 purification, the CD3+ T cells contaminating the purified CD34+ precursor cells before injection, the patient's PBL on the day the biopsy was performed, and the BIL.

TCRBV expression by the different T cell populations mentioned above was first studied by two color flow cytometry using an anti-CD3 mAb (red fluorescence) and mAbs specific for a large set of TCRBV regions. On Table II is shown the composition of the T cell repertoire detected in the different populations. Total percentage of CD3+ cells recognized by the panel of TCRBV-specific mAbs are indicated in the last column. As one can see, on the day the biopsy was performed, 20 mAbs directed at variable TCRBV regions recognized altogether only 8.7% of patient CD3+ PBL, compared to 62.6 and 57.5% of donor CD3+ PBL and CD3+ T cells contaminating the CD34+ preparation, respectively. This proportion increased with time and reached 38.0% on day 39 after transplantation probably as a consequence of ongoing T cell repopulation. Among BIL T cells, most TCRBV subsets were barely detectable except for two which were clearly overexpressed (TCRBV6S7, 9.6% and TCRBV22S1, 11.3%).

Taken together, these preliminary results indicated that repertoire diversity among T cells contaminating the purified CD34+ cell preparation was extensive and apparently not biased by the purification procedure. Of note, the two over-represented subsets among BIL T cells (TCRBV6S7 and TCRBV22S1) were only barely detectable among the patient's PBL tested on the day the biopsy was performed (PBLb on Table II).

The T cell repertoire of these different T cell populations was further investigated by analyzing the distribution of TCR CDR3 length with a recently described technique termed Im-

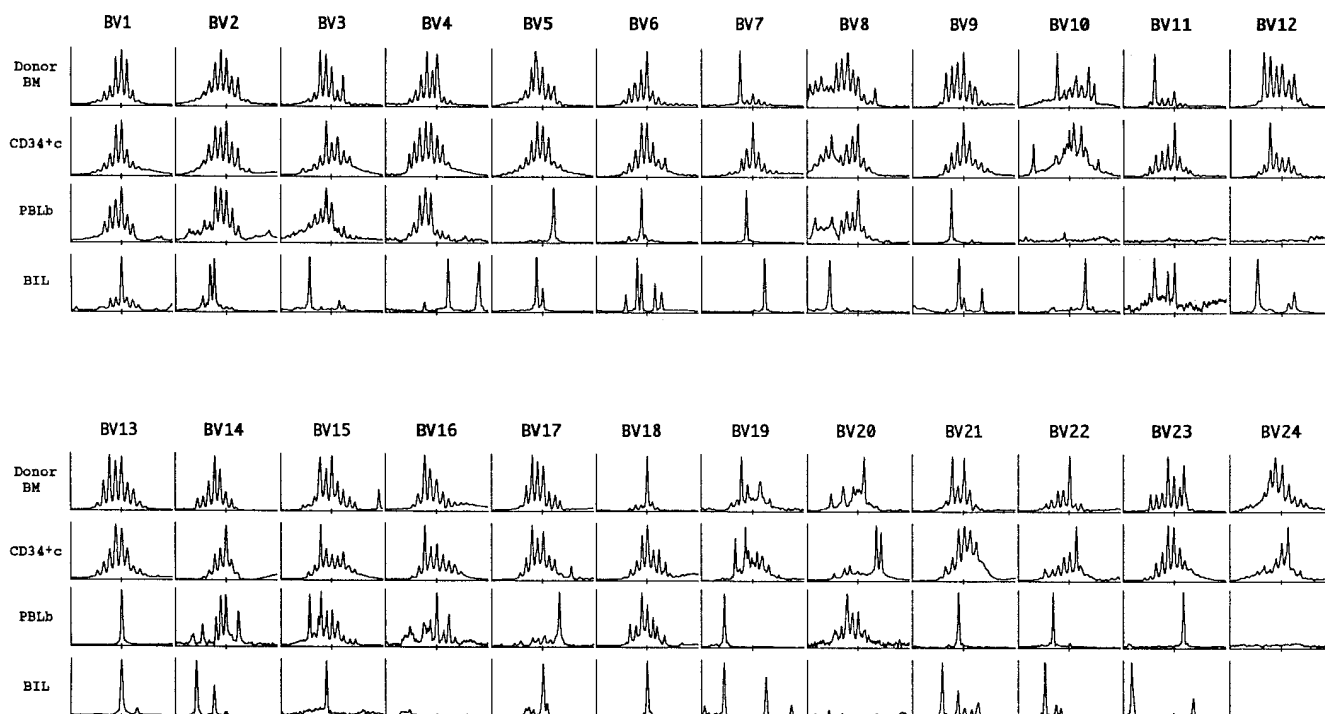


Figure 1. Distribution of CDR3b size (immunoscope profiles) within donor BM cells (*Donor BM*), T cells contaminating the CD34 inoculum (*CD34+c*), patient PBL harvested the day the biopsy was performed (*PBLb*) and within the skin biopsy derived T cell line (*BIL*). Note that HLA-DPB1*0501-specific T cell clones were derived from the BIL TCRBV5S2, 6S7, 13S1, and 22S1 T cell subsets and that the peak observed for Vb13 within PBLb and BIL most likely corresponded to the HLA-DPB1*0501-specific T cell clone derived from the BIL and having the same CDR3 size (data not shown).

munoscope (14). This sensitive technique, which involves a combination of PCR and run off reactions using pairs of V β /C β primers followed by size determination of the elongation products, allows efficient detection of oligoclonal T cell expansion within each of the TCRBV subsets studied. As shown on Fig. 1, a Gaussian-like distribution of CDR3 length was observed for most TCRBV chain genes expressed by either donor BM or CD34 contaminating T cells, demonstrating a high degree of diversity within these T cell populations. In contrast, dramatic alterations in CDR3 length distribution were detected for both the patient PBLb and the BIL T cell-derived cDNA amplified with several primers, e.g., BV5, BV6, BV7, BV9, BV13, BV17, BV19, BV21, BV22, and BV23, for the PBL, and all of them for the BIL T cells. These data confirmed and extended the flowcytometry results obtained with TCRBV-specific mAbs. It is noteworthy that isolated peaks with the same CDR3 length were detected for TCRBV13 and TCRBV19 among both PBLb and BIL T cells. As already suggested by others (17, 18), these peaks most likely represented the same T cell subsets or clones, although a formal demonstration would require direct sequencing of their TCR VDJB junctions. In contrast, most of the other BIL subsets with restricted CDR3 length could not be spotted by this sensitive technique among the patient's PBL. Consequently, all T cell repertoire alterations found among PBL do not necessarily reflect T cell expansions occurring concomitantly at the site of aGVHD.

Origin and specificity of clones derived from BIL T lymphocytes. We then analyzed the specificity of skin-infiltrating T cells at the clonal level. Clones were derived from BIL by limiting dilution as described in Methods. 62 clones were

screened for CD4/CD8 and TCRBV usage with specific mAbs (see Table II). Clones were derived from the following subsets: TCRBV5S2 ($n = 2$), TCRBV6S7 ($n = 7$), TCRBV8S1 ($n = 9$), TCRBV17S1 ($n = 1$), TCRBV13S1 ($n = 2$), and TCRBV22S1 ($n = 7$), (squared values on Table II). 23 clones (referred to as TCRBVX) were not recognized by the available TCRBV-specific panel of mAbs. Among the TCRBVX host-specific T cell clones only one was kept for further functional study. Clones belonging to a given TCRBV subset were particularly homogeneous since they expressed the same co-receptor and had the same reactivity pattern (Table III). Indeed all BV6+, BV13+, BV22+, and BV5+ clones were host reactive (as well as the single BV17+) while none of the BV8+ recognized the host BLCL (Table III). Together with data from immunoscope analysis which evidenced a unique or dominant peak within each of the BIL TCRBV subsets (Fig. 1), these results strongly suggested that each set of clones belonging to the same TCRBV family derived in fact from a single or a few T cell clones. These findings are also in agreement with our previous reports showing that T cells infiltrating the skin at the onset of GVHD were oligoclonal, and for a majority of them, host specific (11, 12). Consequently, one clone representative of each subset was kept for further characterization.

First, DNA from TCRBV5S2, TCRBV6S7*1, TCRBV8S1, TCRBV13S1, TCRBV22S1, and three TCRBVX T cell clones were compared to DNA from host or recipient BLCL using RFLP analysis to determine whether they originated from host or donor. Results, shown on Fig. 2, demonstrated that all clones tested originated from the donor.

We next tested these clones against host and donor BLCL

Table III. Screening of Clones Derived from BIL T Cells

Clone #	Stimulator BLCL (cpm)				TCRBV	CD4/8
	none	HOM2	HOST	DAB		
none	—	407	1536	578	—	
3	534	588	25778	1108	TCRBV6S7	CD4
6	291	407	10409	951	"	"
7	2994	3023	29992	5124	"	"
17	255	453	8284	1022	"	"
23	530	398	16389	1253	"	"
26	307	873	12156	3217	"	"
32	295	363	33857	819	"	"
35	691	980	12938	1898	"	"
40	5658	5377	30056	5616	"	"
46	160	327	9088	881	"	"
52	6663	3137	28112	2524	"	"
57	283	556	10624	1269	"	"
61	86	54	21818	942	"	"
20	170	373	1161	896	TCRBV8S1	CD4
21	570	500	1182	936	"	"
24	96	375	1387	974	"	"
39	150	317	1066	1110	"	"
44	253	381	1166	873	"	"
51	188	548	1112	733	"	"
56	237	327	735	988	"	"
63	160	277	943	992	"	"
65	1458	265	3450	2858	"	"
12	1586	1281	11436	1803	TCRBV13S1	CD4
48	851	773	6631	1560	"	"
22	727	994	4801	1000	TCRBV17S1	CD4
4	395	426	7230	881	TCRBV22S1	CD4
11	395	431	11065	1076	"	"
25	202	281	5896	751	"	"
33	122	345	8077	821	"	"
34	128	351	21764	805	"	"
38	3522	6470	21986	6947	"	"
58	363	263	13965	914	"	"
18	11177	4770	15828	6331	TCRBV5S2	CD8
55	1872	1462	20809	2904	"	"

Each T cell clone obtained by limiting dilution from BIL culture was screened for CD4, CD8, and for the five TCRBV-specific mAbs recognizing the most frequent subsets among BIL cells (see Table II). Clones were tested in a 72-h proliferation assay against host and two unrelated BLCL (HOM2 and DAB).

as well as against a panel of genotyped BLCL bearing or not the HLA-DPB1*0501 mismatched allele in the GVHD direction. As shown in Fig. 3 each clone tested specifically recognized host BLCL and, among allogeneic ones, only those bearing the HLA-DPB1*0501 allele (Fig. 3, top, and Table I for extensive HLA typing of host donor and target BLCL). Accordingly, proliferation of each clone against a stimulating BLCL was blocked by an HLA-DP-specific mAb but remained unaffected by the addition of mAb against HLA-DQ or -DR. Of note, reactivity against HLA-DPB1*0501 of the CD8+ TCRBV5S2 T cell clone was also demonstrated using

this large panel of BLCL. Moreover, this CD8+ TCRBV5S2 as well as the CD4+ TCRBV22S1 T cell clone were found cytotoxic against HLA-DPB1*0501 bearing BLCL (data not shown). No other reactivity than the one directed at HLA-DPB1*0501 was evidenced.

Discussion

We have recently documented the presence of HLA-DP-specific T cells in the skin lesions of patients who developed GVHD after semiallogeneic BMT (11, 12). In these previous examples, other HLA class I and class II incompatibilities were present in addition to the HLA-DP mismatch. Thus, these other mismatches could have contributed to the initiating of the response against HLA-DP. To the best of our knowledge, no biological data were yet available concerning the ability of an isolated HLA-DPB1 mismatched allele to induce a specific alloreaction in vivo. In the present paper we provide evidence that after an allogeneic transplantation with a single HLA-DPB1*O501 mismatch between donor and recipient, a large proportion of the skin-infiltrating T cells were HLA-DPB1*O501-specific. These data conclusively demonstrate the occurrence of an in vivo alloreaction against this isolated mismatched allele. Because these T cells clones were derived from a skin lesion, our data also strongly suggest a direct involvement of HLA-DP in the pathogenesis of GVHD. Nevertheless, it has long been established that alloreactivity against MHC molecules is not the only factor conditioning GVHD since the 1–3% occurrence of DR/DQ and DP recombination cannot account for the 20–50% of GVHD reported between genotypically identical siblings (1). Moreover, the contribution of minor histocompatibility antigens to the increased risk of GVHD has been recently demonstrated (19). Because minor histocompatibility antigens disparity is bound to be significantly greater between unrelated than between

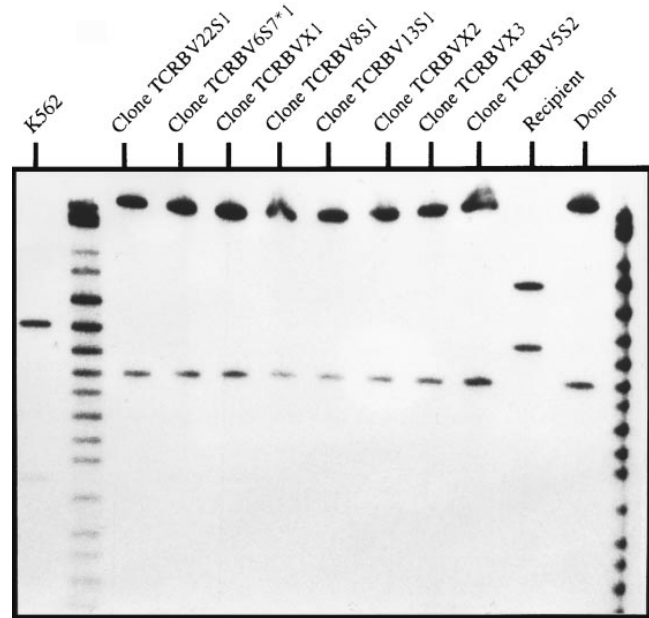


Figure 2. RFLP analysis of clones belonging to each of the TCRBV subsets detected among skin infiltrating T cells. Donor and recipient DNA were clearly identified by alleles of different sizes; all clones are of donor origin.

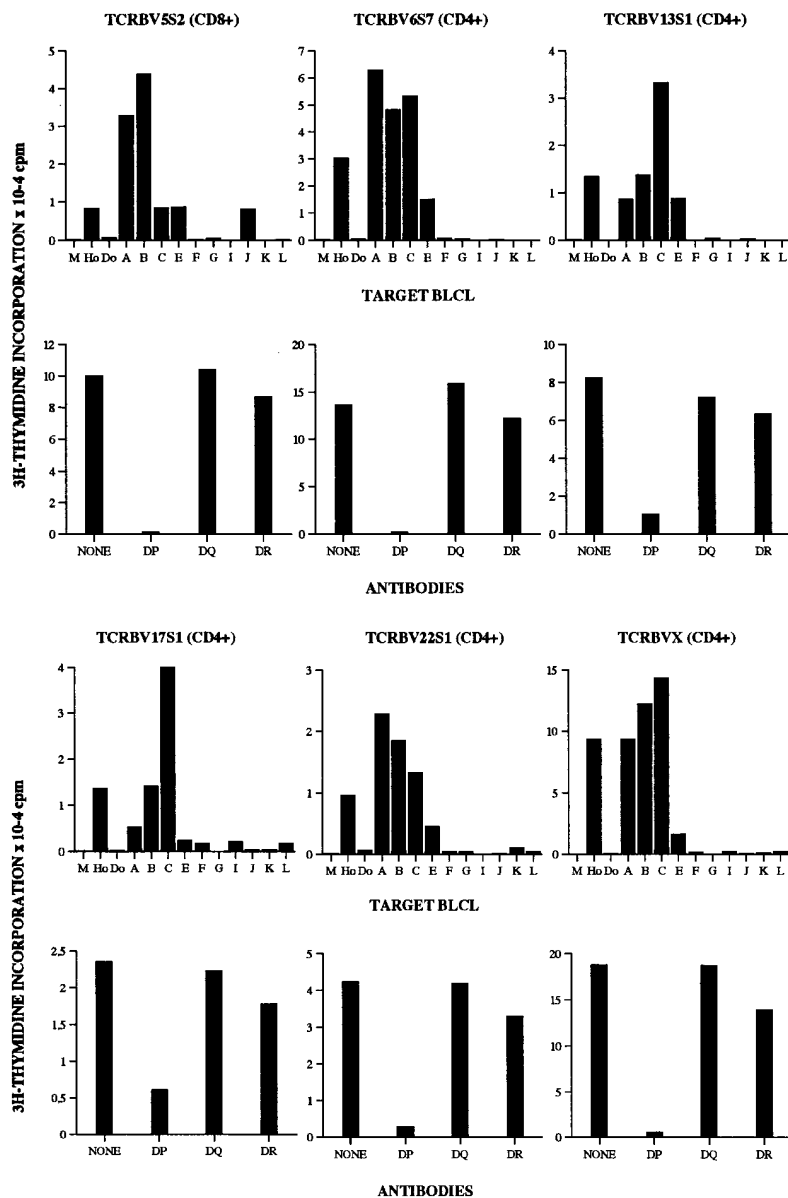


Figure 3. Determination of specificity for TCRBV selected clones: each clone recognized host BLCL (*Ho*) but not the donor BLCL (*Do*). Moreover they also recognized the 4 HLA-DPB1*0501-positive BLCL (*A*, *B*, *C*, and *E*, see Table I for HLA typing) but not the HLA-DPB1*0501-negative BLCL *F*, *G*, *I*, *J*, *K*, *L* except the TCRBV5S2 CD8+ clones which also cross-react with *J* (*top*). Recognition of target BLCL was blocked by mAb against HLA-DP (B7.21) but not by mAb against HLA-DQ (Leu-10) or HLA-DR (GSP41).

genotypical individuals (20), it is likely that specific T cells directed against minor antigens also contribute to the increased risk of GVHD after unrelated bone marrow transplantation. Thus, it is difficult to ascertain that the HLA-DPB1*0501-specific T cells we described were the only ones to participate in the GVHD process. In our protocol, the T cell clones derived from the skin biopsy were screened for their ability to proliferate against host BLCL. Because it has been shown that BLCL might not present MHC class II restricted minor antigens adequately (21), it is possible that some of the T cell clones found negative against host BLCL were in fact specific for host MHC class II restricted minor antigens. Aside from their specificity, the pathological consequences of host recognition by T cells is dependent not only on the total number of cells involved and the strength of the effector-target interaction, but also on the "context" in which such recognition occurs (underlying disease, preparative regimen, treatments). The case presented demonstrates that a single HLA-DP mismatch is able to initiate an *in vivo* alloreaction and therefore

contributes to that part of the pathological process which depends upon the specific T cell response.

It is now clear that GVHD is not the only consequence of host cells recognition by donor T lymphocytes. This alloreaction also has a beneficial effect termed graft-versus-leukemia reaction (GVL). Evidence for GVL comes mainly from clinical studies showing the balance between these two aspects of the allogeneic reaction: patients who experienced GVHD had fewer relapses than patients without GVHD, but only patients with a mild degree of GVHD had a survival advantage (22–24). Although GVH and GVL are both mediated by donor T lymphocytes, it is yet impossible to discriminate the subsets that are responsible for one or the other. As a consequence, most of the recent efforts to control the alloreaction (i.e., to keep the benefit from the GVL effect while avoiding the mortality and morbidity due to GVHD) have focused on the transplanted T cell as a whole (25); for example, some groups have considered the reinjection of graded doses of donor T cells (26), and more recently it was proposed to transduce donor

T cells with the herpes-simplex thymidine kinase (TK) gene before grafting (27). Because the viral TK is able to phosphorylate the nucleoside analogue ganciclovir, resulting in the production of metabolites toxic for mammalian cells, its transfer into T cells renders them sensitive to ganciclovir and allows their destruction through ganciclovir treatment (28). In line with these concerns, how can we take advantage of these two apparently paradoxical observations: HLA-DP incompatibility does not significantly influence the risk of acute GVHD (9, 10), yet HLA-DP specific T cells can be isolated from skin biopsies at the onset of GVHD each time an HLA-DP mismatch is present (11, 12, and this report). If an HLA-DP mismatch is able to induce an allogeneic response in vivo it should be able to trigger both a GVH and a GVL effect (as long as the leukemic cells express the HLA-DP antigens, of course). Indeed, we are currently considering the use of HLA-DPB1-specific T cell clones, transfected with the viral TK gene and directed against a HLA-DPB1 mismatch allele in the GVH direction (to spare the new hematopoiesis from the graft) to generate an allogeneic GVH-GVL effect in the context of a T cell-depleted BMT. Targeting an HLA-DP mismatch in the GVH direction to generate an allogeneic effect has two main advantages. Firstly, because 70% of HLA-A, -B, -DR identical BMT are HLA-DP mismatched, HLA-DP targeting would allow the consideration of a phase I clinical trial in an otherwise immunologically classic BMT. Moreover, although one would spontaneously tend to use autologous T cell clones for such an application, it may become possible to consider the use of third party specific T cell clones in this very particular clinical situation, because of the drastic immunodepression associated with a T cell-depleted BMT. In addition, immune recognition of the clone by T cells arising from the graft can also be diminished if the clones of interest are derived from individuals homozygous for widely represented HLA molecules such as HLA-A1, -B8, -DR3, -DQ2. If third party T cell clones prove useful, the second advantage comes from the fact that the five most frequent HLA-DPB1 alleles (HLA-DPB1*0401, 402, 101, 201, 301) cover > 80% of the Caucasian population. Consequently, a few clones would allow the treatment of a majority of patients. In line with this approach, we have recently demonstrated the feasibility of generating HLA-DPB1*0401-specific CD4+ cytotoxic T cell clones transfected by electroporation with a neutral vector containing the herpes-simplex TK gene. These clones retained their specificity, their function, and their sensitivity to ganciclovir treatment (29). In conclusion, we propose that such clones could be considered as potential therapeutic tools to drive and control a GVH-GVL reaction.

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References

- Ferrara, J.L.M., and H.L. Deeg. 1991. Graft-versus-host disease. *N. Engl. J. Med.* 324:667-674.
- Beatty, P.G., J.A. Hansen, G.M. Longton, E.D. Thomas, J.E. Sanders, P.J. Martin, S.I. Bearman, C. Anasetti, E.W. Petersdorf, E.M. Mickelson, et al.

1991. Marrow transplantation from HLA-matched unrelated donor for hematologic malignancies. *Transplantation (Baltimore)*. 51:443-447.
3. Ash, R.C., J.T. Casper, C.R. Chitambar, R. Hansen, N. Bunin, R.L. Truitt, C. Lawton, K. Murray, J. Hunter, L.A. Baxter-Lowe, et al. 1990. Successful allogeneic transplantation of T-cell depleted bone marrow from closely HLA-matched unrelated donors. *N. Engl. J. Med.* 322:485-494.
4. Anasetti, C., P.G. Beatty, R. Storb, P.J. Martin, M. Mori, J.E. Sanders, E.D. Thomas, and J.A. Hansen. 1990. Effect of HLA incompatibility on graft-versus-host disease, relapse and survival after marrow transplantation for patients with leukemia or lymphoma. *Hum. Immunol.* 29:79-87.
5. Beatty, P.G., R.A. Clift, E.M. Mickelson, B. Nisperos, N. Flournoy, P.J. Marin, J.E. Sanders, R. Storb, E.D. Thomas, and J.A. Hansen. 1985. Marrow transplantation from related donors other than HLA-identical siblings. *N. Engl. J. Med.* 313:765-771.
6. Hansen, J.A., C. Anasetti, P.G. Beatty, P.J. Martin, J.E. Sanders, R. Storb, and E.D. Thomas. 1990. Treatment of leukemia by marrow transplantation from HLA-incompatible donors. Effect of HLA-disparity on GVHD, relapse and survival. *Bone Marrow Transplant.* 6:108-111.
7. Storb, R., H.J. Deeg, J. Whitehead, F. Appelbaum, P.B. Beatty, W. Bensinger, C.D. Buckner, R. Clift, K. Doney, V. Farewell, J.A. Hansen, et al. 1986. Methotrexate and cyclosporine compared with cyclosporine alone for the treatment of acute graft versus host disease after marrow transplantation for leukemia. *N. Engl. J. Med.* 314:729-735.
8. Beatty, P.G., C. Anasetti, J.A. Hanse, G.M. Longton, J.E. Sanders, P.J. Marin, E.M. Mickelson, S.Y. Choo, E.W. Petersdorf, M.S. Pepe, F.R. Appelbaum, et al. 1993. Marrow transplantation from unrelated donors for treatment of hematologic malignancies: effect of mismatching of one HLA locus. *Blood.* 81:249-253.
9. Moreau, P., and A. Cesbron. 1994. HLA-DP and allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 13:675-681.
10. Petersdorf, E.W., A.G. Smith, E.M. Mickelson, G.M. Longton, C. Anasetti, S.Y. Choo, P.J. Martin, and J.A. Hansen. 1993. The role of HLA-DPB1 disparity in the development of acute graft-versus-host disease following unrelated bone marrow transplantation. *Blood.* 81:1923-1932.
11. Gaschet, J., B. Mahe, N. Milpied, M.C. Devilder, B. Dreno, J.D. Bignon, F. Davodeau, M.M. Hallet, M. Bonneville, and H. Vié. 1993. Specificity of T cell invading the skin during acute graft versus host disease after semi allogeneic bone marrow transplantation. *J. Clin. Invest.* 91:12-20.
12. Gaschet, J., M.A. Trevino, M. Chereil, R. Vivien, A.G. Sahuquillo, M.M. Hallet, M. Bonneville, J.L. Harrousseau, R. Bragado, N. Milpied, and H. Vié. 1996. HLA-target antigens and TCR diversity of activated T cells invading the skin during acute graft versus host disease. *Blood.* 87:2345-2353.
13. Gaschet, J., C. Denis, N. Milpied, M.M. Hallet, F. Davodeau, A. Necker, F. Romagne, M. Bonneville, and H. Vié. 1995. Analysis of T-cell receptor Vb expression among Va subsets. Evidence for tight regulation of the combinatorial diversity. *Ann. NY Acad. Sci.* 756:84-86.
14. Even, J., A. Lim, I. Puisieux, L. Ferradini, P.Y. Dietrich, A. Toubert, T. Hercend, F. Triebel, C. Pannetier, and P. Kourilsky. 1996. T cell repertoire in healthy and diseased human tissues analysed by T cell receptor b chain CDR3 size determination: evidence for clonal expansions in tumors and inflammatory diseases. *Res. Immunol.* 146:65-80.
15. Pannetier, C., S. Delassus, S. Darche, C. Saucier, and P. Kourilsky. 1993. Quantitative titration of nucleic acids by enzymatic amplification reactions run to saturation. *Nucleic Acids Res.* 21:577-583.
16. Pannetier, C., M. Cochet, S. Darche, A. Casrouge, M. Zoller, and P. Kourilsky. 1993. The size of the CDR3 hypervariable regions of the murine T cell receptor b chain vary as a function of the recombined germline segments. *Proc. Natl. Acad. Sci. USA.* 90:4319-4323.
17. Dietrich, P.Y., A. Caignard, A. Diu, C. Genevee, J.L. Pico, M. Henry-Amar, E. Angevin, F. Triebel, and T. Hercend. 1990. Analysis of T cell receptor variability in transplanted patients with graft-versus-host disease. *Blood.* 80:2419-2424.
18. Dietrich, P.Y., A. Caignard, Y. Lim Chung, J.L. Pico, C. Pannetier, P. Kourilsky, T. Hercend, and F. Triebel. 1994. In vivo clonal amplification at time of acute graft-versus-host disease. *Blood.* 84:2815-2820.
19. Goulmy, E., R. Shipper, J. Pool, E. Blokland, J.H.F. Falkenburg, J. Vossen, A. Gratwohl, G.B. Vogelsang, H.C. van Houwelingen, and J.J. van Rood. 1996. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N. Engl. J. Med.* 334:281-285.
20. Martin, P.J. 1991. Increased disparity for minor histocompatibility antigens as a potential cause of increased GVHD risk in marrow transplantation from unrelated donors compared with related donors. *Bone Marrow Transplant.* 8:217-225.
21. van Lochem, E.G., A. Bakker, S. Snijder, M. Aarts, G.C. de Gast, and E. Goulmy. 1995. Epstein-Barr virus infection abrogates the stimulatory capacity of B cells to a major histocompatibility complex class II-restricted proliferative T cell clone. *Hum. Immunol.* 42(2):137-144.
22. Apperley, J.F., F.R. Mauro, J.M. Goldman, W. Gregory, C.K. Arthur, J. Hawks, W. Arcese, G. Papa, F. Mandelli, D. Wardle, et al. 1988. Bone marrow transplantation for chronic myelogenous leukemia in first chronic phase: Importance of a graft-versus-leukemia effect. *Br. J. Haematol.* 69:239-246.

23. Weiden, P.L., K.M. Sullivan, N. Flournoy, R. Storb, and E.D. Thomas. 1981. The Seattle Marrow Transplant Team: antileukemic effect of chronic graft-versus-host disease. Contribution for improved survival after allogeneic marrow transplantation. *N. Engl. J. Med.* 304:1529–1532.
24. Horowitz, M.M., R.P. Gale, P.M. Sondel, J.M. Goldman, J. Kersey, H.J. Kolb, A.A. Rimm, O. Ringden, C. Rozman, B. Speck, et al. 1990. Graft-versus-leukemia reaction after bone marrow transplantation. *Blood.* 75:555–562.
25. Kolb, H.J., A. Schattenberg, J.M. Goldman, B. Hertenstein, N. Jacobsen, W. Arcese, P. Ljungman, A. Ferrant, L. Veronck, D. Niederwieser, et al. 1995. For the European group for blood and marrow transplantation. Graft-versus-leukemia effect of donor lymphocyte transfusion in marrow grafted recipients. *Blood.* 86:2041–2050.
26. MacKinnon, S., E.B. Papadopoulos, M.H. Carabasi, L. Reich, N.H. Collins, F. Boulal, H. Castro-Malaspina, B.H. Childs, A.P. Gillio, N.A. Kernan, et al. 1995. Adoptive immunotherapy evaluating escalating doses of leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood.* 86:1261–1268.
27. Tiberghien, P., C.W. Reynolds, J. Keller, S. Spence, M. Deschaseaux, J.M. Certoux, E. Contassot, W.J. Murphy, R. Lyons, Y. Chiang, et al. 1994. Ganciclovir treatment of herpes simplex thymidine kinase-transduced primary T lymphocytes: an approach for specific in vivo donor T-cell depletion after bone marrow transplantation. *Blood.* 84:1333–1341.
28. Borelli, E., R. Heyman, M. Hsi, and R.M. Evans. 1988. Targeting of an inducible toxic phenotype in animal cells. *Proc. Nat. Acad. Sci. USA.* 85:7572–7576.
29. Gallot, G., M.M.H. Hallet, J. Gasset, J.M. Moreau, R. Vivien, M. Bonneville, N. Milpied, and H. Vié. 1996. Human HLA-specific T cell clone with stable expression of a suicide gene: a possible tool to drive and control a GVH-GVL reaction. *Blood.* In press.