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Graft-versus-host disease associated T helper cell responses specific for minor histocompatibility antigens are mainly restricted by HLA-DR molecules

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Summary:

Graft-versus-host reactions are mediated by subpopulations of donor T cells and can be attributed to host specific minor histocompatibility (mH) antigens. We isolated strong anti-host mH antigen proliferative T cell lines, LG2, PN2, and LH3, from three patients suffering from acute graft-versus-host disease (GVHD). To study the role of the different major histocompatibility complex (MHC) molecules in the anti-host mH antigen specific proliferative response, the reactivities of the three T cell lines were analysed in primed lymphocyte test (PLT) assays against panels of stimulator cells obtained from unrelated blood donors. LG2 and LH3 stimulating determinants were commonly detected in the unrelated panel, whereas the PN2 T cell line recognized a rare specificity. The responses were associated with the presence of self HLA-DR molecules on the stimulator cells, although not all DR sharing stimulator cells were recognized. The proliferative responses of LG2, LH3 and PN2 cells could be blocked by monoclonal antibodies (MoAbs) against HLA-DR, but not by MoAbs against HLA-A/B/Cw, HLA-DQ or -DP. At the responder cell level, depletion of CD4 cells as well as blocking with CD4 specific MoAbs abrogated the specific responses of the three T cell lines. Our findings suggest that anti-host Th cell responses activated in the acute phase of GVHD are directed against both frequent and rare mH antigens, are mediated by CD4+ve class II restricted Th cells, and use the HLA-DR molecule as a common restriction element for mH antigen presentation.

Minor histocompatibility (mH) antigen disparities between donor and recipient may increase the risk of graft-versus-host disease (GVHD) after allogeneic HLA genotypically identical bone marrow transplantation (BMT)[1,3] In the anti-host T cell reaction, presumably directed against several mH antigens, both major histocompatibility complex (MHC) class I and II self antigens serve as presenting molecules for mH antigens to subpopulations of donor T cells MHC class I products function as major restriction molecules for mH antigen specific cytotoxic T cell (CTL) responses in patients suffering from GVHD[3–7] The role of MHC class II antigens, which are clearly induced on the target tissues of GVHD[8,9], as restriction molecules in the cellular anti-host response is as yet not definitely identified Reinsmoen *et al* reported that T cells isolated from affected skin lesions of patients with acute GVHD and specifically proliferating to host but not donor cells, were most probably restricted by products of the HLA-D region [10] Although the latter study included a small number of cases it indicated the functional role of both proliferative T lymphocyte (Th) cells and MHC class II antigens in local GVH reactions In line with this observation, we recently demonstrated in 16 patients that anti-host Th cell responses isolated from the patients' peripheral blood were significantly higher in patients having acute GVHD than in patients without GVHD [11] Assuming that these anti-host proliferative responses would be MHC class II restricted, we aimed in this study to define whether a particular MHC class II molecule dominated as the restriction determinant From three patients suffering from acute GVHD, T cell lines with proliferating capacity against host but not donor cells were established and the mH antigen specificity of these T cell lines was analysed in the patients' families and in the unrelated population Using monoclonal antibodies (MoAbs) specific for HLA-DR, -DQ, or -DP molecules and highly purified T cell subsets obtained by antibody-coated magnetic beads, we identified the major phenotype and MHC restriction determinant usage of the Th cells activated in the acute phase of GVHD

Materials and methods

Patients

Patients LG, PN and LH underwent non-T cell-depleted BMT from their HLA-identical MLC non-reactive sibling donors as treatment for acute myeloid leukemia The preparative regimen was total body irradiation (8 Gy) and cyclophosphamide (60 mg/kg/ × 2) To prevent acute GVHD, patients received cyclosporin A (CSA), started on day −1 as a continuous infusion (3 mg/kg/day) followed by oral administration (9 mg/kg/day) The dose of CSA was adjusted according to the clinical course, renal function and plasma concentration, tapered off slowly over a period of months, and discontinued at 3–4

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months after BMT Acute GVHD was diagnosed at day 12 for patient LG (grade III), at day 11 for patient PN (grade III), and at day 12 for patient LH (grade I), and was treated with high dose methylprednisolone

Blood samples

Heparinized blood samples were obtained from patients before and after BMT, from sibling marrow donors, family members and unrelated healthy blood donors Peripheral blood lymphocytes (PBL) were isolated by Ficoll–Isopaque density gradient centrifugation, washed and resuspended in 10% RPMI–1640–dimethylsulfoxide for cryopreservation in liquid nitrogen The HLA typing of the three patients was as follows, patient LG HLA-A1, -A3, -B7, -B8, -Cw7, -DR2, -DR3, -DQw1, -DPw4, patient PN HLA-A3, -A32, -B7, -B44, -Cw7, -DR2, -DQw1, -DQw2, -DP4, and patient LH HLA-A2, -A3, -B7, -B37, -Cw6, -Cw7, -DRw6, -DRw10, -DPw2 Oligonucleotide typing for HLA-DR2 subtypes Dw2, Dw12 and Dw21 was performed by techniques essentially as described by Kenter *et al* [12] The DR2 B5 genes, carrying the allele specific sequences of Dw2, Dw12 and Dw21 were amplified using a set of selected primers Hybridization occurred with the following oligonucleotides, DRB5001, specific for nucleotide residues 109–128 (DRB5) of Dw2, Dw12 and Dw21, DRB5002, specific for nucleotide residues 190–209 (DRB5) of Dw2, and DRB5003, specific for nucleotide residues 161–180 (DRB5) of Dw12 and Dw21

Tissue culture medium

The cell cultures were performed in RPMI–1640 supplemented with antibiotics (gentamicin) and either 15% heat inactivated human serum (T cell lines and proliferation assays) or 10% fetal calf serum (Epstein–Barr virus transformed B cell lines [EBV-LCL])

T cell lines

Anti-host proliferative T cell lines 'LG2, PN2, and LH3' were initiated with PBL obtained from patient LG at 90 days post-BMT, from patient PN at 43 days post-BMT, and from patient LH at 92 days post-BMT The cells were used as responder cells and were cultured with 30 Gy irradiated patients' pre-BMT PBL at a ratio of 1×10^6 post-BMT PBL for 1×10^6 irradiated pre-BMT PBL At day 6, these cells were specifically restimulated with pre-BMT PBL in the presence of 2% highly purified IL-2 (Lymphocult-HP, Biotest) Thereafter, the T cell lines were grown by weekly addition of specific feeder cells alternated by fresh medium containing 15% IL-2 (Lymphocult-T, Biotest)

T cell clones

Three anti-HLA-class II reactive T cell clones specific for HLA-DR2 (clone 2616), HLA-DQw1 (clone 2604) and HLA-DPw3 (clone 2712), kindly provided by Dr A Termijtelen, were used as control reagents in blocking assays

Proliferation assay

Responder cells, $5–10 \times 10^3$, were cultured with 10^5 20 Gy irradiated PBL or with 2.5×10^4 75 Gy irradiated EBV-LCL in flat bottom microtiter plates for 64 h Sixteen hours before harvesting the cultures were labeled with 1 μCi tritiated thymidine Isotope incorporation was measured in a liquid scintillation counter The results (i e mean c p m of triplicate cultures) are either expressed as % of relative response (RR),

$$\%\ RR = \frac{c\ p\ m\ experimental - c\ p\ m\ of\ responders\ alone}{c\ p\ m\ in\ presence\ of\ host\ cells - c\ p\ m\ of\ responders\ alone} \times 100\%$$

or as stimulation index (SI)

$$SI = \frac{c\ p\ m\ experimental}{c\ p\ m\ responders\ alone + c\ p\ m\ stimulators\ alone}$$

SI equal to or greater than 3 are considered as positive

Monoclonal antibodies

The antibodies OKT3, OKT4, and OKT8 react with CD3, CD4, and CD8 T cell markers respectively (purchased from Ortho) The antibody W6/32 (obtained from Sera-Lab Ltd, Sussex) recognizes an HLA-class I monomorphic determinant The antibodies PdV5 2, and B8 11 2 were produced in our department and are specific for a conformational determinant of HLA-DR/DQ/DP and HLA-DR respectively [13] Antibody SPV-L3 is reactive with HLA-DQ (kindly provided by Dr H Spits, DNAX, Palo Alto, CA), antibody B7/21 is specific for HLA-DP (Beckton and Dickinson)

Fluorescence analysis

T cell lines were stained for CD3, CD4, and CD8 expression by a standard double immunofluorescence technique, using phycoerythrin or fluorescein isothiocyanate conjugated CD3 (Leu4), anti-CD4 (Leu3a), and anti-CD8 (Leu2a) monoclonal antibodies The samples were assayed on a fluorescence activated cell sorter

Separation of T cell subsets

CD4 and CD8 T cell subsets were separated by positive and negative selection using antibody-coated monosized magnetic microspheres (Dynabeads, Dynal) [14] Briefly, $2–3 \times 10^6$ T cells were incubated with CD4- or CD8-coated beads at a beads to cell ratio of 20 1 at 4°C and left for 2 h while rotating Then, positive and negative fractions were obtained using a magnetic device Positively selected cell fractions were used in the primed lymphocyte test without interference of attached beads [15]

Results

Generation of LG2, PN2, and LH3 cells in acute phase of GVHD

T cell lines LG2, PN2 and LH3 were induced from three patients suffering from acute GVHD by stimulation of

Table I Proliferative responses of LG2, PN2, and LH3 cells in the presence of stimulator cells collected from specific hosts, bone marrow donors, related and unrelated blood donors

Cell line	*Stimulator cells[a] from*	*c p m* ± SD	*RR[b]*
LG2	Bone marrow recipient LG	6322 ± 1075	*100*
	Bone marrow donor DG	100 ± 20	−2
	HLA-haploidentical sibling CG	19 886 ± 3579	*323*
PN2	Bone marrow recipient PN	10 217 ± 1533	*100*
	Bone marrow donor JN	100 ± 13	−1
	HLA-haploidentical sibling GN	23 273 ± 233	*230*
LH3	Bone marrow recipient LH	45 084 ± 496	*100*
	Bone marrow donor DH	717 ± 93	1
	HLA-identical blood donor RV	10 255 ± 103	*22*

[a]Stimulator cells were EBV-LCL [LG, DG, PN, LH, DH] or PBL [CG, GN, RV]
[b]% relative response The positive reactions (RR > 20%) are in italics

their post-BMT lymphocytes with irradiated patients' pre-BMT cells as outlined in Materials and Methods. After the third and following stimulations, the T cell lines showed specific proliferative responses in PLT assays in the presence of pre-BMT patients' cells without proliferating significantly in the presence of cells of their bone marrow donors (Table I). As indicated in Table I, significant responses of LG2 and PN2 cells could also be induced with cells from an HLA haplo-identical sibling CG (LG2) and GN (PN2); LH3 cells responded in the presence of cells from an unrelated HLA-A, -B, -Cw, -DR matched donor RV.

Anti-host Th cell lines proliferate in the presence of allogeneic DR-sharing stimulator cells

Since pre-BMT patients' cells were not the only cells stimulating LG2, PN2, and LH3, further assays were performed to define the MHC restriction determinants and specificities of these Th cell lines. LG2, PN2, and LH3 cells were tested against a panel of more than 30 healthy unrelated donors. The results (Table II) show that among the 20 donors who share HLA-DR2, or -DR3 with LG2, 13 could stimulate these cells. Although no self HLA specificities other than HLA-DR (i.e. HLA-A, -B, -Cw, -DQ, -DP) were found to be associated with positive responses of LG2 (not shown), two out of 11 non-DR2 or -DR3 positive donors (i.e. JM and HA) were able to stimulate LG2 cells. The latter two stimulator cells shared DPw4 with LG2.

As is shown in Tables III and IV, only HLA-DR matched panel donors were able to stimulate PN2 and LH3 cells. Of the 18 donors sharing DR2 with PN2 cells only three were stimulatory; non-DR-sharing cells ($n = 18$) did not stimulate (Table III). A higher panel reactivity was found for LH3 cells when tested against 24

Table II Frequency analysis and MHC restriction element usage of LG2

Stimulations			*HLA-DR*	*SI[a]*
A	Controls	Patient LG ($n = 6^b$)	2,3	*27 4 ± 10.8*
		Donor DG ($n = 6$)	2,3	0 8 ± 0.2
B	DR sharing	KL	*2*,w6[c]	*26 6*
		KV	*2*,5	*20.5*
		ST	*3*,w6	*127 6*
		PH	*2*	141 6
		HE	1,*2*	*100 3*
		GO	*2*,w6	*20 4*
		GI	*3*,4	*10 1*
		GJ	*3,3*	*5 2*
		DB	*2*,w6	*12 7*
		NY	*2*,W6	*305 8*
		JE	*3*,4	*122 2*
		BL	1,*3*	*16 7*
		AA	*3*,w6	*10.5*
		$n = 5^d$	*3*	1 4 ± 0 8
		$n = 2$	*2*	0 6 ± 0 5
C	DR non-sharing	JM	7,8	10 7
		HA	1,w10	*38.3*
		$n = 9$	non 2,3	1 8 ± 1 1

[a]Stimulation index The positive reactions (SI > 3) are in italics
[b]Number of experiments
[c]HLA-DR sharing with LG2 cells is in italics
[d]Number of panel cells tested

Table III Frequency analysis and MHC restriction element usage of PN2

Stimulators			*HLA-DR*	*SI*[a]
A	Controls	Patient PN ($n=4$)[b]	2 (Dw2)[c]	*7 6±2 1*
		Donor LN ($n=4$)	2 (Dw2)	0 7±0 2
B	DR sharing	KL	*2* (Dw2),w6[d]	*5 8*
		MB	*2* (Dw2),w6	*58 3*
		MA	*2* (Dw2),w10	*14 0*
		$n=15$[e]	*2* (Dw2)	0 8±0 4
C	DR non-sharing	$n=18$	Non 2	0 6±0 3

[a]Stimulation index The positive reactions (SI > 3) are in italics
[b]Number of experiments
[c]The specificities in brackets were determined by oligonucleotide typing
[d]HLA-DR sharing with PN2 cells is in italics
[e]Number of panel cells tested

DR-sharing donors; 11 of the DRw6- or DRw10-sharing donors stimulated LH3 cells, 14 non DR-sharing donors were negative (Table IV).

LG2 cells and PN2 cells discriminate between HLA-identical siblings in the patients' families

To further analyse the stimulating determinants, LG2 and PN2 cells were tested against cells from patients' families (no cells were available from family members of patient LH). As can be deduced from patient LG's pedigree (Figure 1), LG2 cells discriminated between various HLA-identical siblings. Thus, LG2 cells specifically recognized determinant(s) from patient LG (02) but not from donor DG (03, haplotypes a/c), from sibling 05 but not from sibling 06 (haplotypes a/d), and from patient's child 52 but not 53 (haplotypes a/r). Cells of the patient's mother (00, a/b)) did not stimulate. Paternal cells were not available for testing.

The reactivity of PN2 cells in the patient's family is shown in Figure 2. As is clear from this pedigree, PN2 cells discriminated between HLA-identical siblings 02 and 04 (patient and donor, haplocytes b/c), between siblings 06 and 07 (haplotypes b/d), and between siblings 03, 05, and 09 (haplotypes a/c). The maternal cells (00, a/b)) were stimulatory as well. Cells of the father were not available.

LG2, PN2, and LH3 Th cell responses are blocked by anti HLA-DR and anti-CD4 monoclonal antibodies

Several MoAbs specific for T cell markers CD3, CD4 and CD8, and specific for HLA-A/B/Cw, -DR, -DQ or -DP backbone structures were tested for their capacity to inhibit the responses of LG2, PN2 and LH3 cell lines. The MoAbs (1:100, 1:400; 1:1600) as well as the responder cells were tested at different concentrations (data not shown). From representative experiments

Table IV Frequency analysis and MHC restriction element usage of LH3

Stimulators			*HLA-DR*	*SI*[a]
A	Controls	Patient LH ($n=2$)[b]	w6,w10	*7 2±1 1*
		Donor DH ($n=2$)	w6,w10	1 7±1 0
B	DR sharing	JB	2,*w6*[c]	*7 8*
		IZ	4,*w10*	*35 4*
		ST	3,*w6*	*4 0*
		BR	3,*w6*	*4 0*
		BD	7,*w10*	*11 2*
		TU	5,*w10*	*11 2*
		GU	5,*w6*	*11 6*
		RV	*w6,w10*	*25 0*
		VD	4,*w6*	*9 2*
		GO	2,*w6*	*5 1*
		NK	5,*w6*	*10 3*
		$n=10$[d]	*w6*	1 3±0 9
		$n=3$	*w10*	1 6±0 4
C	DR non-sharing	$n=14$	Non w6,w10	1 5±1 1

[a]Stimulation index The positive reactions (SI > 3) are in italics
[b]Number of experiments
[c]HLA-DR sharing with LH3 cells is in italics
[d]Number of panel cells tested

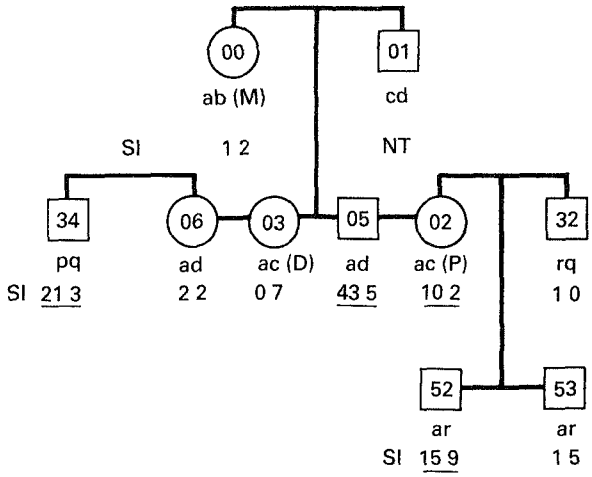

Figure 1 Proliferation pattern of LG2 cells in patient LG's family Underlined responses represent positive reactions (SI > 3) P, patient, D, marrow donor M, mother The HLA haplotypes are as follows a, A1,B8,DR3, b, A29,B44,DR8, c, A3,B7,DR2, d, A1,B7,DR2, p, A3,B7, DR2,q, A1,B8,DR3,r, A2,B44,DR4

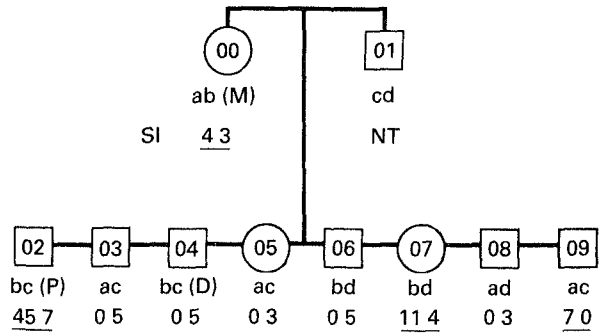

Figure 2 Proliferation pattern of PN2 cells in patient PN's family Underlined responses represent positive reactions (SI > 3) P, patient, D, marrow donor M, mother The HLA haplotypes are as follows a, A29,B44,DR7,b, A32,B44,DR2,c, A3,B7,DR2,d, A3,B44,DR5

shown in Table V we can conclude that the OKT3 antibody (CD3) was a potent inhibitor of LG2, PN2, and LH3 responses, as well as the antibodies OKT4 (CD4), PdV5 2 (HLA-DR, -DQ, -DP), and B8 11 2 (HLA-DR) No blocking of LG2, PN2, and LH3 responses was observed in the presence of the antibodies OKT8 (CD8), w6/32 (HLA-A, -B, -Cw), SPV-L3 (HLA-DQ) and B7/21 (HLA-DP)

LG2 and PN2 proliferative responses are mediated by the CD4+ population

FACS analysis of the LG2 and PN2 cultures revealed fluctuations in their CD4/CD8 subset constitution, depending on the number of restimulation cycles of the lines To define the T cell subset responsible for the proliferative activity, T cell subset depletion studies using CD4 or CD8 antibody coated dynabeads were performed Separation using CD8 coated beads yielded in all cases 98–100% pure populations of CD4+ cells as the negatively selected fraction In the positively selected fraction, thus containing all the CD8+ cells, a variable non-specific binding of CD4+ cells could be observed (1–29%), which appeared to depend on the viability of the cell cultures Representative experiments using CD8 coated beads (Table VI, expts 1–5) indicate that the proliferative responses of PN2 and LG2 could be attributed to the highly pure negatively isolated CD4+ fractions and not to the CD8 enriched positively selected fractions, even in a case where the unseparated cell line was not itself responsive (expt 4) To exclude the possibility that CD8+ cells did not respond due to the presence of the dynabeads, we also performed a cell separation with CD4 coated dynabeads (expt 5) The proliferative response of the CD4+ fraction was not inhibited by the presence of the beads, whereas no response of the CD8+ fraction was found in the absence of beads

Table V Inhibition of proliferation of Th cell lines LG2, PN2 and LH3

		Responder cells					
		LG2		*PN2*		*LH3*	
Monoclonal antibody	*Specificity*	*Expt 1*[a]	*Expt 2*	*Expt 3*	*Expt 4*	*Expt 5*	*Expt 6*
None	–	100[b]	100	100	100	100	100
OKT3	CD3	*8*	*5*	*20*	—	*9*	*7*
OKT4	CD4	*50*	*47*	*11*	*18*	*16*	*31*
OKT8	CD8	114	139	110	96	89	102
w6/32	HLA-A, -B, –Cw	76	124	84	80	81	84
PdV5 2	HLA-DR, –DQ, -DP	*21*	—	*13*	*6*	—	—
B8 11 2[c]	HLA-DR	*17*	*29*	*3*	*1*	*19*	*16*
SPV L3[c]	HLA-DQ	111	146	106	103	77	86
B7/21[c]	HLA-DP	115	—	97	114	—	96

[a]Proliferation was measured in the presence of specific stimulator cells i e EBV-LCL from patient LG (Expts 1,2), PBL from blood donor MA (Expts 3, 4), and EBV-LCL from patient PN (Expts 5, 6)

[b]Relative responses measured in the absence of MoAbs (100%) were 41 580 c p m (Expt 1), 21 797 c p m (Expt 2), 14 481 c p m (Expt 3), 14 682 c p m (Expt 4), 45 657 c p m (Expt 5), and 38 100 c p m (Expt 6) RR < 75% in the presence of MoAbs (1 300) represent inhibition (in italics)

[c]The MoAbs SPV-L3 and B7/21 significantly inhibited the proliferative responses of two control alloreactive T cell clones specific for HLA-DQ and HLA DP respectively, whereas MoAb B8 11 2 did not aspecifically block these clones (data not shown)

Table VI Functional analysis of PN2 and LG2 isolated T cell subsets

Expt	*Cell line*	*Method of selection*	*CD4/CD8*	*SI*[b]
11	PN2	None	70/29[a]	*28 2*
		CD8 beads + fraction	1/95	0 6
		CD8 beads − fraction	98/2	*25 3*
2	PN2	None	84/16	*6 1*
		CD8 beads + fraction	7/94	0 9
		CD8 beads − fraction	99/< 1	*7 4*
3	LG2	None	83/18	*10 2*
		CD8 beads + fraction	29/71	0 8
		CD8 beads − fraction	100/0	*20 7*
4	LG2	None	15/85[c]	1 3
		CD8 beads + fraction	2/97	1 0
		CD8 beads − fraction	99/1	*29 3*
5	LG2	None	93/7	*9 3*
		CD8 beads + fraction	19/81	1 5
		CD8 beads − fraction	100/0	*5 4*
		CD4 beads + fraction	95/5	*5 0*
		CD4 beads − fraction	10/90	1 1

[a]Percentage of positive cells
[b]Stimulation index Responses are measured against host EBV-LCL as stimulator cells Responses in italics represent positive reactions (SI > 3)
[c]Remarkable predominance of CD8 + cells

Discussion

The Th cell lines LG2, PN2 and LH3 were generated from the PBL of three patients suffering from acute GVHD after HLA genotypically identical BMT These T cell lines proliferated specifically in the presence of recipient but not of donor cells Since the LG2, PN2 and LH3 cells were initiated between patients' post-transplant (i e donor derived) cells as responders and patients' own pre-transplant cells as stimulators, these T cell lines are almost certainly directed against non-HLA or mH antigens The finding that LG2 and PN2 cells discriminated between various HLA-identical siblings in the patients' families is in agreement with the latter supposition The male specific mH antigen H-Y seemed not to be involved in LG2, PN2 or LH3 recognition, since female as well as male stimulator cells were recognized (data not shown)

The reactivity patterns of LG2, PN2 and LH3 cells against a panel of unrelated donors indicated that some, but not all, HLA-DR matched donors were stimulatory (Tables I–IV) The role of HLA-DR was further confirmed by inhibition studies using MoAbs (Table V), only the HLA-DR specific MoAb B8 11 2 and the class II backbone specific MoAb PdV5 2 could block the LG2, PN2 and LH3 responses, whereas MoAbs against HLA-DQ or -DP were inactive On the responder cell level the HLA-DR restricted mH antigen specific responses seemed to be mediated exclusively by CD4 + ve T cells This was concluded from the inhibitory effect of anti-CD4 MoAbs (Table V) and of physical elimination of CD4 + ve cells using magnetic beads (Table VI)

The mH antigen determinants defined by LG2 and LH3 cells were commonly detected in the HLA-DR matched panel (65% and 46% respectively) In contrast, the PN2 cells recognized only 17% of the HLA-DR2 matched panel donors HLA-DR2 can be divided into Dw2, Dw12 and Dw21 subspecificities To test the possibility that PN2 cells used a non-frequent Dw subtype as a restriction molecule, DR2 subtyping was performed using DR2 subtype specific oligonucleotides It was found that PN2 cells as well as the majority of the panel donors expressed the common Dw2 subtype Thus, PN2 cells use HLA-Dw2 as a restriction molecule and are specific for (a) non-frequent mH antigen(s)

The usage of HLA-DR as a restriction determinant for anti-host proliferative T cells isolated from GVHD skin lesions was reported earlier by Reinsmoen *et al* [10] The HLA-DR restriction molecules identified in our study were DR2 (LG2, PN2), DR3 (LG2), DRw6 (LH3), and DRw10 (LH3) Other HLA-DR specificities reported to be involved in mH antigen Th cell responses include HLA-DR2,[16] -DR3,[10 16] -DR4,[5] and -DR5 [17] These accumulating results point at a rather broad usage of HLA-DR alleles to present mH antigens to Th cells Nevertheless, a possible 'minor' role for other class II molecules cannot be excluded An earlier study suggested that the HLA-DP locus could restrict mH antigen responses [10] We observed that LG2 cells were stimulated by the HLA-DR2 and -3 negative panel donors JM and HA (Table II), suggesting a role for HLA-DPw4 However, the host specific proliferative response of LG2 cells could not be blocked by the HLA-DP specific MoAb B7/21 One possible explanation could be that not HLA-DP antigens but other as yet non-identified class II determinants may restrict the latter response Another option is that HLA-DP restricted cells form but a relatively small part of the LG2 effector cell population, blocking of which by anti-HLA-DP MoAbs might not significantly affect the overall response Studies at the clonal level are currently being undertaken to discriminate between these possibilities

Anti-mH antigen Th cells are poorly documented in GVHD Previous *in vitro* studies on GVH reactions

mainly emphasized the role of class I restricted mH antigen-specific CTL.[3-7] Recently, however, we found evidence suggesting that, probably in addition to CTL activity, ongoing anti-host Th cell activity may be a risk factor for acute GVHD.[11,18] Here we show that in three patients the former reactivity could be mainly attributed to the recognition of host mH antigens by CD4+ ve post-BMT recipient cells, and that HLA-DR sharing is a prerequisite for this response.

The possible significance of our findings may relate to recent observations that HLA-DR, and also -DQ and -DP molecules are strongly induced on keratinocytes and enterocytes in acute GVHD.[8,9,19] Therefore, these cells could act as antigen presenting cells in GVHD. So far, however, *in vitro* studies on allo- and hapten specific T cell activation in mouse and man have not been able to settle this issue because class II positive keratinocytes either failed to function,[20,21] functioned poorly,[22,23] or could only function after stimulation with interferon gamma[24] as antigen presenting cells. In view of our present findings, it is tempting to propose that through the induction of HLA-DR on the target tissues of GVHD, mH antigens are presented to the patient's immune system. Hence, local HLA-DR expression may play a role in the perpetuation of mH antigen specific Th cells. Yet, very little is known about the identity of the local target and effector cells involved in GVHD. Further studies unravelling the cellular mechanisms of GVHD are needed to define the role of HLA-DR restricted Th cell responses to mH antigens.

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