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BRIEF COMMUNICATION

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Dendritic cells induce HLA-DP-specific T-cell proliferation between MLR-negative siblings

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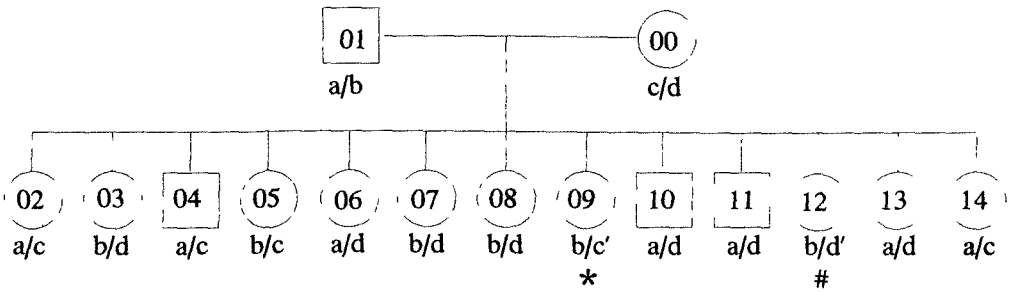

Fig. 1 Pedigree and *HLA* genotypes of the family members. *HLA* haplotypes: *a: A2, B35, Cw4, DR12, DQ1, DPB1*0402; b: A2, B35, Cw4,* DR11, *DQ3, DPB1*0401; c: A2, B44, Cw5,* DR8, *DQ4, DPB1*1901; d: A11, B51, Cw2,* DR2, *DQ1, DPB1*0401; c'*: A2, B44, *Cw5, DR8, DQ4, DPB1*0401; d': A11, B51, Cw2, DR2, DQ1, DPB1*1901.* * 09: a recombination occurred between the maternal *DQ* and *DP* loci in the *c'* haplotype. # 12: a recombination occurred between the maternal *DQ* and *DP* loci in the *d'* haplotype

The MHC genes encoding class II HLA-DP antigens map centromerically of the *HLA-DQ* loci on the short arm of chromosome six (Shaw et al. 1981). These HLA-DP antigens have originally been identified by cellular typing reagents (Shaw et al. 1980; Pawelec et al. 1982). At present, different molecular genotyping techniques are available to explore the *DPB1* locus polymorphism. More allelic sequences are currently recognized for the *DPB1* locus than for the *DQA1* or *DQB1* loci. *DPB1* is herewith the second most polymorphic MHC class II gene after the *DRB1* locus (Marsh and Bodmer 1994). HLA-DP molecules can function as restriction molecules in antigen presentation (Eckels et al. 1983). They have been indicated to act as transplantation antigens in kidney allografting (Bonneville et al. 1988). In bone marrow transplantation, incompatibilities for HLA-DP between HLA-matched bone marrow donor and recipient have been shown to correlate with acute graft versus host disease (Odum et al. 1987; Eiermann et al. 1992). HLA-DP disparities can be detected following *secondary* T-cell stimulation protocols. The contribution of HLA-DP products to stimulation in a *primary* mixed leucocyte reaction (MLR) is, however, still debatable. In the absence of strong immunogenic HLA-DR differences, the effect of HLA-DP differences on the MLR has been reported to be weak or negligible by some authors (Shaw et al. 1980; Eiermann et al. 1992) but rather significant by others (Schroeijers et al. 1988; Olerup et al. 1990).

To elaborate on the assumption that the latter discrepancy could lie on the level of the antigen presenting cell (APC), we investigated the stimulatory capacity of dendritic cells (DC) in a primary MLR between an MLR-negative HLA-DP-disparate sibling pair. DC are known to be strong APC (Knight et al. 1987) and are highly efficient in the initiation of primary in vitro T-cell responses such as in allogeneic MLR (Steinman et al. 1978). Here we describe the induction of an HLA-DP-specific proliferative T-cell response which could not be detected either in a primary or in a secondary MLR.

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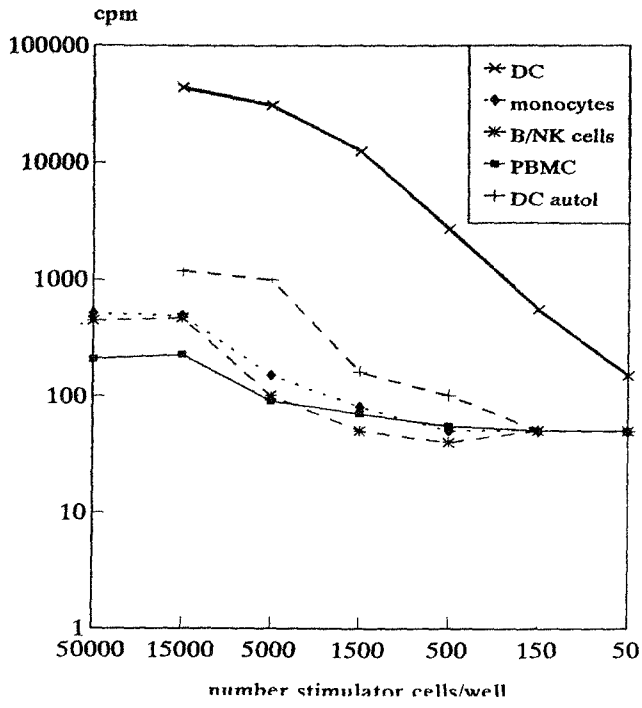

Fig. 2 MLR with T cells of 09 as responder cells and different APC of 05 as stimulator cells. The stimulatory capacity of different APC (PBMC, B/NK cells, monocytes, and DC) of individual 05 was compared in a MLR. Concentrations of 5×10^4 to 50 cells/well were used to stimulate 5×10^4 responder T cells of individual 09. ^{3}H-Thymidine incorporation was measured after 5 days of culture. The relative response in a standard MLR between 05 and 09 was 1%

A family of 13 siblings and their parents were typed for HLA-A, -B, -C, -DR, and -DQ by complement-dependent microcytotoxicity and for HLA-DP by sequence-specific oligonucleotide typing on polymerase chain reaction (PCR)-amplified DNA (PCR-sequence-specific oligonucleotides (SSO); Fig. 1). Both serological and PCR-SSO typing were performed twice, using different blood samples from each individual. The PCR-SSO typing for *DPB1* could be confirmed by sequencing of amplified DNA with *DPB1*-specific primers (Versluis et al. 1993). PCR-SSO typing and sequencing for *DPB1* revealed recombinations between maternal *HLA-DP* and *-DQ* loci both for sibling 09 and sibling 12 (Table 1). The *HLA-DQ:DP* recombination for sibling 09 resulted in an HLA-DP disparity between sibling 05 and 09. Since this disparity for HLA-DPB1*1901 did not give rise to any reactivity in a standard MLR (data not shown), we evaluated the capacity of DC to initiate primary DPB1*1901-specific T-cell responses. Hereto peripheral blood DC were enriched from individual 05 and 09 according to the method described by Freudenthal and Steinman (1990) with minor modifications. Monocytes and B/NK cell fractions were also rescued in order to compare the antigen presenting capacity of DC versus other APC. All fractions were analyzed on a FACScan (Becton Dickinson Mountain View, CA) for purity with monoclonal antibody (mAb) to lineage-specific markers. Purified monocytes were >95% CD14+, B/NK cell fractions contain 60% CD19+ and 35% CD16+/CD56+ cells. Expression of high levels of MHC class II molecules in

Table 1 DPB1 PCR-SSO typing of the family on PCR-amplified DNA

			Sequence-specific oligonucleotides[a]																						
			2	0	0	0	0	3	3	5	5	5	5	6	6	6	6	7	7	7	8	8	8
			4	9	9	9	9	5	5	5	5	5	5	5	9	9	9	6	6	6	5	5	5
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			1	1	2	3	4	1	2	1	2	3	4	2	2	3	6	1	2	3	1	2	3
00[b]	*DPB1*0401*[c]	*DPB1*1901*	+	+	0	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	+	0	+
		*DPB1*0401*	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0
		*DPB1*1901*	+	+	0	0	0	0	+	0	0	+	0	0	+	0	0	0	0	+	0	0	+
01	*DPB1*0401*	*DPB1*0402*	+	+	0	0	0	+	+	+	+	0	0	0	0	0	+	+	0	0	+	0	0
A	*DPB1*0401*	*DPB1*0402*	+	+	0	0	0	+	+	+	+	0	0	0	0	0	+	+	0	0	+	0	0
B			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
05	*DPB1*0401*	*DPB1*1901*	+	+	0	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	+	0	+
A		*DPB1*0401*	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0
B		*DPB1*1901*	+	+	0	0	0	0	+	0	0	+	0	0	+	0	0	0	0	+	0	0	+
08		*DPB1*0401*	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0
A		*DPB1*0401*	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0
B			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
09		*DPB1*0401*	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0
A		*DPB1*0401*	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0
B			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	DPB1*0401	*DPB1*1901*	+	+	0	0	0	+	+	+	0	+	0	0	+	0	+	+	0	+	+	0	+
A		*DPB1*0401*	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0
B		*DPB1*1901*	+	+	0	0	0	0	+	0	0	+	0	0	+	0	0	0	0	+	0	0	+

[a] Oligonucleotides used for typing (Histocompatibility Workshop and Conference 1991); + = positive, 0 = negative with oligonucleotide

[b] Designation of the different individuals of the family (see Figure 1)

[c] Generic amplification

[d] Group-specific amplification, using polymorphism at amino acid position 85 of the second exon of the *DPB1* gene

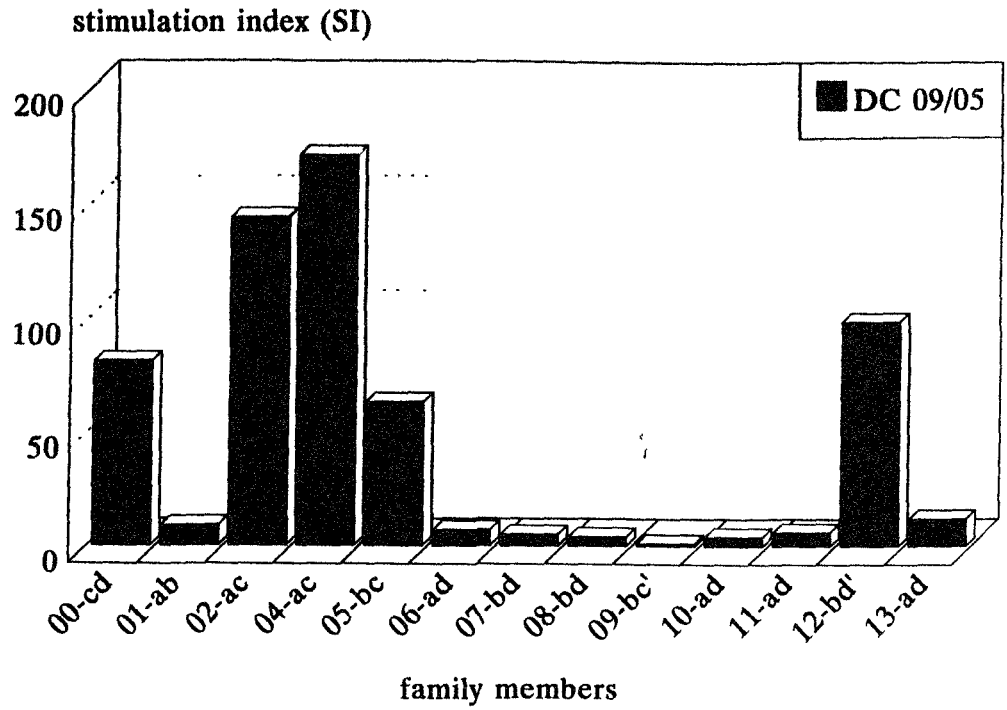

Fig. 3 HLA-DPB1*1901-specific proliferation. PBMC of family members (00 and 01: mother and father, respectively, 02-13: children) were tested in a PLT for stimulation of T-cell line DC 09/05. PBMC of sibling 03 were not available for testing as stimulator cells. Responder T cells (1×10^4) were cocultured with 1×10^5 3000 rad irradiated PBMC for 64 h. One μCi ^{3}H-thymidine was added to the cultures for a period of 16 h. Specific proliferation was expressed in stimulation indices (SI); SI = *cpm experimental* [cpm responder cells alone + cpm stimulators cells alone] SI <4 was considered as negative; SI ≥4 was considered as positive. *HLA* haplotypes of the family members are depicted in Figure 1

addition to the absence of lineage specific markers were used to distinguish the DC (Knight et al. 1987). DC-enriched fractions were more than 50% pure (data not shown).

Unseparated peripheral blood mononuclear cells, monocytes, B/NK cells, and DC were subsequently used as stimulator cells in an MLR at concentrations varying from 5×10^4 to 50 stimulator cells/well. As shown in Figure 2, T cells of sibling 09 responded to sibling 05 only when DC were used as stimulator cells. Even in the highest concentration, none of the APC but the DC were capable of inducing T-cell proliferation between this sibling pair (Fig. 2).

In order to confirm the HLA-DPB1*1901 specificity of the DC-induced T-cell response, a T-cell line designated DC09/05 was generated by restimulating the responding T cells with PBMC of sibling 05 in the presence of 1% leucoagglutinin and 20 units/ml rIL-2. T-cell line DC09/05 displayed significant proliferative activity in the primed lymphocyte test (PLT) assay to PBMC of all HLA-DPB1*1901-positive family members (i.e., 00, 02, 04, 05, 12, 14: Fig. 3). The *HLA-DQ:DP* recombination for sibling 12 resulted in the expression of HLA-DPB1*1901 and consequently the recognition by T-cell line DC09/05. None of the HLA-DPB1*1901-negative individuals of the family were recognized by T-cell line DC09/05. Moreover,

Table 2 HLA-DPB1*1901-specific proliferation of DC09/05 (panel study)

Ind[a]	HLA types[b]						cpm[c]
	A	B	C	DR	DQ	DPB1	
Al	1,2	7,8	w7	15,3	2,6	*0401*	113
Ba	2,11	51,55	w3	15,9	6,9	*0301, 0501*	336
Bo	3	7,62	w3, w7	15	1	*0501*	476
Eg	1,3	8,62	w3, w7	3,13	2,6	*0601*	259
El	11,31	62,57	w3, w6	4,7	7,9	*0201, 0401*	365
Es	1,2	18,27	w2	14,8	4,5	*0301, 0401*	142
Gr	2,3	7,6	w7	1	5	*0301, 0401*	128
Ka	1,32	8,44	w7	3,7	2	*0401, 1101*	114
Kl	2,24	38,35	w3	15,13	6	*0401*	295
Ko	24	39,27	w3	4,12	7	*0401, 0501*	139
Kr	2,3	27,37	w2, 26	4,10	5,8	*0601, 1901*	20419
Mu	1,3	7,8	w7	2,3	1,2	*0401, 0601*	62
Na	1,2	8,44	w7	15,3	2,6	*0401, 1301*	504
St	2,30	7,58	w7	1,14	5	*0402, 1901*	8487

[a] Designation of the healthy unrelated individuals whose PBMC were used as stimulator cells in a standard PLT with DC09/05 as responder
[b] All individuals were serologically typed for HLA-A, -B, -C, -DR, and -DQ; and by PCR-SSO typing for HLA-DPB1
[c] Proliferation of T cell DC09/05 was expressed in amounts of ^{3}H-thymidine incorporation (counts per minutes; cpm)

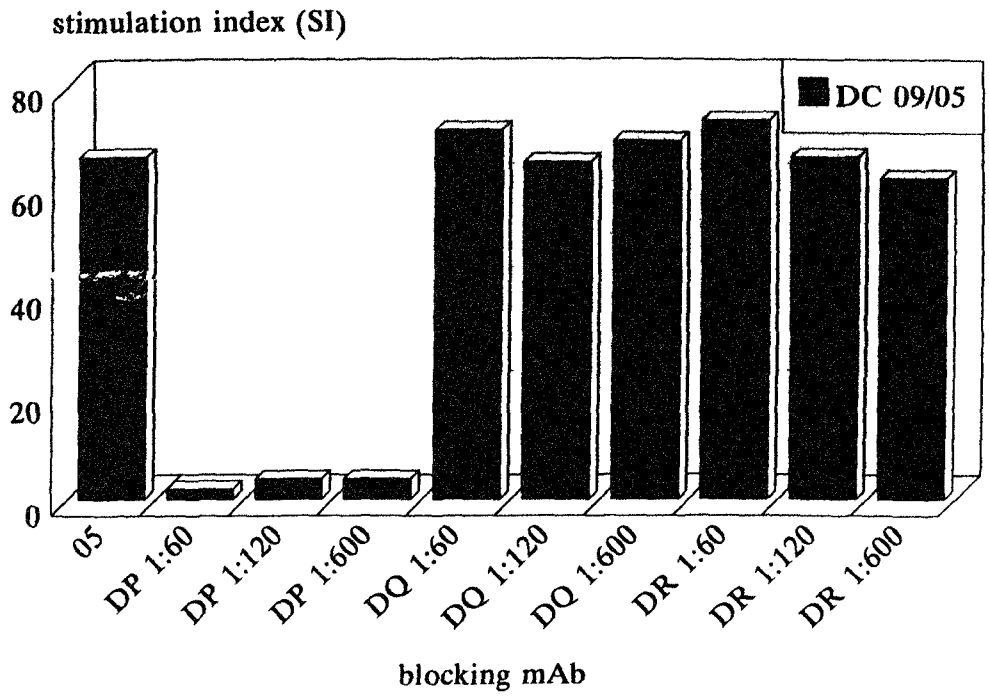

Fig. 4 Blocking of the HLA-DP-specific proliferation Three MHC class II-specific monoclonal antibodies were used to block the proliferation of T-cell line DC 09/05 upon stimulation with PBMC of individual 05· the DP-specific B7 21 (Becton Dickinson), the DQ-specific SPvL3 (Spits et al 1984) and B8 11 2 (Rebai et al 1983) against the DR backbone The DR- and DQ specific antibodies were ascitic fluids and about three times more concentrated than the purified culture supernatant (DP)

when this T-cell line was tested against a panel of HLA-DPB1 PCR-SSO-typed donors representing different *DP* alleles, two HLA-DPB1*1901-positive individuals were stimulatory while none of the *HLA-DPB1*1901*-negative panel donors (n = 12) was recognized (Table 2) No crossreactivity of the T-cell line was found with products of other *DP* alleles, the T-cell line DC 09/05 appeared specific for HLA-DPB1*1901 Blocking of the proliferative activity of T-cell line DC 09/05 in a PLT with mAb to HLA-DP, but not to HLA-DR or -DQ, confirmed its DP reactivity (Fig 4)

This HLA-DPB1*1901 disparity could not be demonstrated in a standard MLR, nor did we observe a significant stimulation index in the standard PLT assay in this particular 09/05 responder/stimulator combination Determination of the HLA-DPB1*1901-specific frequency of helper T-lymphocyte precursors (HTLp) in the responder 09/ stimulator 05 combination however revealed a low number of HTLp (33 HTLp/10^6 cells)

Our results emphasize the superiority of DC compared with other APC in the initiation of T-cell responses As potent APC, DC are characterized by high MHC class II expression and are furnished by the important costimulatory molecules such as B7-1 and B7-2 Both these characteristics of DC may contribute to the potency to induce a T-cell response to an HLA-DP disparity which was undetectable in a standard MLR and PLT The DC appears to function as an appropiate stimulator cell to initiate HLA-DP-specific T-cell lines and clones which could be useful for functional in vitro analyses or eventually for HLA-DP typing

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