Table 1. DR, DQ and DP β Chains from a Panel of DRw8 B Cell Lines

WS ID	Cell Name	DR/Dw	DQ*	DP†	DRβ	DQβ	DPβ
#9066	TAB089	w8/DB7	w1	7	β1	β1	β4
#9067	BTB	w8/w8	w4	4	β1	β2	β5
#9068	BM9	w8/w8	w4	2B?	β1	β2	β2
#9069	MADURA	w8/w8	w4	4	β1	β2	β5
#9070	LUY	w8/8 3	w7	1,4	β1	β3	β3,β5
#9071	OLGA	w8/8 2	w4	3,4?	β2	β2	β2,β4
#9072	SPACH	w8/8 2	w4	4B?	β2	β2	β4
#9100	OLL	w8/	w4	3 4B?	β2	β2	β4 β?

*DQ types locally determined, †DP types by Eckels

(TAB", DRβ1/DQβ1) or DQw3 ("LUY", DRβ1/DQβ3) The "OLGA" type (DRβ2/DQβ2) might be generated by mutations of DRβ1 into DRβ2 from "BTB" since both DR β chains carry the DRw8 epitope and are most likely evolutionally related

Another interesting finding is that four different DR/DQ haplotypes perfectly correlated with HLA D types, "TAB" correlated with DB7, "BTB" with Dw8, LUY" with Dw8 3, and "OLGA" with Dw8 2 In the primary MLR in one combination of these HTC cells, DR molecules are different and may be stimulatory to each other, while in another combination DQ molecules are different and may be stimulatory Thus, DQ molecules as well as DR molecules appear to be responsible for the HLA D specificity on the DRw8 carrying haplotype

These DR/DQ haplotypes were found differently with one or two DP molecules isolated by B7/21 The tentative nomenclature was given to these DP molecules as previously published (2), where five distinct DP β chains (DPβ1-DPβ5) were described Four DPβ chains (DPβ2-DPβ5) were identified (Table 1) A correlation between DPβ5 and the cellularly defined DPw4 antigen was confirmed However, the other three DP β chains were not correlated with any DP antigens in this study

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Identification of a Cellularly Defined DRW8 Subtype

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Cellular mechanisms involved in the allograft rejection process remain highly controversial Using monoclonal antibodies, several studies have demonstrated convincingly that most of the infiltrating mononuclear cells are T lymphocytes Moreover, several investigators have developed techniques for culturing T cells out of various human allografts in order to delineate functions of intragraft T cell populations

From a rejected human kidney graft a limiting dilution technique (1) was used to clone a large number of graft invading cells with clonal efficiency of 1/13 Out of 55 clones, 20 were tested for 1) proliferative activity in primed lymphocyte typing (PLT), 2) cytotoxic activity in cell-mediated lympholysis (CML), and 3) cytotoxic activity inhibited by monoclonal antibodies (MAb) A large panel of well HLA-defined cells (PBL PHA-blasts, and B lymphoblastoid cell lines – LCL) were used as stimulator or target cells This panel included all DRW8 positive cells from workshop homozygous B LCL (BM9, TAB 089, MADURA, BTB, OLGA, LUY, S PACHEO, OLL, SPL), seven heterozygous DRW8 positive cells from Blood Bank of Leiden (Netherlands), and cells from recipient BER (DR2 DRW6) and donor DAB (DR5-DRW8) The following MoAbs were used to inhibit cytotoxicity experiments against donor-BLCL W6 32, LEU 10, NDS 10, 1A3, B7/21, 2D6, GSP 4 1 NDS 13 UK 8 1, CHE 249 2, MAD 88 (all workshop reagents), and BT 2 9 (anti class II), D1 12 (anti DR) VI 15 (anti DR), and BM 50 (anti DR)

Clone "1D9" was selected for its ability to proliferate with and to kill only but not all cells bearing DRW8 phenotype (Table 1) This clone was significantly

Table 1. Proliferative and Cytotoxic Activities of Clone 1D9

Stimulators (PTL) or Targets (CML)	HLA DR/DQ	Clone 1D9 Responder/Effector in PLT (cpm)*	Clone 1D9 Responder/Effector in CML (% lysis)†
Le #15 (BLCL)	7-W8/W2	21040	25/22
Le #16 (BLCL)‡	1 W8/W1	3285	-/
Le #24 (PHA blasts)	W8-W13/W1	10383	-/
Le #26 (PHA blasts)	1-W8/W1	11010	/
Le #28 (PHA blasts)	W8-W14/W1	10121	/
Le #29 (PHA blasts)	W8-W13/W1	13327	/
Le #31 (BLCL)‡	3-W8/W2	5055	-/-
LUY (workshop) (BLCL)	W8/W3	18573	24/12
BER (recipient BLCL)	2-W14/W1	2100	/
DAB (donor BLCL)	5-W8/W3	20600	16/17

*Results expressed as mean (triplicate) cpm of 3H−TdR uptake of clone cultured 72 hours with stimulating cells, †% specific 51 Cr release calculated at two effector/target ratios (20 1/4 1), ‡Leiden #16 and #31 were not recognized by 1D9

inhibited by broad anti-class II and anti-monomorphic DR MoAb, but not by anti-DQ or anti-DP MoAb Monoclonal antibody MAD 88 (anti-DRW8) elicited against DRW8 positive cell MADURA, which was lysed by clone "1D9," did not block "1D9" cytotoxicity directed against donor cell DAB (DRW8 positive) But this antibody labeled in immunofluorescence the Leiden #16 cell (DRW8 positive), which in its turn was not lysed by "1D9," clearly suggesting that "1D9" and MoAb MAD 88 recognized two different epitopes on the β chain of the DRW8 molecule

In order to identify at the DNA level this cellularly defined DRW8 subtype, a restriction fragment length polymorphism (RFLP) study was carried out to distinguish a DNA polymorphism of these DRW8 cells according to their sensitivity to clone "1D9 " Genomic DNAs from nine DRW8 positive homozygous workshop cells, from two recognized heterozygous DRW8 positive cells (Leiden #15 and donor DAB), and from a non-recognized heterozygous DRW8 positive cell (Leiden #16) were digested with Eco RI, Bam HI, Taq I, and Hind III and then hybridized with workshop DR β, DQ α, and DQ β probes With DR β probe and all enzymes tested, DRW8 haplotype revealed a characteristic pattern distinct from those of others DR specificities Moreover a specific fragment of 8 9 Kb was noted with Taq 1 But no polymorphism was found at the DNA level because the same RFLP pattern was observed for all DRW8 positive cells including the non-recognized Leiden #16 cell All the restriction enzyme used to hybridize the DQ α and DQ β probes revealed three different DQ patterns These results were concordant with those previously reported by serology (2)

In summary, clone 1D9 specific for the kidney donor cells is described It killed neither K562 nor autologous BLCL On a large panel of well HLA-characterized cells it recognized a majority of DRW8 positive cells (15/17 cells) On the other hand, all DRW8 negative cells (N = 22) were not recognized Proliferative and cytotoxic activities were concordant RFLP analysis of DRW8 positive cells either recognized or not by 1D9 did not revealed differences with DR β probe Hybridization with DQ α and DQ β probes exhibited three different patterns without any relationship to 1D9 reactivity 1D9 T-cell clone might be directed against a DRW8 subtype which would need further investigations (other restriction enzymes and probes) at the DNA level Alternatively, the DRW8 molecule might be the restriction element for some yet unknown minor alloantigens

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