Products of HLA Class I and Class II (B,C,DP,DQ,DR) Genes All Contribute to Induction of Recipient Anti-Donor Responses in Rejected Kidneys

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T LYMPHOCYTES play a major role in the development of cellular responses leading to rejection of an allograft. We previously described a limiting dilution technique allowing the cloning and expansion of about 10% of mechanically harvested kidney graft invading T lymphocytes. Fifty-five alloreactive T cell clones (ATLCs), studied phenotypically and functionally, were shown to react specifically with kidney donor-derived B lymphoblastoid cell line (BLCL).[1] In the present study, we have analyzed the recognition repertoire of 20 of 55 ATLCs.

MATERIALS AND METHODS

The clinical status of the recipient has already been described elsewhere [1] Mononuclear cells infiltrating the irreversibly rejected kidney allograft were cloned by limiting dilution and cultured with irradiated donor BLCL in RPMI 1640 supplemented with human serum and recombinant IL 2 (0 94 n mol/L) as previously described [1] We tested the cytotoxic and proliferative activities of ATLCs against a set of allogeneic cells (characteristics given in Table 1) Cytotoxicity was assessed by a standard ^{51}Cr release assay[1] and proliferation by ^{3}H-thymidine uptake after a three-day culture with irradiated stimulator cells Positive and negative

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Table 1. HLA Phenotype of Male and Female Panel Cells Used in Cytotoxic and Proliferative Assays

No	Sex	Origin	HLA						
			A	B	C	DR	DQ	DP	LBQ
1	M	PBL	*2*	44	5	4	*w3*	—	—
2	F	PBL	*2*, 3	35, 57	4	1, 7	w1, *w3*	—	—
2'	F	PBL	*2*	51	2	2, 7	w1, w2	—	—
3	M	PBL	3, *11*	8, 35	4 *7*	3, 6	w1, w2	—	—
4	F	PBL	1, *11*	62, 37	2, 4	4	*w3*	—	—
5	M	PBL	3, 29	*18*, 44		6, 9	w1, *w3*	—	—
6	F	PBL	1, 30	8, *18*	5, *7*	3	w2,	—	—
7	M	PBL	24 31	41, *55*	*3*	6, 7	w1, *w3*	w4, w5	—
8	F	PBL	1 24	49, *55*	*3 7*	6, *11*	w1, *w3*	—	—
9	M	PBL	1	8 60	*3 7*	2, 7	w1, *w3*	—	—
10	F	PBL	1, 32	57, 60	*3*, 6	2, 7	w1, *w3*	—	—
11	M	PBL	3, 33	7, 14	—	2, 3	w1, w2	—	—
12	F	PBL	3, 32	7 12	—	2 7	w1, w2	—	—
13	M	BLCL	25	*18*		*11*	*w3*,	w2	1
14	F	BLCL	25	*18*		*11*	*w3*,	w2	1
15	M	BLCL	1 3	7 13	6, *7*	7, *8*	w2,	—	—
16	F	BLCL	24, 26	56, 58	1	1, *8*	w1,	—	—
17	M	BLCL	24	7	*7*	2	w1	—	—
18	F	BLCL	23	7	*7*	2	w1	—	—
19	F	BLCL	*2* 3	7, 51	*7*	2, 14	w1,	—	—
20	M	BLCL	*2, 11*	*18, 55*	*3 7*	*8, 11*	*w3*	—	—
21	M	BLCL	28, 30	13 *55*	6, *7*	6, 7	w1, w2	—	—

NOTE BLCLs No 19 and No 20 were derived from kidney recipient (autologous) and donor B lymphocytes respectively Italic type denotes compatibility with donor's antigens

reactions were defined by means of the cluster analysis program of Caroll et al [2] Monoclonal antibodies (MoAbs) used in blocking experiments are indicated in Table 2 The blocking effect of MoAbs (Wilcoxon statistical analysis $P < .05$) was studied at optimal MoAb concentrations and an effector to target ratio of 20 1

RESULTS AND COMMENTS

Twenty ATLCs were tested for proliferation against a panel of irradiated PHA blasts and BLCLs In addition 15 ATLCs (previously shown to lyse donor BLCL) were studied for their lytic activity against a panel of ^{51}Cr labelled panel cells Proliferation and cytotoxic assays were concordant and various recognition patterns were observed (Table 2) In general no precise characterization of the HLA specificity recognized could be done as targets generally shared several HLA antigens with donor cells Therefore we carried out blocking experiments using MoAbs directed against HLA class I and II molecules Cytotoxicity of three clones (40 2 6 2F7 and 1B4) was abrogated by addition of anti-HLA class I MoAb during CML assay indicating that 40 2 6 recognized a HLA Bw55 specificity Clone 1B4 reacted with cells No 13 and No 14 sharing B18 and DRw11 with donor BLCL and probably recognized the B18 specificity ATLC 2F7 recognized an HLA class I specificity shared between cells 3,5 6 8 9,16 20 and 21 Six of eight reactive target cells were Cw7 positive whereas on the other two reactive cells a C blank could not be excluded On the other hand four of 13 non reactive cells were Cw7 + and all of them were also B7+ Since two subtypes of Cw7 have been described one of which is less

Table 2 Functional Characteristics of ATLCs

ATLC	CD	CML	Reactive Panel Cells*	Blocking MoAbs†	Comments
40 2 6	CD8	yes	7 8 20 21	HLA I	anti Bw55
2E5	CD8	yes	7 8 9 10 20	not tested	ant Cw3
1B4	CD8	yes	13 14 20	HLA I	anti B18
2F7	CD8	yes	3 5 6 8 9 16 20 21	HLA I	anti Cw7 subtype
1D9	CD4	yes	15 20	Broad HLAII DR	anti DRw8 subtype
1B8	CD4	yes	15 16 20	Broad HLAII DR	anti DRw8
1E7	CD4	yes	15 16 20	DR	anti DRw8
2C7	CD4	yes	20	Broad HLAII	anti DR private
2C5	CD4	yes	20	Broad HLAII DR	anti DR private
2C3	CD4	yes	15 16 20	DQ	*anti DQ blank*
1C7	CD4	yes	13 14 15 16 20	DQ	anti DQ new
1E3	CD4	yes	13 14 20	DP	anti DP
1B5	CD8	yes	13 14 20	DP Broad HLAII	anti DP
1F5	CD8	yes	13 14 20	Broad HLAII	anti HLA II
1F2	CD4	yes	13 14 15 16 20	Broad HLAII	anti HLA II
2D11	CD4	no	13 14 20	Broad HLAII	anti HLA II
2D9	CD4	no	15 16 20	Broad HLAII	anti HLA II
1D3	CD4	no	15 16 20	Broad HLAII	anti HLA II
1F3	CD4	no	15 16 20	Broad HLAII	anti HLA II

NOTE All ATLCs were CD2 + CD3

*Reactive panel cells were defined as cells able to significantly trigger ATLC proliferation and to be significantly lysed by cytotoxic effector cells

†In cytotoxicity blocking experiments the following anti HLA MAbs were used HLA class I (W6/32) [3] Broad HLA class II (BT2/9 [4] DR (BM50) [5] DQ (Leu10)[6] and DP (B7 21) [7]

frequently seen in HLA-B7 individuals [8] it is possible that the nonB7Cw7 subtype is recognized by clone 2F7. Cytotoxic activity of the majority of the ATLCs tested could be inhibited by MoAbs directed against HLA class II structures. Similarly, concomitant analysis of data from panel and blocking experiments led in general to the precise characterization of the HLA class II specificity recognized by these ATLCs (Table 2).

In this paper, we have investigated the recognition repertoire of 20 of 55 ATLCs derived from cells infiltrating a rejected kidney. Results summarized in Table 2, indicate that cells committed against almost all HLA specificities mismatched between kidney donor and recipient (including HLA C and DP generally ignored in clinical transplantation) could be evidenced. In this view, it should be noted that graft invading cells were directly cloned after their isolation prior to any bulk culture thus ruling out possible preferential in vitro expansion of ATLCs sensitized against a few antigens. The fact that all ATLCs which were assumed to be representative of graft invading cells, recognized HLA molecules emphasizes the role of HLA recognition in the rejection process. However we cannot exclude the possibility that cells not recruited in the cloning procedure using donor BLCL were committed against non HLA molecules not borne by stimulating cells.

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