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Y-antigen killing by T cells of women is restricted by HLA

Cytotoxic T cells are important in graft rejection and in the control of virus infections, but the mode of interaction of these cells with their targets, particularly in man remains unclear. It has been shown in mice that products of genes in the major histocompatibility complex (H-2) are involved in these interactions even when the cytotoxic T cells are specific for viral or non-H-2 antigens. This involvement is seen as the requirements that the target cell must express the specific non-H-2 antigen and in addition the same H-2, D or K region antigens as were present on the cells which initiated the immune response[1–4]. Cytotoxic T cells specific for viral or non-H-2 antigens are thus restricted in the targets that they can kill by the H-2 antigens on the targets. For example, cytotoxic T cells from H-2^b female mice suitably primed to the Y antigen of H-2^b males, will only kill cells from male mice carrying an H-2D region derived from H-2^b (refs 5, 6). This report is to our knowledge the first clear demonstration that a similar restriction occurs in man. We describe a situation in man where cytotoxic reactions specific for non-HLA antigens can only occur when target cells carry both the HLA-A2 antigen of the original sensitising cell and the non-HLA target determinant(s). The strong association with maleness suggests that one of the specific antigens involved was coded for by the Y chromosome.

Our study began with an observation that a female patient (Reef) who had been suffering from aplastic anaemia rejected a bone marrow graft from her HLA-identical brother. Before this rejection, a transient chimaerism of peripheral blood cells indicated that the graft had initially taken. Rejection was accompanied by a spontaneous recovery of the patient's bone marrow haemopoietic function, thus allowing further investigations. Peripheral blood lymphocytes from the patient were used as effector cells to measure the level of cytotoxicity against target cells from the patient's family and an unrelated panel. The patient's lymphocytes were first restimulated *in vitro* by incubating them for 6 d with irradiated stimulator cells from her HLA identical brother. Cytotoxicity was measured using an isotope release assay[7] with target cells which had been incubated with phytohaemagglutinin for 3 d and then labelled with sodium ^{51}chromate. Effector cells (70×10^4) and target cells (1×10^4) in RPM-1640 plus 20% heat-inactivated human AB serum were incubated for 4 h in round-bottomed microtitre plates. Plates were centrifuged for 5 min at 200*g* and the supernatant removed and counted in a γ-counter. Each cell combination was tested in triplicate. After subtraction of background the percentage of isotope release was calculated according to the following formula:

$$\frac{\text{experimental mean} - \text{mean of spontaneous release}}{\text{freeze–thaw mean (100\% release)} - \text{mean of spontaneous release}} \times 100 = \% \text{ kill}$$

In our initial studies performed 31 weeks after grafting, cytotoxicity was demonstrated against the donor's lymphocytes even when the patient's cells had not been restimulated *in vitro* (Table 1). This suggested that killing was to a non-HLA target

Table 1 Killing pattern of cells from patient Reef. against her own target cells and those from other family members

Targets			HLA compatibility with patient	% Killing of CML	
Donor	♂	ac*	yes	63†	18‡
Patient	♀	ac	yes	−1	NT
Sib 5	♂	ac	yes	63	NT
Sib 3	♂	ad	no	53	13
Sib 4	♀	ad	no	31	NT
Mother	♀	cd	no	4	0

Table 1 shows percentage of chromium release of tests in which the patient's cells were tested against members of her family. NT, not tested.
*HLA haplotypes: a, A2, BW40, W6, CW3; b, A2, BW40, W6, CW3; c, A2, B12, W4, —; d, AW31, BW35, W6, CW4.
†Results after *in vitro* sensitisation to donor.
‡Results without previous *in vitro* sensitisation. MLRs between donor, patient and sib 5 were sufficiently low to exclude the possibility of a recombination between HLA-B and HLA-D.

Table 2 Positive and negative killing by cells from patient Reef against target cells from unrelated individuals

		HLA Phenotypes HLA-A		HLA-B		HLA-C		Sex	% Kill
(Expt 1):	1	2*	W31	W35	W40	CW3	CW4	♂	30
week 42	2	2	11	W15	W40	—	CW4	♂	33
	3	1	2	8	W40	—	NT	♂	28
	4	1	28	W17	W22	—	CW1	♂	−1
	5	9	11	5	W21	—	CW1	♂	−1
	6	2	W31	12	W35	—	CW4	♀	0
	7	1	2	8	W22	—	—	♀	−1
	8	—	11	—	27	—	CW1	♀	−3
	9	11	W26	W16	W35	—	CW4	♀	−2
	10	1	W31	8	W40	—	CW3	♀	−1
Positive control (donor)		2	2	12	W40	—	CW3	♂	60
(Expt 2) week 52	11	1	2	8	W40	—	CW3	♂	44
	12	2	3	7	12	—	—	♂	47
	13	3	W30	W35	W40		NT	♂	9
	14	2	3	7	W40		NT	♀	4
	15	2	3	7	W40		NT	♀	−1
	16	2	3	7	W40		NT	♀	3
	17	2	9	5	W40	—	CW3	♀	15
	18	1	2	8	W40	—	CW3	♀	6
	19	1	2	5	8	—	—	♀	−1
	20	2	3	7	12	—	—	♀	3
	21	—	9	12	W40	—	CW3	♀	6
	22	11	W31	W15	W17	—	CW3	♀	−1
Positive control (donor)		2	2	12	W40	—	CW3	♂	48
(Expt 3)	23	—	3	18	27	CW2	CW4	♂	−3
Week 72	24	1	9	8	27	—	CW1	♂	0
	25	—	3	W17	18	—	—	♂	−3
	26	1	2	5	5	—	CW4	♀	−2
	27	2	W32	8	W15	—	CW3	♀	−3
	28	3	W26	—	W35	—	CW4	♀	1
	29	10	28	W35	HR	—	CW4	♀	0
	30	—	9	7	W15	—	CW3	♀	1
	31	1	3	W25	W40	CW2	CW4	♀	−2
	32	1	9	18	W35	—	CW4	♀	−1
	33	1	3	—	W35	CW3	CW4	♀	−1
	34	1	28	5	8	—	—	♀	−3
	35	3	W30	7	W17	—	CW3	♀	−3
	36	1	9	8	W40	—	CW3	♀	0
Positive control (donor)		2	2	12	W40	—	CW3	♂	13

NT, not tested.
*The HLA antigens compatible with patient Reef are underlined.

as the donor was HLA identical to his sister. Three further experiments were performed using effector cells from the recipient sensitised to the donor and using cells from an unrelated panel as targets. HLA phenotypes are shown in Table 2. In several cases positive reactions were observed. The level of killing seemed to decline with time, and the gradual waning seemed to be associated with the patient's recovery. Family segregation studies were performed on the relatives of positive panel members. In three such studies the killing pattern failed to segregate with the HLA haplotype, again confirming that killing was directed to a non-HLA target or targets (Table 3).

We have searched for HLA restriction of killing, and a subsequent retrospective analysis revealed a strong association with HLA-A2. All targets that were killed carried the HLA-A2 antigen, although not all HLA-A2 cells were killed. The possibility that a split in HLA-A2 specificities was being recognised was excluded by the family studies (families 2 and 3, Table 3). Here the HLA-A2-bearing haplotype was associated with killing when expressed in one cell, but when expressed on another cell this haplotype was not associated with killing (family 2—c haplotype, family 3—a haplotype). Also, in family 3, no killing was associated with the antigen HLA-A28 which by serology was highly cross-reactive with HLA-A2.

Further analysis revealed that 15 out of 17 targets that were killed were male. Target cells from all fifteen of the HLA-A2 males were killed (Table 4) but cells from males not carrying HLA-A2 were not killed. Cells derived from two females were killed—both females were also HLA-A2 positive, but the level of killing was at a lower level than that observed with other positive reactions in the same test. This suggested that at least two specificities were involved, one associated with the Y chromosome exclusively and the other carried by a proportion of A2 females. One of the females that gave target cells showing positive killing was a sister of the patient (Table 1). The presence of the second system in the males will have been masked by the killing specific for the Y antigen. A clear example of the independent inheritance of an HLA-A2 gene product and the presumed Y product was shown in family 3 (Table 3). Here the HLA-A2 specificity inherited by the male child C1 was from the mother's a-haplotype. Cells from the two other male children were not killed.

Table 3 Pattern of killing of a non-HLA target by effector cells (raised between the patient Reef and her donor) tested on 3 families

Family 1 (week 64) Target		HLA haplotypes	Sex	% Kill
Mother	ab	1, 8, W6/2, W22, W6	♀	0
Father	cd	1, 8 W6/2, W40, W6	♂	23
C1	ac		♀	0
C2	ad		♂	19
C3	bc		♂	23
Family 2 (week 64)				
Mother	ab	3, 7, W6/1, 8, W6	♀	−1
Father	cd	2, 12, W4/2, W40, W6, CW4	♂	42
C1	ac		♂	34
C2	bc		♀	5
C3	ad		♂	41
C4	ac		♀	1
C5	bc		♀	3
Family 3 (week 72)				
Mother	ab	2, W40, W6, CW3/28, 12, W4	♀	1
Father	cd	1, 8, W6/2, W35, W6, CW4	♂	13
C1	ac		♂	15
C2	bc		♂	−1
C3	bc		♂	−2

In family 2 the female C2 inherited the A2 from the father and was not killed, but her brother who inherited the same A2 was killed. In family 3 the male C1 inherited the A2 from the mother and was killed, but C2 and C3 did not inherit A2 and were not killed. The weeks represent the time after bone marrow grafting that the family studies were performed. Compatibility with patient is underlined.

Table 4 Y chromosome linkage of non-HLA target and restriction of killing to HLA-A2 positive targets

		Sex of HLA-A2 targets ♂	♀
CML	+	15	2*
	−	0	17

*Level of killing was much lower than other positive targets in same experiment.

These results demonstrate that the cell-mediated cytotoxic reactions of the patient's lymphocytes are specific for non-HLA antigens but restricted by an HLA product in as much as only targets carrying HLA-A2 are lysed. This is very similar to the restriction seen with murine cytotoxic T cells reacting with non-H-2 antigens[1–6].

We have not yet unambiguously identified the effector mechanism in this human cell-mediated cytotoxicity system but feel it is not an antibody-dependent system as the cytotoxic reactions occurred in 20% heat-inactivated human AB serum. In these conditions it has been impossible to measure antibody dependent cell-mediated cytotoxicity (L. Doyer, personal communication). This is important as serum from the patient Reef, when tested against the panel members (Table 2) using the two-colour fluorescence technique[8], gave a positive reaction with some target cells that were positive in the cytotoxicity test.

Our observations of male-associated killing are analogous to those of Gordon *et al.* in the mouse[5,6]. In their case, cytotoxicity was only observed after *in vivo* sensitisation with a male skin allograft followed by *in vitro* priming. The clinical relevance of the Y-associated killing phenomenon is that it is probably an *in vitro* reflection of the known influence of sex on the incidence of graft against host disease in bone marrow transplantation between HLA identical siblings[9]. In kidney grafting, several workers have observed that female recipients of male grafts survived longer than female recipients of female grafts (ref. 10, and Kissmeyer-Nielsen, Opelz and Terasaki, personal communication). This was interpreted by Oliver to indicate that the Y-linked histocompatibility antigen may actively induce a prolongation of renal allograft survival in man. Although these results contrast with our own, they should be interpreted with caution as HLA-A2 positive combinations were not separately analysed, and some modes of immunisation to the Y-linked histocompatibility antigen may result in protective immunity.

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