

NON-HLA IMMUNOLOGICAL FACTORS IN EIGHTEEN HLA-IDENTICAL RENAL ALLOGRAFTS

J B Dossetor, T Kovithavongs

University of Alberta, Edmonton, Alberta, Canada

Introduction

Although there is much evidence that HLA antigen matching, for both A, B, C and DR factors, is of value in predicting cadaveric graft (CD) survival it does not seem to be as important as previous blood transfusion [1] and certain other factors such as recipients' inherent immune responsiveness [2]. Whereas HLA haplotype matching is prognostic for survival of grafts from living related donors (LRD), the importance of other factors is evident from the fact that untreated HLA-identical skin allografts are rejected within 17–22 days, presumably because of oligo-allelic alloantigen systems of low immunogenicity.

Cytotoxic T cells, generated either in vivo as a consequence of blood transfusion [3] or in vitro by a mixed leucocyte reaction, (MLR) [4], are directed against HLA-ABC antigens of the incompatible stimulator as well as non-HLA factors, but the latter have not received much attention.

Non-HLA factors have been detected, but not defined, by immunological studies in recipients of HLA-identical renal allografts from sibling donors whose lymphocytes fail to stimulate the recipients' in MLR. Eighteen such allografts form the data base for this report. Evidence for four immunological systems is presented:

- a) Non-HLA lymphocytotoxic mechanisms mediating rejection.
- b) Non-HLA suppressor mechanisms, mediated by lymphocytes, which are donor specific.
- c) The role of viral antigen in rejection (a possible example of the Zinkernagel phenomenon [5], and
- d) Examination for the postulated effect of Y-chromosomal male-specific factors in those who share HLA-A2, or A2,B7 [6].

Though incompatible endothelial-monocyte alloantigens are of importance in clinical transplantation [7] we have no evidence of this in the 18 patients in this report.

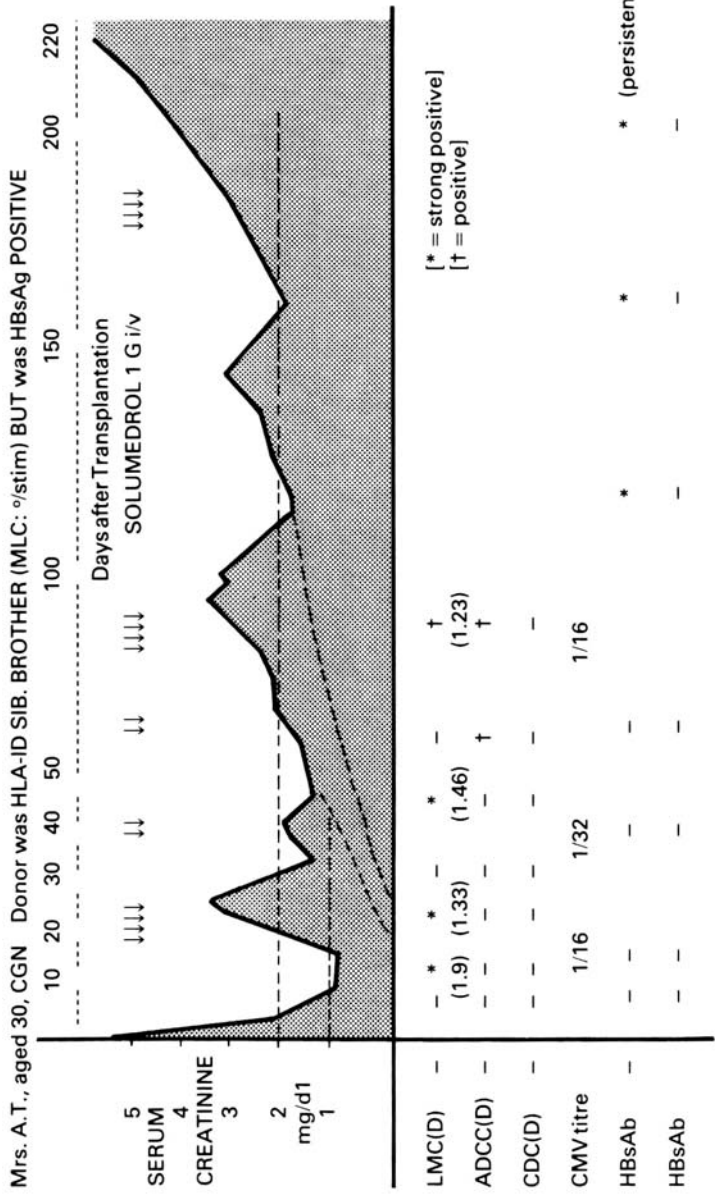


Figure 2. Rejection of an HLA-identical graft, with hepatitis B transmission

Methods

Lymphocytotoxicity by in vivo generated cells we term lymphocyte mediated cytotoxicity or LMC, as illustrated in Figures 1 and 2. Similar reactions by in vitro generated cytotoxic T-cells we term cell mediated lympholysis or CML. Both tests react putative killer cells with ^{51}Cr -labelled target cells, usually PHA-blasts at a 40:1 effector-target cell ratio. Incubation for LMC or CML is carried out for four to six hours at 37°C by techniques previously reported by this laboratory [8]. Owing to small cell numbers only on certain occasions has killing been more accurately quantitated by using a range of E:T ratios and expressing results as lytic units [9].

Our technique for detection of post-transplant donor-specific suppressor cells has also been previously described [9,10]. The simplest system is to use six day MLRs to induce cytotoxic T-cells in pre-transplant recipient cells, R(-T), (reclaimed from liquid nitrogen) by stimulating them with mitomycin-treated donor cells, Dm, and then determining if this induction is suppressed by mitomycin-treated post-transplant recipient cells, R(T+)m, as symbolised by: [R(-T)/Dm + R(T+)m]. In studies in HLA-identical siblings, because recipient and donor cells do not stimulate in MLR or induce cytotoxic T-cells, it is necessary to use an unrelated third-party cell, X, as the responder population in MLR. Donor-specificity is revealed if only R(T+)m cells will inhibit killer cell induction in [X/Dm] cultures, i.e. if only [X/Dm + R(T+)m] cultures are suppressed when subsequently assayed in CML.

Methodology for complement mediated cytotoxicity or antibody dependent cellular cytotoxicity are routine. When done against reclaimed donor lymphocytes these are symbolised as CDC(D) and ADCC(D) (Figure 2).

Results

Non-HLA rejection episodes with coincident LMC(D)

For these 18 patients the incidence of major rejection episodes (defined as doubling in serum creatinine within a few days when other causes or renal impairment are excluded) is summarised in Table I (where the patients are further subgrouped according to sex of donor and recipient and degree of sharing of HLA-A2 or A2,B7 as will be discussed below). Five of 18 patients had major rejection episodes in the first two post-transplant months, but only one was irreversible; the other four all recovered excellent function which continued long term (as did 12 of 13 who had no early major rejections).

Displayed in Figure 1 is sequential data for serum creatinine and lymphocyte mediated cytotoxicity, LMC(D), of female patient DB who received a renal allograft from her HLA-identical brother. Both waves of clinical rejection are closely preceded by detection of cytotoxic T-cells in the peripheral blood against donor lymphocytes. When ^{51}Cr release of recipient against donor in LMC is more than 1.2 times control (donor against donor) LMC is positive.

The specificities being recognised by these cytotoxic T-cells have not been determined, but they are non-HLA.

TABLE I. Analysis of rejections in 18 HLA-identical renal allografts

HLA-identical sibling renal allografts (18)	Number	Sharing A2 or A2, B7		Not sharing A2 or A2, B7	
		No rejection Minor rejection	Major rejection	No rejection Minor rejection	Major rejection
Male to female	3	0	2*	1	0
Female to female	5	5	0	-	-
Female to male	4	3	0	1	0
Male to male	6	1	1	2	2

*Rejection of one of these was irreversible, donor was HBsAg positive

Donor-specific non-HLA suppressor cells

We have previously published one example of this phenomenon [10] which is further illustrated by the data in Figure 3. In MLR reactions where cell X is stimulated by donor cells (mitomycin-treated) there is good MLR proliferation and generation of cytotoxic cells which is strongly suppressed by post-transplant recipient cells, but not by pre-transplant recipient cells. Critically important is the further observation that post-transplant recipient cells do not inhibit cytotoxic T-cell generation to an unrelated cell, Y. (The shaded areas in Figure 2 denote the range of results with the same cell populations, used repeatedly as controls, for tests done with successive new populations of recipient cells).

Suppressor cell studies were done in six of 18 HLA-identical transplants. Five studies showed suppressor cells developing soon after transplantation, as in Figure 3; in a sixth (the patient who had a major rejection in the male-to-male, A2 or A2B7-sharing subgroup in Table I) suppressor cells were not found, but such evidence is insufficient to claim that their absence was a factor in rejection.

Although it is evident that these cells are donor-specific they cannot be developed as a result of HLA antigen disparity, in these five instances. The specificity to which they are directed cannot as yet be determined.

Irreversible rejection, possibly mediated by viral antigen

Figure 2 shows sequential data from a female recipient of a kidney from her HLA-identical brother. The situation was complicated by the clinicians being unaware, until after transplantation, that the donor was HBsAg positive; he also had the 'e' antigen in his blood. The recipient was negative for hepatitis B antigens pretransplant.

Several rejection episodes occurred with partial response to courses of i.v. Solumedrol (in 1g doses), but kidney function thereafter progressively deteriorated. During early rejection episodes LMC(D) was strongly positive; later ADCC(D) became positive. Later still, more than three months after transplantation, hepatitis B surface antigen was detected in the blood and, thereafter, was persistently positive. Because of risk to laboratory personnel, we then discontinued immunological studies.

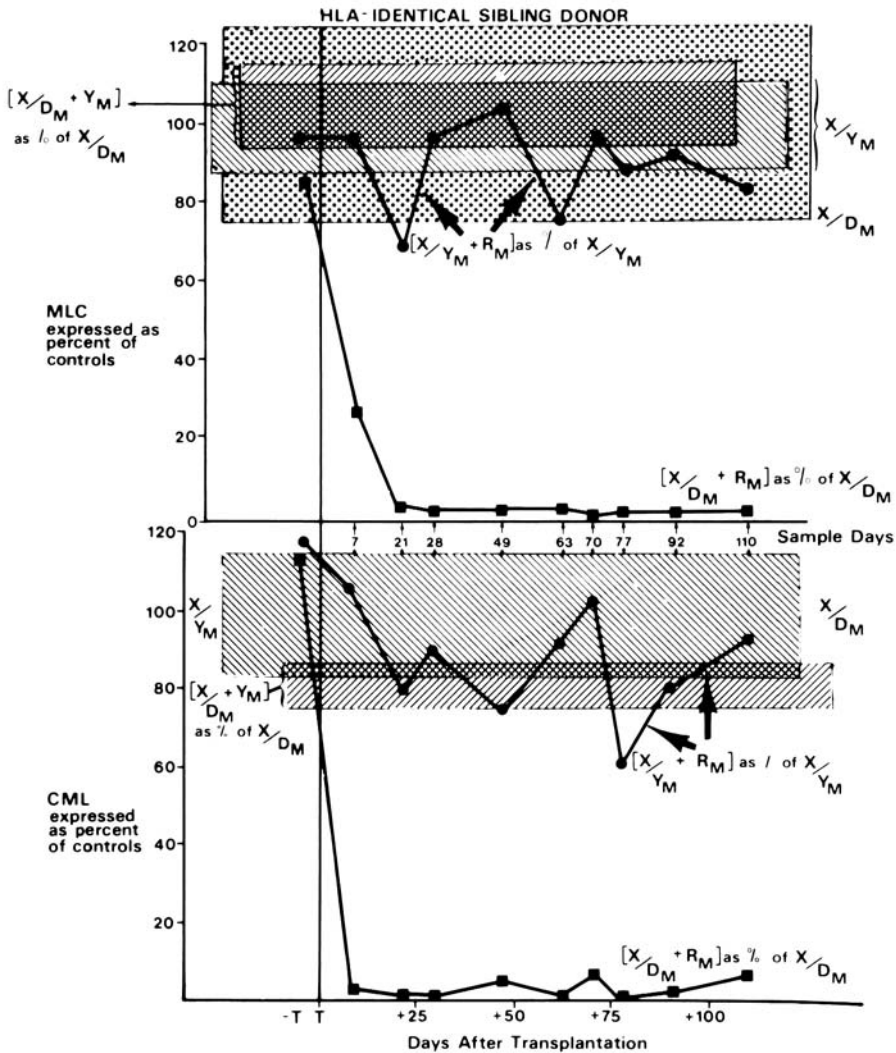


Figure 3. Suppressor cell study in a case with no rejection episodes

Loss of an HLA-identical renal graft by rejection is a rare event. Clearly HLA incompatibility is not the cause and, in this instance, it is strongly suspected that cytotoxic T-cells were directed against hepatitis B antigens expressed on renal cells of the allograft. This would be more effective in rejecting the kidney if donor and recipient were closely matched for HLA-ABC antigens as shown in Zinkernagel's experiments in an animal model [5]. It could be speculated that a CMV seronegative recipient of a CMV infected graft may have rejections on a similar basis, though the evidence in man is still fragmentary and inconclusive.

Inconclusive evidence for Y-chromosome influence in rejection in HLA-identicals

Table I shows an analysis to see if there is support for the claim of Pfeffer and Thorsby [6] that the Y-chromosome may carry histocompatibility factors which may occasionally determine rejection activity. Although there were major rejections in both male-to-female grafts which shared A2 or A2B7 and none in the subsequent two groups which shared these antigens (which data favour the hypothesis) it is also evident that there is insufficient data to draw any conclusion, especially as the male-to-male groups have three major rejections. Also the only irreversible rejection is the case considered in Figure 2.

Acknowledgments

This work was supported by MT 5901 of Medical Research Council of Canada.

References

- 1 Opelz G, Graver B, Terasaki PI. *Lancet* 1981; *i*: 1223
- 2 Opelz G, Mickey MR, Terasaki PI. *Lancet* 1972; *i*: 868
- 3 Wunderlich JR, Rogentine GN Jr, Yankee RA. *Transplantation* 1972; *13*: 31
- 4 Kristensen T. *Transplant Proc* 1978; *10*: 319
- 5 Zinkernagel RM, Doherty PC. *Nature* 1974; *251*: 547
- 6 Pfeffer PF, Thorsby E. *Transplantation* 1982; *33*: 52
- 7 Cerilli GJ, Brasile L, Galouzis T et al. *Transplantation* 1981; *32*: 495
- 8 Kovithavongs T, Schlaut J, Pazderka V et al. *Transplant Proc* 1978; *10*: 547
- 9 Dossetor JB, Liburd EM, Kovithavongs T et al. *Transplant Proc* 1981; *13*: 1645
- 10 Dossetor JB, Liburd EM, Kovithavongs T et al. *Transplant Proc* 1979; *11*: 1260

Address for correspondence: Clinical and Transplant Immunology Group, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada