Brief Definitive Report

DIFFERENCES BETWEEN PIG TISSUES IN THE EXPRESSION OF MAJOR TRANSPLANTATION ANTIGENS: POSSIBLE RELEVANCE FOR ORGAN ALLOGRAFTS*

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In the mouse, qualitative differences occur between cell types in the expression of gene products of the *H-2* major histocompatibility complex (MHC). In particular, antigenic products of the central *(I)* region (Ia antigens) are expressed by B lymphocytes and a few other cell types (1), whereas the *K-* and *D-* region antigens are present on practically all cells. Since strong transplantation antigens are also determined by the *I* region (2), the expression of its gene products by different tissues may influence the survival of such tissues as allografts.

In the pig, the survival of kidney allografts between siblings of known MHC genotype (as defined by mixed lymphocyte culture and lymphocytotoxicity serotyping) shows a strong gene dose effect (3). However, liver allografts survive any degree of mismatching (4) and confer donor-specific protection to subsequent renal grafts (5). We have used the approach of Davies and Alkins (6) to investigate the possibility that these findings may be due to antigenic differences between liver and kidney tissues.

Materials and Methods

Animals. Two partly inbred lines of pigs are available; one homozygous for the "1" MHC haplotype and the other for the "5" haplotype, as defined serologically. These are referred to as S1/S1 and S5/S5.

Antiserum. No. 4454 is one of a bank of alloantisera used to type the Babraham pig herd. Microcytotoxicity testing using peripheral blood leukocyte (PBL) targets from 145 offspring of backcross matings and from randomly chosen pigs showed 4454 to be specific for an MHC haplotype designated 1 (7). Cells from S5/S5 pigs were used as controls in absorptions and cytotoxicity tests.

PBL. These were separated from citrated blood by the Ficoll-Hypaque method, and those for use as target cells were stored viable at $-196°C$. PBL for absorption were used immediately after preparation.

Kidney Cells. These were from lines established from the kidneys of S1/S1 and S5/S5 pigs. They synthesized large concentrations of metallothionein on exposure to cadmium salts: a property specific for renal and hepatic parenchyma (8). Three lines of S1/S1 and two of S5/S5 cells were available.

Liver Cells. These were from lines obtained from the livers of 5-day-old S1/S1 or S5/S5 pigs;

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they produced metallothionein on exposure to cadmium salts. Nonparenchymal cells were eliminated by initial cultivation in an arginine-deficient, ornithine-supplemented medium (9). Four lines of S1/S1 and one of S5/S5 cells were available.

Platelets.   These were isolated from citrated blood by differential centrifugation (10); a typical preparation contained 1 lymphocyte per 2×10^4 platelets.

Absorption Methods.   Kidney and liver parenchymal cells, washed free of serum, were scraped from the surface of culture bottles. All packed cell volumes were determined by centrifugation at 10^4 g. Antiserum was diluted 50-fold with phosphate-buffered saline (PBS), pH 7.2, and 1 ml of dilute serum was added to 40–50 μl packed cells which were resuspended and maintained thus at room temperature for 30 min. Cells were then removed by centrifugation and the antiserum was reabsorbed by the same procedure.

Elution of Antibody from Platelets.   Platelets carrying adsorbed antibodies were washed thoroughly with PBS then suspended in glycine/hydrochloric acid buffer, 0.2 M, pH 3.0 for 30 min at room temperature. After centrifugation, the supernate was dialyzed exhaustively against PBS.

Cytotoxicity Tests: PBL Targets.   Antisera were titrated against cells labeled with $Na_2 \, ^{51}Cr \, O_4$ (CJS 4; The Radiochemical Centre, Amersham, Great Britain) using standard methods (11). Rabbit serum detoxified by absorption with pig erythrocytes in the presence of EDTA (12) was the source of complement (C). Controls for antibody toxicity, C toxicity, and spontaneous isotope release were included.

Kidney and Liver Targets.   Confluent monolayers in the wells of Nunclon-Delta microtest plates (NUNC UK Ltd., Stafford, Great Britain) each received 1 μCi $Na_2 \, ^{51}Cr \, O_4$ in 25 μl phosphate-buffered Waymouth's medium MB 752/1 containing 5% fetal calf serum. After incubation at 37°C for 1.5 h followed by five washes, each well received medium (25 μl) antiserum (25 μl) and C (25 μl). In controls, medium replaced antiserum, C, or both. Total releasable isotope was determined by incubation of monolayers with distilled water (75 μl). After 1.25 h incubation at 37°C, 25 μl of supernate from each well was counted in a Packard 3002 gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) and the counts expressed as a percentage of total releasable isotope. All assays were done in triplicate.

Results

Absorptions with Platelets.   Unabsorbed 4454 antiserum (UNABS), cytotoxic towards >95% PBL of S1/S1 type (Fig. 1 A; f) as shown by high isotope release and confirmed by dye exclusion, was absorbed serially, fourfold, with S1/S1 platelets. The absorbed serum (ABS) was still cytotoxic towards a proportion of S1/S1 PBL (Fig. 1 A; a–d). Two subpopulations of leukocytes (L_1 and L_2), are therefore distinguished. Absorbed antibodies, eluted from platelets (ELU), and concentrated fourfold, were as cytotoxic as UNABS (Fig. 1 A; e, compare f). ELU therefore recognizes antigens present on platelets and all PBL; ABS recognizes antigens present on L_2 but absent from platelets and L_1.

Absorptions with Liver Cells.   Absorption of ELU with S1/S1 liver cells removed essentially all cytotoxicity for S1/S1 PBL (Fig. 1 A; g). This suggests that liver cells express all antigens present on platelets. Serial fourfold absorption of ABS with liver cells removed little or no cytotoxic antibody (Fig. 1 B; a–d, and e), and similar absorption of 4454 UNABS yielded an antiserum of restricted cytotoxicity for S1/S1 PBL, (Fig. 1 C; a–d, and e) apparently the same as ABS, as would be expected if platelets and liver cells possess an identical array of antigens.

Absorption with S1/S1 Kidney Cells or PBL.   All cytotoxic antibodies in 4454 UNABS (Fig. 1 D; a) were removed by serial fourfold absorption with S1/S1 kidney cells (Fig. 1 D; b–e) or with S1/S1 PBL (Fig. 1 D; f). Kidney therefore possesses not only those antigens expressed by liver cells, platelets, and L_1, but also those antigens restricted to L_2.
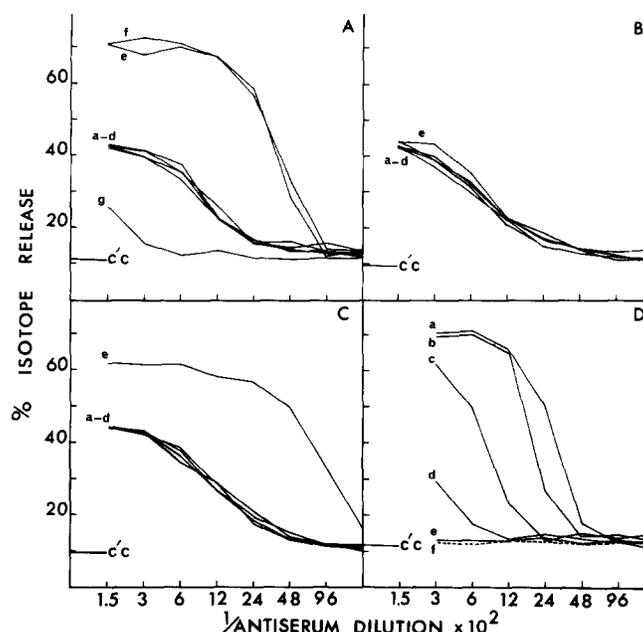
FIG. 1. Cytotoxicity for S1/S1 PBL targets. (A) a–d, antiserum 4454 absorbed four times with S1/S1 platelets. Titrations made after successive absorptions gave overlapping lines (a–d); e, antibodies eluted from platelets (ELU); f, 4454 UNABS; and g, ELU reabsorbed with S1/S1 liver cells. (B) a–d, ABS reabsorbed four times with S1/S1 liver cells; and e, ABS. (C) a–d, 4454 UNABS absorbed four times with S1/S1 liver cells; and e; 4454 UNABS. (D) a, 4454 UNABS; b–e, 4454 UNABS absorbed four times with S1/S1 kidney cells; and f, 4454 UNABS after three absorptions with S1/S1 PBL. C'C, C toxicity.

Specificity Controls.   4454 UNABS was not cytotoxic towards S5/S5 PBL, even at high concentrations (Fig. 2 A; b). The same target cells were totally lysed by an antiserum specific for the S5 haplotype, (Fig. 2 A; a). When 4454 UNABS was serially absorbed threefold with S5/S5 PBL, cytotoxicity towards S1/S1 PBL was unaffected, (Fig. 2 B; a, and b); similar threefold absorption with S1/S1 PBL removed all cytotoxicity (Fig. 2 B; c).

Cytotoxicity of 4454 UNABS, ABS, and ELU Towards Liver and Kidney Cell Monolayers.   When S1/S1 monolayers from the same animal were used as targets, 4454 UNABS and ELU were cytotoxic for both liver and kidney (Fig. 3 A and B; a, and c) while ABS was cytotoxic for kidney but had no measurable effect on liver (Fig. 3 A and B; b). Identical results have been obtained with all available cell lines (three kidney and four liver) derived from different S1/S1 pigs. These results confirm the assignments of antigen expression based on the absorption studies described.

Unexpectedly, however, 4454 UNABS and ABS have always shown definite cytotoxicity towards "nonspecific" S5/S5 kidney monolayers, (Fig. 3 D; a, b, c, and d) which may be removed by absorption with S5/S5 kidney cells (Fig. 3 D; e, and f). This also reduced cytotoxicity for S1/S1 kidney monolayers, (Fig. 3 C; compare c, and d: e, and f). This nonspecific killing contrasts with the lack of cytotoxicity of 4454 UNABS for S5/S5 PBL derived from the same animal (Fig. 2 A; b), even at a much higher concentration, and despite the fact that PBL are
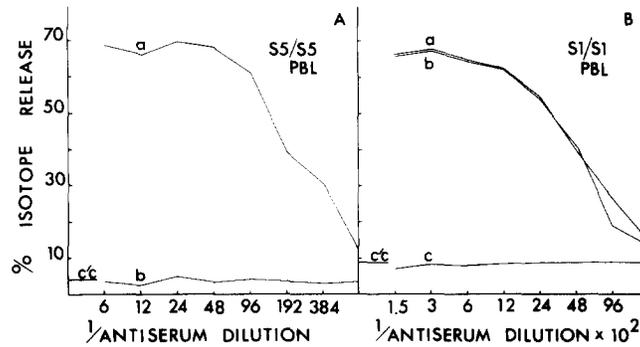
FIG. 2. Specificity controls. (A) S5/S5 PBL targets: a, effect of anti-S5 serum; and b, effect of 4454 (UNABS). (B) S1/S1 PBL targets: a, effect of antiserum 4454 (UNABS); b, after three absorptions with S5/S5 PBL; and c, after three absorptions with S1/S1 PBL.
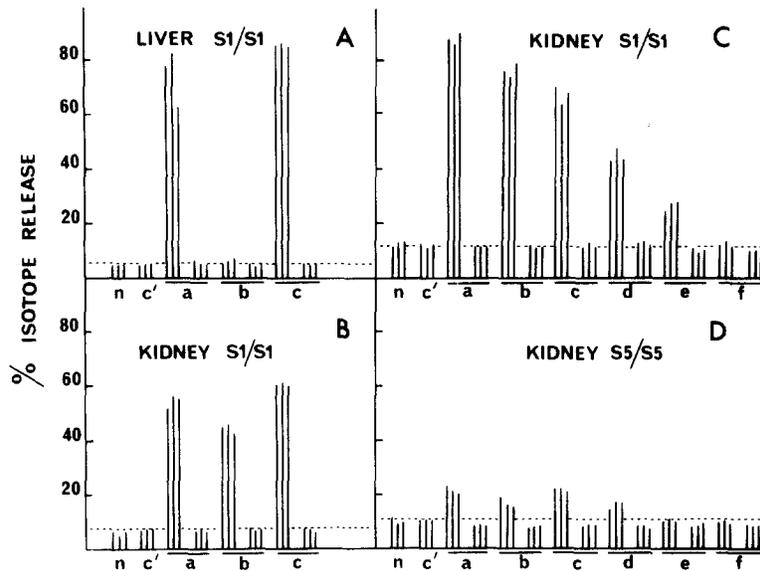

FIG. 3. Cytotoxicity for kidney and liver cell monolayers: (triplicate assays; in each group, left block contains antibody plus C; right contains antibody plus medium). (A and B) S1/S1 liver and kidney from the same animal: a, 4454 (UNABS) at $^1/_{150}$ dilution; b, 4454 ABS at $^1/_{150}$ dilution; and c, ELU at $^1/_{150}$ equivalent concentration. (C and D) S1/S1 and S5/S5 kidney: 4454 UNABS diluted (a) $^1/_{150}$; and (b) $^1/_{450}$. ABS diluted (c) $^1/_{150}$; and (d) $^1/_{450}$. ABS after four absorptions with S5/S5 kidney cells: diluted (e) $^1/_{150}$; and (f) $^1/_{450}$. n, release of isotope by cells in medium only; c', C control (cells plus C plus medium).

more sensitive targets. Preliminary evidence shows that absorption of 4454 with S5/S5 kidney cells does not affect its cytotoxic titer for S1/S1 PBL but absorption of 4454 with S1/S1 PBL, which removes all cytotoxicity for the same target cells, fails to affect the nonspecific killing of S5/S5 kidney monolayers. Nonspecific cytotoxicity for S5/S5 liver cells has not been found. These results indicate that some alloantigens present on kidney cells and skin (since antiserum 4454 was raised by skin grafting) are absent from PBL, platelets, and liver cells.

Discussion

In mice, the K, I, and D regions of the MHC all determine transplantation antigens and there appears to be a cumulative effect between K- and I-region differences on the rate of skin graft rejection (2). In rats, the fate of kidney allografts is determined quantitatively by the degree of disparity between donor and recipient for gene products of the MHC (13). In man, similar observations have been made for skin grafts between related individuals genotyped for HL-A antigens (14). In pigs, the fate of kidney allografts between siblings depends on the degree of disparity of MHC gene products, but liver allografts survive even when totally disparate. This may be due either to unique properties of liver, unrelated to its antigenicity, or to antigenic deficiencies relative to kidney. The latter could arise either through a relative deficiency of liver cells in antigens common to all tissues, or through an absence of certain antigens from liver. The present study supports the second possibility by showing that liver cells do lack antigens present on kidney cells. Some of these antigens are lymphocyte alloantigens of the MHC and resemble Ia antigens of the mouse in showing restricted expression on a lymphocyte subpopulation. Other antigens, less well characterized, are apparently shared by skin and kidney but absent from leukocytes and liver.

It is proposed that the acceptance of liver allografts despite MHC disparity is due to the presentation by the graft of a limited array of alloantigens insufficient to provoke rejection. Primary vascularization of the graft and the release of soluble antigen may also favor the induction of tolerance or enhancement. Subsequent transplantation of a kidney from the same donor would then confront the recipient with yet another limited array of alloantigens, those present on kidney but absent from liver. This would in turn favor unresponsiveness and the donor-specific prolongation of kidney allografts already reported (5).

Summary

Evidence is presented that there are differences between pig liver and kidney parenchymal cells in the expression of major histocompatibility complex antigens. Analogues of I-region antigens of the mouse are detectable on kidney cells and some peripheral blood leukocytes (PBL) but not on liver, platelets, and other PBL. Such differences between liver and kidney may explain (a) the fate of these organs as allografts and (b) the donor-specific protection of kidney by liver. Other antigens peculiar to skin and kidney have been detected but require further characterization.

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