Transplantation of metanephroi across the major histocompatibility complex in rats

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Rogers, Sharon A., Helen Liapis, and Marc R. Hammerman. Transplantation of metanephroi across the major histocompatibility complex in rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R132–R136, 2001.—To determine whether transplanted metanephroi grow, differentiate, and function in hosts that differ in major histocompatibility complex loci (RT1 loci in rats) from donors in a defined way, we implanted metanephroi from embryonic day (E) 15 PVG (RT1c) rat embryos into the omentum of nonimmunosuppressed uninephrectomized PVG-RT1avl (host) rats. By 4 wk posttransplantation, metanephroi had grown and differentiated such that glomeruli, proximal and distal tubules, and collecting ducts had normal structure and ultrastructure. At 12 wk posttransplantation, weights of metanephroi were 54 ± 8 mg. Inulin clearances were 0.9 ± 0.3 μl·min⁻¹·100 g rat wt⁻¹. In vitro, splenocytes from PVG rats stimulated the proliferation of cells originating from both PVG-RT1avl rats in which a transplant had been performed and PVG-RT1avl rats with no transplant. Full-thickness PVG-RT1avl skin engrafted normally on PVG-RT1avl rats in which PVG metanephroi had been previously implanted and metanephroi retained a normal appearance. In contrast, skin from PVG rats sloughed, and the tubular architecture of metanephroi was obliterated by a mononuclear cell infiltrate consistent with acute rejection. Here we show for the first time that functional chimeric kidneys develop from metanephroi transplanted across the MHC from embryonic day (E) 15 PVG (RT1c) (2, 3, 6, 24) rat embryos into the omentum of nonimmunosuppressed uninephrectomized PVG-RT1avl (host) rats. Our data are consistent with a state of peripheral immune tolerance secondary to T cell “ignorance” (4, 14, 18, 19, 25) permitting their survival.

METHODS

Metanephroi were surgically dissected from E 15 embryos under a dissecting microscope using previously described techniques (20) and implanted within 45 min in the omentum of anesthetized 6-wk-old female (host) rats. During the same surgery, host rats had one kidney removed. Four weeks after transplantation, end-to-end ureteroureterostomy was performed with a microvascular technique (interrupted 10–0 suture) between the ureter of a metanephros implanted in the omentum and the ureter of the kidney that had been removed. Eight weeks later all remaining native renal tissue (the contralateral kidney) was removed from host rats, after which inulin clearances were measured in conscious rats after placement of an indwelling bladder catheter and intravenous line exactly as in previous studies (20). Baseline measurements for inulin were performed on urine and blood samples obtained before beginning the inulin infusions. These “background” values were subtracted from measurements performed after beginning the inulin infusion. Infusion of inulin was begun only after removal of all remaining native renal tissue and drainage of all urine remaining in the bladder (10–20 μl). Only the implanted metanephros re-
mained connected to the bladder. As before, rats received no immunsuppression (20). Metanephroi or kidneys were fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin exactly as in previous studies (20).

Electron microscopy was performed using techniques previously described by Liapas et al. (13). Mixed lymphocyte reactivity assays were performed as previously described (5) except the RPMI 1640 was supplemented with 1% glutamine, 1% sodium pyruvate, 1% nonessential amino acids, 0.1% penicillin-streptomycin, 0.1% 2-mercaptoethanol, 10% fetal calf serum, and 1 mM HEPES. The number of stimulator cells used in assays was none or 2 × 10^6. The number of responder cells was 0.5 × 10^6.

RESULTS

Shown in Fig. 1A is a photograph of two metanephroi originating from PVG rat embryos 4 wk after implantation in the omentum of a PVG-RT1avl host. Shown in Fig. 1, B–E, are hematoxylin and eosin-stained sections of similarly transplanted PVG metanephroi. Transplanted developed metanephroi are kidney shaped (Fig. 1A). A ureter is present (Fig. 1B). A cortex and a medulla are present. As would be expected for rodent kidneys (20), developed metanephroi have a single papilla (Fig. 1C). Cortices contain mature-appearing glomeruli and proximal and distal tubules (Fig. 1D). The medulla contains mature collecting ducts (Fig. 1E).

Inulin clearances were measured at 12 wk posttransplantation in metanephroi that had been transplanted from PVG rat embryos into the omentum of PVG-RT1avl or PVG hosts. Weights of metanephroi (n = 5 transplants) were 54 ± 8 mg and urine volumes were 84 ± 21 μl/h. Clearances were 0.9 ± 0.3 μl·min⁻¹·100 g rat wt⁻¹ or 34.0 ± 8.0 μl·min⁻¹·g kidney (metanephros) wt⁻¹, several times higher than those measured previously in Sprague-Dawley→Sprague-Dawley transplants (20). Inulin clearances were comparable to those measured in PVG rats in which metanephroi from PVG embryos (isografts) were transplanted (Table 1).

Clearances were ~0.2% of the mean per single kidney from normal rats (n = 3) expressed as microliters per minute per 100 gram rat weight (460 μl·min⁻¹·100 g⁻¹) and 9% of those measured per single kidney of normal rats expressed as microliters per minute per g kidney weight (375 μl·min⁻¹·g⁻¹).

Electron microscopy of metanephroi transplanted 4 wk previously was performed (Fig. 2). Shown in Fig. 2A is a glomerular capillary loop containing two red blood cells. Labeled is the nucleus of a normal-appearing endothelial cell, a visceral epithelial cell, and epithelial podocytes. A basement membrane of normal appearance (10) is delineated. Figure 2B shows a lower-power view of a glomerulus. Mesangial cells and parietal

Table 1. Weights of metanephroi, urine volumes, and inulin clearances

<table>
<thead>
<tr>
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<th>PVG→PVG (n = 6)</th>
<th>PVG→PVG-RT1avl (n = 5)</th>
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<tbody>
<tr>
<td>Weight, mg</td>
<td>47 ± 4.7</td>
<td>54 ± 8.0</td>
</tr>
<tr>
<td>Urine vol, μl/h</td>
<td>89 ± 18</td>
<td>230 ± 58</td>
</tr>
<tr>
<td>Inulin clearance, μl·min⁻¹·100⁻¹ g kidney wt⁻¹</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>35 ± 6.4</td>
<td>34 ± 8.0</td>
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Data are expressed as means ± SE.
epithelial cells are shown. Figure 2C shows a proximal tubule with a relatively immature brush border, consistent with what would be expected in a young rat (1, 10). A distal tubule is shown in Fig. 2D, and a collecting duct in Fig. 2E.

Normally, T cells are activated via the cross-linking of their antigen receptor after the recognition of target antigen/MHC complexes presented on appropriate cells (4). There is evidence that peptides may be presented in the context of MHC class I on cells that are unable to trigger any response from T cells with the appropriate T cell receptor. T cells apparently “ignore” the presentation of antigen by these “nonprofessional” transplant antigen presenting cells (APCs) and under some circumstances cannot recognize transplant antigen presented by host dendritic cells. The T cells are able to respond if the appropriate transplant antigen is later presented by a transplant “professional” APC, such as a dendritic cell and, once stimulated in this way, the T cells are able to mediate effector functions on the previously ignored antigen source (4, 14, 18, 19, 25).

To determine whether a state of peripheral tolerance secondary to T cell ignorance is present in PVG-RT1avl hosts, we first evaluated the reactivity of responder splenocytes obtained from PVG-RT1avl rats in which no transplant had been performed and the reactivity of splenocytes obtained from PVG-RT1avl rats in which a transplant had been performed toward stimulator splenocytes from PVG rats (Table 2).

As a control, we determined that splenocytes from PVG-RT1avl rats did not stimulate the proliferation of splenocytes from either transplanted or nontransplanted PVG-RT1avl rats above baseline (no stimulator; none). In contrast, splenocytes from PVG rats stimulated the proliferation of cells originating from both PVG-RT1avl rats in which no transplant had been performed and of cells originating from PVG-RT1avl rats in which a transplant had been performed (Table 2).

To shed light on why metanephroi originating from PVG rats survive in PVG-RT1avl hosts under circumstances where reactivity in vitro of splenocytes from PVG-RT1avl rats toward splenocytes from PVG rats can be demonstrated, we performed full-thickness skin grafts from PVG or PVG-RT1avl donors onto PVG-RT1avl rats into which a metanephros from a PVG rat had been implanted 3 wk previously and had been developing normally as judged by visual observation. Ten days later, transplanted metanephroi were removed from PVG-RT1avl rats.

In contrast to the appearance of engrafted skin grafts 10 days postremoval from PVG-RT1avl donors (Fig. 3A), skin grafts from PVG donors had sloughed, leaving an open wound (Fig. 3B). Metanephroi from PVG-RT1avl rats transplanted into PVG-RT1avl rats that received skin from PVG-RT1avl rats into which a metanephros from a PVG rat had been implanted 3 wk previously and had been developing normally as judged by visual observation. Ten days later, transplanted metanephroi were removed from PVG-RT1avl rats.

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Table 2. Mixed lymphocyte reactivity

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>(PVG-RT1avl)</th>
<th>(PVG-RT1avl + Transplant)</th>
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<tr>
<td>None</td>
<td>700 ± 181</td>
<td>915 ± 374</td>
</tr>
<tr>
<td>PVG</td>
<td>8,033 ± 1,637</td>
<td>18,452 ± 366*</td>
</tr>
<tr>
<td>PVG-RT1avl</td>
<td>1,065 ± 547</td>
<td>566 ± 135</td>
</tr>
</tbody>
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Data are means ± SE of triplicate measurements. Multiple comparisons were made using Bonferroni’s test. Responder PVG-RT1avl.

*PVG > None, P < 0.05; *PVG > PVG-RT1avl, P < 0.05. Responder PVG-RT1avl + transplant: †PVG > None, P < 0.001; *PVG > PVG-RT1avl, P < 0.001.
DISCUSSION

If an allograft or xenograft is devoid of professional APCs (dendritic cells) of donor origin, then T cell ignorance could result in acceptance (4, 14, 18, 19, 25). Such a mechanism has been invoked to explain the acceptance in mice of rat pancreatic islets that have been placed in culture to rid them of APCs (11). It would be expected that acceptance could be overcome by host exposure to donor-type professional APCs.

Although the technique of APC removal via culturing pancreatic islets is effective in rodents (11), it is more difficult to achieve the same results with whole organs such as kidneys (12, 15). However, it is possible to deplete dendritic cells from donor kidneys by the combination of total body irradiation and cyclophosphamide pretreatment of donors and, in doing so, prolong graft survival in RT1 incompatible hosts (15). Also, long surviving “immunologically enhanced” (dendritic cell depleted) kidneys transplanted into RT1 incompatible hosts do not elicit strong primary T cell-dependent alloimmunity after transplantation into a secondary recipient of the same genotype as the original host (12). “Immunological enhancement” is accomplished by injecting hosts with donor-strain spleen cells before transplantation and host anti-donor antisera before transplantation and at the time of transplantation (12).

In contrast to mature organs, a developing organ, such as an E 15 metanephros would be expected to be depleted of dendritic cells of donor origin by virtue of the absence of a vasculature from which donor dendritic cells can enter the organ before its removal from the donor embryo (20) and the absence of mature dendritic cells themselves at this stage of rat embryonic development (16). For these reasons, the use of a developing metanephros in lieu of a developed kidney could provide a means by which transplantation of an organ depleted of donor dendritic cells can take place.

The data shown in Table 2 show that splenocytes from PVG-RT1avl hosts that received kidneys from PVG donors are capable of reacting in vitro when mixed with splenocytes from PVG donors. Therefore, T cells from PVG-RT1avl hosts containing PVG transplants can recognize PVG splenocytes (that include PVG dendritic cells) as foreign in vitro.

The ability of PVG-RT1avl hosts to reject full-thickness skin grafts from adult PVG donors is demonstrated in Fig. 3B. Therefore, T cells from PVG-RT1avl hosts containing PVG transplants can recognize PVG tissue as foreign in vivo. Both full-thickness skin from adult PVG donors (Fig. 3B) and transplanted metanephroi (Fig. 3D) are rejected after transplantation of PVG skin that, unlike PVG metanephroi, contains donor dendritic cells (16). The data shown in Fig. 3 suggest that a state of peripheral immune tolerance secondary to T cell ignorance permits the survival and growth of transplanted metanephroi, because after a source of PVG dendritic cells is provided to PVG-RT1avl hosts (skin), both the skin and the transplanted PVG metanephroi are rejected.

Findings similar to those we report were observed after the transplantation of dendritic cell-depleted kidneys from adult rats to MHC incompatible hosts (12). In fact, retransplanted immunologically enhanced kidneys are acutely rejected after hosts are injected with donor dendritic cells (12).

Class II MHC heterodimers on nonlymphoid, renal proximal tubule cells in transplanted metanephroi might be expected to present transplant antigens to host T cells in the absence of donor dendritic cells, resulting in rejection of transplanted kidneys or metanephroi. However, such is not the case (12, 15) (Figs.
1–3). It may be that, as in murine proximal tubule cells (7), class II MHC occurs without coexpression of costimulatory receptors such as B7 and serves as an extra thymic mechanism for the maintenance of immune tolerance (8).

Perspectives

A shortage exists of human kidneys available for transplantation (22). Transplantation of pig kidneys into humans has been suggested as a substitute for allotransplantation. Unfortunately, the transplantation of porcine vascularized organs such as kidneys into humans is rendered problematic, in part because of the reaction of preformed antibodies against antigens present on the vascular endothelium of the pig (hyperacute rejection). Theoretically, hyperacute rejection of pig metanephroi transplanted into humans should be muted as a function of the extent that the developed organ becomes vascularized by the host (22).

There is evidence that both angiogenesis and vasculogenesis contribute during development to the blood supply of the kidney (9, 23). The origin of the vascular endothelium in metanephroi transplanted to ectopic sites depends on the stage of development at which transplantation takes place (9) and possibly on the site (9, 23). In the case of 11-day-old mouse or chick metanephroi grafted onto the chorioallantoic membrane of the quail, the vasculature is derived entirely from the host (23). In the case of 11- to 12-day-old mouse metanephroi grafted into the anterior chamber of the eye, the glomerular microvascular endothelium derives from both donor and host (9). In either case, large external vessels derive from the host (9, 23).

Clearances of transplanted metanephroi shown in Table 1 are too low to sustain life (8, 20, 21). However, clearances can be increased by more than 100-fold by removal of host renal mass at the time of implantation (20), incubating metanephroi with growth factors such as vascular endothelial growth factor before implantation or removal of host renal mass at the time of implantation (8), and by the administration of insulin-like growth factor I to hosts (21).

Given the host origin for much (9) or all (23) of their vasculature, to the extent that a state of T cell ignorance toward transplanted metanephroi can be induced after pig→human metanephros transplantation as it is after PVG→PVG-RT1<sup>av</sup> rat transplantation, the use of xenograft metanephroi for transplantation into humans may prove to be advantageous relative to the use of developed kidneys.

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