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Murine Renal Allografts: Spontaneous Acceptance Is Associated with Regulated T Cell-Mediated Immunity

Alice A. Bickerstaff,* Jiao-Jing Wang,* Ronald P. Pelletier,* and Charles G. Orosz

It was shown >20 yr ago that mice will spontaneously accept renal allografts in the absence of immunosuppression, but the mechanism responsible for this is not understood. We transplanted DBA/2 (H-2d) kidneys into nephrectomized C57BL/6 (H-2b) mice, and the allografts were spontaneously accepted for >60 days without immunosuppression. In contrast, nonimmunosuppressed cardiac and skin allografts in the same strain combination are rejected within approximately 10 days. The accepted renal allografts have a prominent leukocytic infiltrate, suggesting an ongoing, local immune response. At 60 days post-transplant, the recipients of accepted renal allografts display DBA/2-reactive alloantibodies. They also display DBA/2-reactive delayed-type hypersensitivity responses that are actively counter-regulated by DBA/2-induced TGF-β production, but not by IL-10 production. These data suggest that a donor-reactive, cell-mediated immune mechanism involving TGF-β is associated with the spontaneous acceptance of renal allografts in mice. The Journal of Immunology, 2001, 167: 4821–4827.

One long-standing tenant of transplant biology is that MHC-mismatched allografts rapidly initiate the process of acute rejection in the absence of immunosuppression. This is certainly true for murine skin allografts (1), cardiac allografts (2), intestinal allografts (3), islet allografts (4), and hepatocyte allografts (5). However, it is not necessarily true for liver allografts (3) or renal allografts (6). To date, most experimental effort in transplantation has been directed toward identifying the mechanisms of acute allograft rejection, and its subversion by immunosuppressive agents. These studies have demonstrated that numerous pharmacologic agents can block acute rejection, resulting in extended allograft survival.Interestingly, some of these agents can be withdrawn after a relatively brief period of peritransplant treatment with minimal risk for the subsequent development of acute rejection. Such agents include anti-VCAM-1 mAb (7), anti-CD4 mAb (8), anti-CD40 ligand (anti-CD40L) mAb (9), and galium nitrate (GN)3 (10). Many investigators have studied the immunobiology of this phenomenon, but the specific mechanisms by which these agents subvert acute rejection and promote allograft survival after treatment is discontinued remain ill defined.

We have studied the mechanisms by which anti-CD4 mAb, GN, and anti-CD40L mAb influence alloimmunity in murine cardiac allograft recipients. In general, the evidence available to date indicates that the allograft acceptance caused by these agents is not due to drug-induced ignorance of donor alloantigens, since the allograft acceptors continue to make donor-reactive alloantibodies and to generate donor-induced cytokines (11). Instead, allograft acceptance is associated with a shift from a proinflammatory to an anti-inflammatory T cell disposition toward donor alloantigens. This shift can be demonstrated by monitoring donor-reactive delayed-type hypersensitivity (DTH) responses. Nonsuppressed cardiac allograft rejectors mount strong DTH responses to challenge with donor alloantigens, illustrating a proinflammatory T cell disposition toward donor alloantigens (12). In contrast, allograft acceptors fail to mount donor-reactive DTH responses (13). This failure is an active rather than a passive process, since inclusion of anti-TGF-β or anti-IL-10 Abs at the DTH challenge site restores donor-reactive DTH responses (14). Hence, the allograft acceptors are, in fact, sensitized for donor-reactive DTH responses, but fail to mount them because of dominant, anti-inflammatory, donor-reactive mechanisms that rely on the local, alloantigen-induced production of TGF-β and IL-10 to block the proinflammatory donor-reactive T cell responses. Whether the expression of this anti-inflammatory T cell response is responsible for the prolonged allograft survival that develops in allograft acceptor mice remains to be determined.

It has been known since the 1970s that murine renal allografts are often spontaneously accepted (6), yet virtually nothing is known about the immune mechanisms responsible for this acceptance. An attractive hypothesis is that acceptance is due to the spontaneous development of an anti-inflammatory immune regulatory mechanism similar to the one developed by immunosuppressed cardiac allograft acceptor mice. The early studies with renal allograft acceptors were extensive, but they failed to uncover direct evidence of immune regulation (6). DTH studies were not included in this analytic effort. Studies reported in this communication used donor-reactive DTH responses to probe the donor-reactive immune disposition in murine renal allograft acceptors. These studies provided direct evidence that these acceptor mice express an anti-inflammatory, rather than a pro-inflammatory, disposition toward donor alloantigens.

Materials and Methods

Mice

C57BL/6 (H-2b) and DBA/2 (H-2d) mice were obtained from Taconic Farms (Germantown, NY). All mice were housed and treated in accordance with institutional guidelines.
with animal care guidelines established by the National Institutes of Health and Ohio State University.

Murine kidney transplantation

Murine kidney transplantation was performed as described by Zhang et al. (15). Briefly, the donors left kidney was isolated by ligating and dividing the adrenal and testicular vessels with microscure. The aorta and inferior vena cava (IVC) were mobilized at their junction, with the left renal artery and vein. The aorta was ligated below the renal vessel. An elliptical patch of bladder containing the left ureterosvesical junction was excised. The graft was perfused in situ with 0.2–0.4 ml cold, heparinized Ringer’s lactate. Finally, the kidney with vascular supply and ureter attached to the bladder patch were harvested en bloc. The recipient right native kidney was removed immediately before transplantation. The infrarenal aorta and IVC were carefully isolated and cross-clamped. An end-to-side anastomosis between the donor renal vein and the recipient IVC was performed. Following successful anastomosis the kidney graft perfused instantly. Urinary reconstruction was then performed by a bladder-to-bladder anastomosis. The right native kidney was removed 1 wk post-transplantation. Kidney graft survival was followed by daily examinations of overall animal health. The technical success rate was 85%.

Serum creatinine determination

Quantitative serum creatinine levels were determined using kits from Roche Molecular Biochemicals (Indianapolis, IN). Creatinine reagents and a Roche/Hitachi analyzer were used to perform the analysis. Conventional units (milligrams per deciliter) were converted to Systeme International units by multiplying the conventional units by 88.4. The concentration of creatinine in the serum is expressed as micromoles per liter.

Murine cardiac transplantation

Heterotopic cardic transplantation was performed as described by Corry et al. (16). In general, the native hearts from heparinized donor mice (DBA/2) were anastomosed to recipient B6 abdominal aorta and vena cava using microsurgical techniques. Graft survival was assessed by transabdominal palpation.

Immunosuppression with GN

As described previously (12), GN (Galite, Fujisawa, Deerfield, IL.) was administered as an initial s.c. bolus injection of 2.2 mg 24 h before graft implantation. This was followed by 28 days of continuous delivery via osmotic minipumps (model 2002, Alzet, Palo Alto, CA), which delivered 0.5 μl (12.5 μg GN)/h. Circulating levels of GN fall to subtherapeutic levels within 7 days of pump removal (10).

Murine skin transplantation

Split-thickness skin allografts were performed according to the method of Billingham et al. (1). Briefly, the ears were removed from anesthetized DBA/2 mice and placed in cold PBS. Forceps were used to raise a split-thickness epithelial flap from the cartilaginous bed at the base of the ear; the flap was separated along the length of the ear and trimmed appropriately. Oval grafts (8 × 10 mm) were placed on the graft beds prepared on the recipient’s flank. The graft was covered with a protective bandage for 5 days. Rejection was considered to occur at the point when the grafts exhibited dark discoloration, scabbing, and necrotic degeneration.

Subcellular alloantigen

Subcellular alloantigen was prepared according to the method described by Engers et al. (17). Briefly, fresh RBC-depleted DBA/2 splenocytes suspended in PBS were subjected to three rapid freeze/thaw cycles using liquid nitrogen and spun at 13,000 rpm for 30 min to remove the residual debris. The supernatant was adjusted to 3–5 mg protein/ml and used as the source of subcellular alloantigen. For DTH challenge, 25 μl (75–125 μg protein) of this solution was injected into murine pinnae.

Tetanus toxoid (TT)

TT (Wyeth-Ayerst, Marietta, PA) was obtained at a concentration of 10 limits of flocculation (Lf)/ml in PBS. To sensitize mice, 0.1 ml (1 Lf) TT was injected s.c. at least 14 days before DTH challenge. To challenge mice for DTH reactivity, 25 μl (0.25 Lf) TT was injected at the DTH challenge site.

Cytokine Abs used in DTH assay

Polynuclear rabbit anti-TGF-β, polynuclear goat anti-IL-10, control rabbit IgG, and control goat IgG were all obtained from R&D Systems (Minneapolis, MN).

DTH responses

Direct DTH method. Sensitized mice received pinnae injections containing 25 μl challenge Ag using a 30-gauge insulin syringe.

Transfer DTH method. Kidney acceptor mice, skin rejector mice, and cardiac rejector mice were tested for DTH responses between 60–90 days post-transplant using a transfer DTH assay. For this assay, the pinnae of naive B6 mice were injected using a 30-gauge insulin syringe, with 25 μl containing 8 × 10^7 syngeneic splenocytes from the transplanted mice plus challenge alloantigen with or without 25 μg neutralizing cytokine Abs. Changes in ear thickness were measured both before injection and 24 h after injection using a dial thickness gauge (Swiss Precision Instruments, Carlstadt, NJ). For reference, changes in the range of 0–30 × 10^{-4} in represent background swelling due to injection trauma, changes in the range of 40–60 × 10^{-4} in represent moderate DTH responses, and changes in the range of 70–100 × 10^{-4} in represent strong DTH responses.

Alloantibody analysis

The presence of DBA/2- or C57BL/6-reactive IgG Ab was determined by the ability of serum to bind to DBA/2 or C57BL/6 thymocytes. Binding was detected by flow cytometry, using FITC-conjugated goat anti-mouse IgG (γ-chain specific; Pierce, Rockford, IL). Results are shown as the percentage of DBA/2 or C57BL/6 thymocytes that bound detectable alloantibody. Treatment of DBA/2 thymocytes with serum from naive C57BL/6 mice resulted in <2% binding.

Histologic examination of renal tissue

Renal tissues were excised and fixed in 10% neutral buffered formalin, dehydrated in upgraded ethanol (70, 95, and 100%), and embedded in paraffin. For histologic analysis, four to six μ sections were mounted on slides and stained with H&E.

Results

Immune phenotype associated with renal allograft acceptance

Studies presented in this communication are based on the observation that DBA/2→C57BL/6 renal allografts are often spontaneously accepted without immunosuppression. This is illustrated in Fig. 1. In the DBA/2→C57BL/6 strain combination, all cardiac allografts are rapidly rejected within 7–10 days, and all skin allografts are rejected within 15–17 days, yet 80% of the kidney allografts remain functional for >60 days. Indeed, the survival of renal allografts is similar to the survival of renal isografts (Fig. 1). In this model, physiologically effective function of the allograft is assured, since the native kidneys of the transplant recipient are removed, making the graft recipient totally dependent on renal allograft function for survival. Analysis of serum creatinine levels also demonstrates normal levels of renal function in renal allograft recipients (Table 1). Indeed, C57BL/6 mice that have spontaneously accepted DBA/2 kidneys for >60 days display the same serum creatinine levels as normal naive C57BL/6 or DBA/2 mice or C57BL/6 isograft recipients (Table 1).

Several observations suggest that renal allograft acceptance is not simply due to immune ignorance. First, the recipients of accepted renal allografts make donor-reactive alloantibodies. Cytometric analysis revealed that serum obtained from renal allograft recipients at 60 days post-transplant contains IgG that can bind to DBA/2, but not C57BL/6, thymocytes (Fig. 2). Further, alloantibody levels in the recipients of accepted renal allografts are comparable to those in recipients of rejected cardiac allografts within the same strain combination. This indicates that at the very least the immune system of the allograft recipient has recognized and responded to the allogeneic MHC class I molecules of the renal allograft. Thus, functional graft-reactive lymphocytes remain present in the renal allograft acceptor, and the inflammatory responses that develop at the graft site do not occur in the absence of central alloantigen recognition by the allograft recipient.

Second, histologic analysis of tissue sections stained with H&E reveals that the renal allografts undergo significant leukocytic infiltration, which persists for at least 60 days (Fig. 3). At 60 days
post-transplant, renal allografts (Fig. 3A), but not isografts (Fig. 3B), have prominent infiltration by mononuclear leukocytes. In general, there is a cuffing of leukocytes around the vessels and small aggregates of mononuclear cells scattered within the cortex of accepted renal graft tissue. Nevertheless, allografts show no histologic evidence of tissue destruction, nor do they exhibit endothelialitis or tubulitis. Studies suggest that the leukocytic infiltration of accepted renal allografts is immunologically active. Real-time PCR analysis reveals that the mRNA levels for IFN-γ, IL-2, and IL-10 are much higher in the accepted renal allografts than the background cytokine mRNA levels found in normal, nontransplanted DBA/2 kidneys (data not shown). Based on these observations, the accepted renal allografts appear to be the focus of an active immune process. Accepted renal allografts also demonstrate some histopathologic changes not seen in renal isografts (Fig. 3). Trichrome staining reveals renal allografts taken at 70 days post-transplant (Fig. 3, D and G) have noticeably more interstitial fibrosis than renal isografts (Fig. 3, E and H) or normal kidneys (Fig. 3, F and I). Despite this fibrosis and the presence of circulating donor reactive alloantibodies, no intimal formation was observed in the arterial structures of these tissue sections. Thus, accepted renal allografts develop some, but not all, features of chronic allograft rejection within 70 days of transplantation.

Evidence for immunoregulation in renal allograft acceptors

As reported previously, a similar immune phenotype is exhibited by C57BL/6 mice that accept DBA/2 cardiac allografts due to transient immunosuppression with anti-CD4 mAb (10), GN (10), or anti-CD40L. These cardiac allograft acceptors display donor-reactive alloantibodies, but not donor-reactive DTH responses (13). As shown in Fig. 4, nonimmunosuppressed renal allograft acceptors also fail to display DTH responses when challenged 60 days post-transplant with either intact DBA/2 splenocytes (cellular donor Ag) or a freeze/thawed extract of DBA/2 splenocytes (subcellular donor Ag). In contrast, nonimmunosuppressed cardiac or skin allograft rejectors mount potent donor-reactive DTH responses when challenged with either cellular or subcellular donor alloantigens. Thus, neither the direct nor the indirect pathway of T cell alloantigen recognition initiates a proinflammatory, donor-reactive immune response after cutaneous challenge in renal allograft acceptor mice.

Table I. Serum creatinine levels in mice

<table>
<thead>
<tr>
<th>Renal allograft recipients (DBA/2→C57BL/6)</th>
<th>Creatinine (μmol L⁻¹ ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postoperative day 20 (n = 8)</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>Postoperative day 40 (n = 8)</td>
<td>43 ± 28</td>
</tr>
<tr>
<td>Postoperative day 60 (n = 8)</td>
<td>37 ± 8</td>
</tr>
<tr>
<td>Renal Isograft Recipients (C57BL/6→C57BL/6)</td>
<td></td>
</tr>
<tr>
<td>Postoperative day 20 (n = 4)</td>
<td>34 ± 13</td>
</tr>
<tr>
<td>Postoperative day 40 (n = 4)</td>
<td>34 ± 13</td>
</tr>
<tr>
<td>Postoperative day 60 (n = 8)</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>Nontransplanted DBA/2 mice (n = 4)</td>
<td>27 ± 8</td>
</tr>
<tr>
<td>Nontransplanted C57BL/6 mice (n = 4)</td>
<td>29 ± 4</td>
</tr>
</tbody>
</table>

In cardiac allograft acceptors, the failure to mount donor-reactive DTH responses is caused by donor alloantigen-induced counter-regulatory mechanisms that block DTH responses (13). Thus, these mice cannot mount DTH responses to a recall Ag, such as TT, if it is colocalized with donor alloantigens at the DTH challenge site. Renal allograft acceptors exhibit a similar DTH counter-regulatory mechanism (Fig. 5). When the pinnae of naive C57BL/6 mice are injected with splenocytes from tetanus-sensitized renal allograft acceptors plus tetanus, prominent DTH responses develop. In contrast, an injection with the same splenocytes plus either cellular or subcellular donor-derived alloantigens results in no response. Donor-reactive DTH counter-regulation is revealed when the pinnae are injected with acceptor splenocytes plus a mixture of TT and either cellular or subcellular donor alloantigens. Under these conditions, the tetanus-reactive DTH responses are lost. Thus, splenocytes from renal allograft acceptors display a donor alloantigen-induced immune mechanism that interferes with DTH responses. This DTH counter-regulatory mechanism can be induced by indirect, and possibly direct, donor alloantigen recognition.

In cardiac allograft acceptor mice, the mechanism that counter-regulates DTH responses is mediated by both TGF-β and IL-10 (14). This can be demonstrated by including Abs that bind either TGF-β or IL-10 along with donor alloantigens at DTH challenge sites. Under these conditions, donor-reactive DTH responses are restored. Splenocytes from renal allograft acceptor mice display a similar, cytokine-mediated DTH counter-regulatory mechanism (Fig. 6). When the pinnae of naive mice are injected with donor alloantigens plus anti-TGF-β Abs, donor-reactive DTH responses are restored. Interestingly, when they are injected with donor alloantigens plus anti-IL-10 Abs, only background levels of swelling develop. Thus, it appears that the regulation of donor-reactive cell-mediated immunity in nonimmunosuppressed DBA/2 × C57BL/6 renal allograft acceptor mice is mediated by TGF-β, but not by IL-10. This contrasts with the regulation of cell-mediated immunity in immunosuppressed DBA/2 × C57BL/6 cardiac allograft acceptor mice, which is mediated by both TGF-β and IL-10 (14).

**Discussion**

Almost 25 yr ago, Russell and colleagues (6) demonstrated that heterotopic murine renal allografts can survive for long periods (60–100 days) without the need for immunosuppression to block acute rejection. Subsequent studies by others have either confirmed (18–20) or contested (21, 22) this observation. Our studies in the DBA/2 × C57BL/6 strain combination support the original observations of Russell and colleagues by demonstrating the >60- to 100-day survival of renal allografts in the absence of immunosuppression (Fig. 1). This strain combination represents one of the
in ear thickness (mean ± SD) when naive C57BL/6 mice are challenged with cellular DBA/2 alloantigen (n = 4).

FIGURE 4. Untreated kidney allograft acceptors fail to express donor-reactive DTH responses. Kidney allograft acceptor, cardiac allograft rejector, and skin rejector mice were challenged in the pinnae with cellular (□) or subcellular (□) donor alloantigen (DBA/2). DTH responses were measured after 24 h as the change in ear thickness (mean ± SD). The dashed line, background (BKG), represents the mean change in ear thickness + SD when naive C57BL/6 mice are challenged with cellular DBA/2 alloantigen (n = 4).

We note that renal allografts represent an interesting and unusual allograft model in that they are concurrently subject to two types of allograft rejection responses, one that is cardiac-like and one that is skin-like. As immediately revascularized organs, renal allografts are quite similar to cardiac allografts, and one might expect them to induce similar immune responses. However, unlike cardiac allografts, renal allografts require a second anastomosis with the recipient at the level of the bladder. This bladder connection is not immediately revascularized. Rather, it requires a period of angiogenesis during which the microvasculature of the donor and recipient bladder wall must form a functional interface. This component of renal engraftment is similar to early events that occur in skin allografts. The immunologic significance of this component is unclear, but graft-compromising problems with bladder anastomoses are observed in approximately 20% of the allograft recipients around 2–70 days post-transplant (Fig. 1). These are often considered as technical failures. However, they could also result from acute rejection responses in the bladder wall. Indeed, such technical failures are somewhat less common in renal isografts (Fig. 1). Interestingly, the pathobiology of the bladder anastomosis has been completely ignored in favor of the pathobiology within the kidney itself. The concern is that two different immune responses, one in the immediately revascularized kidney and one in the slowly revascularized bladder, may be associated with renal allograft rejection. Although either of these mechanisms could compromise renal allograft function, only one of them is studied, while the other is completely ignored. The possibility that two mechanisms of acute rejection operate in renal allografts raises the possibility that they may be differentially regulated. Clearly, this could complicate studies of immune regulation in this experimental system.

Fortunately, the majority of renal allografts avoid acute rejection regardless of its mechanism. The obvious question is why renal allografts are spontaneously accepted while cardiac allografts are spontaneously rejected despite identical MHC disparities and allograft location within the abdomen. According to current immunologic thinking, there are three possibilities: ignorance (clonal deletion), anergy, or immune regulation. Ignorance due to clonal deletion is excluded because the grafts of the acceptor mice have...
striking leukocytic infiltration (19) (Fig. 2) that involves T cells (19). Further the allograft acceptors exhibit donor-reactive MLC activity (6) and sufficient in vivo donor-reactive T cell function to produce donor-reactive IgG (19) (Fig. 2).

Anergy as a cause of allograft acceptance remains subject to debate. Anergy at the CD4+ T cell level is excluded by the persistence of alloantibody production, as described above. Anergy at the CD8+ T cell level remains a possibility. Early studies indicated that renal allograft acceptors display a significant depression in donor-reactive CTL activity (6), although they did not determine whether normal numbers of CD8+ T cells were present in allograft acceptor mice. Interestingly, a similar depression of donor-reactive CTL has been reported in transiently immunosuppressed cardiac allograft acceptor mice (20, 25). Further, renal allograft acceptor mice can reject donor-matched skin allografts (a process thought to involve both CD4 and CD8 T cells) without losing renal function (6). Thus, the case for T cell anergy as a mechanism of renal allograft acceptance remains open.

In contrast, a case can now be made for immune suppression. Detailed initial studies with renal allograft acceptors uncovered little or no evidence of donor alloantigen-associated immune suppression. In their analytic tests they observed 1) induction of skin allograft rejection by naive mice that received lymph node cells from renal allograft acceptor mice, 2) induction of graft-vs-host disease in neonatal mice after transfer of lymph node and spleen cells from renal allograft acceptor mice, 3) neutralization of donor alloantigen-matched tumor cells in vivo by spleen and lymph node cells from renal allograft acceptor mice, and 4) induction of in vitro donor-reactive CTL generation by mixtures of splenocytes from normal naive mice and allograft acceptor mice (6). Yet, treatment of renal allograft acceptors with donor-sensitized lymphocytes results in transient graft damage, but not in graft rejection (26), suggesting the possible engagement of active suppressor mechanisms. Nevertheless, our studies demonstrated donor alloantigen-linked DTH nonresponsiveness in renal allograft acceptor mice similar to that observed in transiently immunosuppressed cardiac allograft acceptor mice. This DTH nonresponsiveness could be induced by either subcellular donor alloantigen or intact viable donor splenocytes (Fig. 4), suggesting that it involved the indirect, and possibly the direct, pathway of donor alloantigen presentation. Although induction of DTH counter-regulation was donor alloantigen dependent, its effects were not Ag specific. Thus donor-induced DTH inhibition could impair DTH responses to a third party Ag, such as tetanus toxoid (Fig. 5). This is in keeping with the nature of the agent that mediates DTH regulation, TGF-β, which is a nonselective inhibitor of DTH responses (27). The fact that active TGF-β was produced after DTH challenge with donor alloantigens was suggested by the recovery of donor-reactive DTH responses when local TGF-β was neutralized with Abs (Fig. 6).

Given that renal allografts develop immune suppression and thus avoid acute allograft rejection, it becomes necessary to explain how this is associated with their development of at least one feature of chronic rejection, interstitial fibrosis. One possibility is that escape from acute rejection does not reflect the induction of allograft tolerance. Rather, it reflects immune deviation toward an immune response with different pathologic consequences, i.e., chronic tissue remodeling. In this regard it is intriguing that renal allograft acceptors develop donor-reactive IgG (Fig. 2), since such alloantibodies can mediate the development of chronic rejection-like tissue remodeling in cardiac allografts (28). Studies are ongoing to determine the extent of this remodeling that develops over longer periods of time than those evaluated in the reported studies (~60 days). The goal of future studies will be to determine whether the development of immune regulation will lead to stable, long term renal allograft function, or whether it permits eventual compromise of the graft function due to an unregulated immune phenomenon such as chronic allograft rejection.

Several features of this anti-inflammatory response to donor alloantigens are worthy of mention. First is the observation that this anti-inflammatory T cell behavior can develop spontaneously in response to an allograft, and immunosuppressive agents are not necessarily required to artificially force the immune system to adopt this pattern of immune behavior. Apparently both proinflammatory and anti-inflammatory T cell responses are normal options of the murine immune system. This may help to explain why an identical pattern of TGF-β/IL-10-mediated, anti-inflammatory immune behavior develops when cardiac allograft recipients are treated with such disparate immunosuppressants as deleting anti-CD4 mAb (13), deleting anti-CD40L mAb,4 and nondeleting GN (14).

A second feature is the fact that renal allograft acceptors display TGF-β-mediated DTH regulation, but not IL-10-mediated DTH regulation. The mechanistic basis for this is unknown. This pattern makes them different from immunosuppressed cardiac allograft acceptor mice, which concurrently display both DTH regulatory systems. It clearly demonstrates that the TGF-β-mediated and IL-10-mediated regulatory mechanisms can develop independently. Additional support for this comes from studies with B cell knockout mice. These mice can accept cardiac allografts if treated transiently with GN and display only the IL-10-mediated pathway of DTH regulation, not the TGF-β-mediated pathway (A. A. Bickerstaff, manuscript in preparation). Thus, different immune mechanisms may be responsible for the induction or expression of TGF-β-mediated and IL-10-mediated immune regulation. In turn, the differential expression of TGF-β or IL-10 at a graft site may have significant impact on graft pathology. TGF-β and IL-10 have different arrays of biologic activity. For example, TGF-β is fibrogenic, while IL-10 is not. It is interesting to note that accepted renal allografts, which presumably endure prolonged intragraft TGF-β production, also display chronic rejection-like tissue remodeling (6). It might be beneficial to find therapeutic strategies that favor IL-10-mediated regulation and discourage TGF-β-mediated immune regulation.

An outstanding question is why this anti-inflammatory disposition toward donor Ag develops spontaneously in kidney allograft recipient, but needs the help of selected immunosuppressants in cardiac allograft recipients? What is different about the two allograft systems that tips the immune decision-making process in opposite directions? Our current thinking develops from observations made in the anterior chamber-associated immune deviation model (29), where a similar TGF-β-mediated immunoregulatory mechanism develops in response to Ag challenge. There, local conditions define the spectrum of immune responses that are permissible. We postulate that features inherent in kidney tissues promote the development of TGF-β-mediated anti-inflammatory T cell behavior. Either these features are missing from cardiac tissues, or cardiac tissues express other tissue features that promote proinflammatory T cell behavior and are missing from renal tissues. Studies are underway that directly address this issue.

We note that while spontaneous renal allograft acceptance is exhibited by mice and not by humans, the same is not true for the expression of anti-inflammatory T cell behavior. We recently demonstrated that several transplant patients who unilaterally discontinued immunosuppression but retained allograft function display an anti-inflammatory disposition toward donor alloantigens (30), as revealed in the trans-vivo DTH assay (31). Further, we have found that approximately 20% of our renal transplant patients who remain on immunosuppression develop a similar anti-inflammatory disposition toward donor alloantigens (C. G. Orosz,
manuscript in preparation). Thus, the anti-inflammatory DTH phenotype may be relatively common in humans and completely unappreciated. Observations regarding the induction and expression of this phenotype in murine experimental systems may have direct relevance to human transplantation. The critical question that remains is whether this anti-inflammatory phenotype is causal for allograft acceptance or an epiphenomenal event that occurs in transplant recipient under conditions that favor allograft acceptance over rejection. No data yet exist to definitively answer this question.

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