Contribution of androgens to chronic allograft nephropathy is mediated by dihydrotestosterone

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Contribution of androgens to chronic allograft nephropathy is mediated by dihydrotestosterone.

Background. Donor and recipient gender influence long-term allograft outcome after kidney transplantation. Sex hormones are likely to contribute to these gender-related differences. The present study investigated the role of androgens and their inhibition on the development of chronic allograft nephropathy.

Methods. Male or female Fisher (F344) kidneys were orthotopically transplanted into intact male Lewis recipients. Animals were treated either with testosterone, the antiandrogen flutamide, the 5α-reductase inhibitor finasteride, or vehicle. Twenty weeks after transplantation animals were harvested for histology, immunohistology, and molecular analysis.

Results. Testosterone treatment resulted in an increased proteinuria as well as profound glomerulosclerosis, tubulointerstitial fibrosis, and mononuclear cell infiltration that paralleled enhanced intragraft mRNA levels of transforming growth factor-β (TGF-β) and platelet-derived growth factor-A and -B chain (PDGF-A and -B). In contrast, flutamide and finasteride reduced glomerulosclerosis as well as the inflammatory cell infiltration associated with decreased TGF-β, PDGF-A, and -B chain mRNA expression. No gender-related donor differences were noted between the groups.

Conclusions. Our data suggest that dihydrotestosterone mediates the adverse effects of androgens on chronic allograft nephropathy. The inhibition of androgens improves long-term allograft outcome after kidney transplantation.

Chronic allograft nephropathy is the most important cause of late graft loss after kidney transplantation. It is characterized by a progressive deterioration of renal function associated with nonspecific histopathological findings such as glomerulosclerosis, tubular atrophy, interstitial fibrosis, inflammatory cellular infiltration, and intimal thickening of graft arteries [11].

Apart from alloantigen-dependent mechanisms, alloantigen-independent factors are involved in the rejection process [2, 3]. Consistent with this concept, donor and recipient gender may influence renal transplantation. In general, graft survival is enhanced in female recipients while kidneys from male donors have better long-term allograft outcome [4].

For many years, it has been hypothesized that the impact of donor gender on graft survival may primarily result from genetically determined differences in allograft size and structure, as females have smaller kidneys than males. Therefore, transplantation of a female kidney into a male recipient may be functionally inadequate for the demands of the recipient, which results in hyperfiltration-induced glomerular injury and may thus be responsible for the reduced allograft survival of female donors [5, 6].

On the other hand, gender-related differences among renal transplant recipients may be attributed to sex hormones. Sex hormones influence renal hemodynamics, mesangial cell proliferation, extracellular matrix metabolism, as well as synthesis and release of vasoactive agents, cytokines, and other growth factors, which in turn are capable of altering the progression of renal diseases [7]. In males, the presence of the major androgen hormone testosterone is widely believed to promote renal injury in several non-transplant animal models of glomerulosclerosis [8–10]. In addition, various humoral and cellular immune responses are regulated by sex hormones [11].

Utilizing ovariectomized female recipients, we recently demonstrated that in the absence of the main sex hormones, kidneys derived from female donors deteriorate more rapidly than allografts of male origin [12]. Our data indicated that testosterone substitution exacerbates chronic allograft nephropathy in kidneys of male origin.

There remained a number of open questions: Do these results apply to grafts of male origin only? Does testosterone also tamper renal function in intact males? Finally, does therapeutic interference with testosterone receptors prevent allograft damage? Are those growth factors that are commonly associated with chronic allograft nephropathy [transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF)] affected by these changes?

The present study was undertaken to answer these questions.

METHODS

Animals

Naive inbred rats (Charles River, Sulzfeld, Germany), weighing 200 to 250 g, were used throughout the experiments: male and female Fisher (F344, RT1v1) rats served as donors, whereas male Lewis (Lew, RT1) rats were used as recipients. Animals were kept under standard conditions and were fed rat chow and water ad libitum. All experiments were approved by a governmental committee on animal welfare.

Renal transplantation

Under ketamine (Ketamin, 100 mg/kg IP; CP-Pharma, Burgdorf, Germany) and xylacine (Rompun, 10 mg/kg IP; Bayer, Leverkusen, Germany) anesthesia, the left donor kidney was removed, cooled, and positioned orthotopically into the recipient. Donor and recipient renal artery, vein, and ureter were anastomosed end to end with 10-0 Prolene sutures. No ureteral stent was used. To overcome infectious complications caused by operation, rats received Cephtriaxone (Rocephin; 20 mg/kg/day, intramuscularly; Hoffmann-la Roche AG, Grenzach-Wyhlen, Germany) on the first postoperative day. Animals were treated with low-dose cyclosporine A (1.5 mg/kg/day SC; Novartis GmbH, Nürnberg, Germany) over the first ten days after transplantation to overcome an initial episode of acute rejection. The contralateral native kidney was removed on the tenth postoperative day.

Experimental design

Intact male recipients were transplanted either with a male or a female kidney. Animals were assigned to the following eight experimental groups ($N \geq 6$ per group) according to donor gender and treatment: male graft, testosterone treatment (M/T); male graft, vehicle treatment (M/V); male graft, flutamide treatment (M/FL); male graft and finasteride treatment (M/FI); female graft, testosterone treatment (F/T); female graft, vehicle treatment (F/V); female graft, flutamide treatment (F/FL); and female graft and finasteride treatment (F/FI). Testosterone (0.5 mg/kg), testosterone receptor blocker flutamide (25 mg/kg), and 5α-reductase inhibitor finasteride (25 mg/kg) were dissolved in sesame oil and subcutaneously administered every second day (0.1 mL) following transplantation until harvesting. Control animals were given sesame oil alone.

Grafts of male and female gender were used to control for the effects of donor gender. The testosterone receptor antagonist flutamide was applied to block the effects of testosterone at the receptor site. Since testosterone is not the active form of the hormone at many sites of action, we employed the 5α-reductase inhibitor finasteride to control for effects not mediated by testosterone itself. 5α-Reductase converts testosterone to the more potent androgenic steroid dihydrotestosterone.

Functional measurements

Every four weeks, body weight was measured and 24-hour urine samples were collected using metabolic cages with a urine-cooling system. Quantitative urine protein was nephelometrically determined. Serum and urine creatinine levels as well as hormones were measured and creatinine clearance was calculated at the end of the study.

After 20 weeks, rats were anesthetized with diethylether, and immediately thereafter, intra-aortic blood pressure was measured (Sirecust 404, Siemens, Dortmund, Germany). Animals then were bled and the transplanted kidney was removed. Samples were snap frozen in liquid nitrogen for immunohistological staining and molecular analysis or fixed in formalin for light microscopical evaluation.

Histology and immunohistology

For histology, kidney tissues were fixed in 4% buffered formalin, embedded in paraffin, and stained with either hematoxylin/eosin or periodic acid-Schiff (PAS). Glomerulosclerosis was defined as a collapse of capillaries, adhesion of the obsolescent segment of Bowman's capsule and entrapment of hyaline in the mesangium [13]. At least 200 glomeruli were counted per kidney section and the proportion of sclerosed to total glomeruli was expressed as a percentage (glomerulosclerosis index). Glomerulopathy, tubular atrophy, interstitial fibrosis and vascular intimal proliferation were quantified according to the Banff 1997 classification [14] and scored 0 to 3, respectively.

For immunohistology, cryostat sections (4 μm) were fixed in acetone, air dried, and individually stained with primary monoclonal mouse-derived antibodies against monocytes/macrophages (ED1) and CD5+ T-lymphocytes (OX19; Serotec Camon Labor-Service GmbH, Wiesbaden, Germany). After incubation with primary antibody, sections were incubated with rabbit anti-mouse IgG and thereafter with the alkaline phosphatase antialkaline phosphatase (APAAP) complex (Dako A/S, Hamburg, Germany). Cells staining positive were counted and expressed as cells per field of view (cells/fv). At least 20 fields of view per section and per specimen were evaluated at ×400 magnification.

RNase protection assay

Total RNA was extracted from the kidney tissues according to the modified guanidine-isothiocyanate preparation [15] and stored at −80°C until further processing. Intragraft mRNA expression specific for transforming

growth factor-β1 (TGF-β1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined by RNase protection assay with the use of in vitro transcription and RPA Kit (Pharmingen, Becton Dickinson GmbH, Hamburg, Germany) according to the protocol provided by the manufacturer. Briefly, antisense RNA probes were prepared with the use of T7 RNA polymerase transcription in the presence of [α32P]UTP. Radiolabeled RNA probes were then extracted with Tris-saturated phenol and chloroform:isoamyl alcohol and precipitated with ethanol. Probe activity was measured in a scintillation counter. Thereafter, 10 μg total RNA from each samples were hybridized together with a specific radiolabeled antisense probe (activity >3 × 10⁵ cpm) at 56°C overnight. After purification and precipitation, protected fragments were separated by electrophoresis on a 5% polyacrylamide gel. Intensities of the protected bands were quantified by a phosphorimaging analyzer (Fuji-BAS 1500, Düsseldorf, Germany), and the ratios of the investigated genes to GAPDH (internal control) were calculated.

Reverse transcriptase-polymerase chain reaction

Isolated RNA was amplified by reverse transcription (RT) with an Oligo(dT)$_{12-18}$ primer (GIBCO/BRL, Karlsruhe, Germany). One microgram of total RNA was added to 0.5 μg of primer. A reaction mixture containing the RT buffer solution [(GIBCO/BRL); adenosine, thymidine, guanosine, and cytosine triphosphate each in a concentration of 0.2 mmol/L/L (Boehringer Mannheim GmbH, Mannheim, Germany); 0.5 μL of 40 U/μL of recombinant ribonuclease inhibitor (Promega) and 0.5 μL of 200 U/μL M-MLV reverse transcriptase (GIBCO/BRL)] was added and the first chain reaction allowed to proceed (42°C, 1 hour). The reaction was halted by heating to 95°C for five minutes followed by cooling on ice.

Specific cDNA products corresponding to mRNA for the PDGF-A chain [16], PDGF-B chain [17], and β-actin [18] were amplified using the polymerase chain reaction (PCR). One microliter from RT reaction was taken for PCR, which was performed in PCR buffer (Qiagen GmbM, Hilden, Germany) using 0.2 mmol/L/L of each deoxynucleoside triphosphates, 1 μmol/L/L of both primers (Eurogentec, Köln, Germany), and 2.5 U thermus Aquaticus (Taq) DNA polymerase (Dianova). A Perkin-Elmer Thermal Cycler (Model 9600; Perkin-Elmer, Norwalk, USA) was used for amplification allowing 30 to 35 cycles for each primer.

The amplified PCR product was identified by electrophoresis on 1.5% agarose gel stained with ethidium bromide. Cytokine cDNA was semiquantitated by densitometric comparison with β-actin (internal control) from the same sample after the positive image was digitized by video for computerized densitometry. The results are given as the ratio of intensity of growth factors to β-actin mRNA.

Statistical analysis

Data are presented as mean ± SEM. Parametric data were compared using one-way analysis of variance, followed by a multiple pair-wise comparison according to the Newman-Keuls test. Nonparametric data were tested using the Kruskal-Wallis one-way analysis of ranks. A *P* value of less than 0.05 was considered significant.

RESULTS
Functional data

Proteinuria did not differ significantly between vehicle-treated animals (M/V, F/V) according to the origin of the kidney by week 20 (Fig. 1). In contrast, animals treated with testosterone had developed an increased urinary protein excretion. The level of protein excretion was comparable throughout the period observed between flutamide-, finasteride-, and vehicle-treated rats independent of donor gender.

Serum creatinine levels, creatinine clearance, hematocrit, and mean arterial blood pressure did not significantly differ between the groups (Table 1). However, there was a tendency towards an increased blood pressure in animals that had received a female kidney as compared with those with a graft of male origin.

With respect to sex hormones, there was a trend towards increased serum testosterone levels in testosterone- and flutamide-treated animals as compared with vehicle-treated rats. Finasteride did not significantly influence these levels.

At the beginning as well as during follow-up, body weights were comparable in all groups (Table 2). Similarly, the change in body weight between transplantation and harvesting did not differ among the groups. At the time of transplantation, the donor kidney weight/recipient body weight ratio (kw/bw) was lower in recipients of a female kidney as compared with animals with a kidney of male origin. However, by the end of the follow-up period, the kw/bw ratio increased to the same level in all groups irrespective of whether animals had received a graft of male or female origin.

By week 20, prostate and vesicle were profoundly enlarged in testosterone-treated animals (M/T and F/T), whereas flutamide and finasteride resulted in a significant regression of both organs.

Histology and immunohistology

At the end of the follow-up period, vehicle-treated animals (M/V, F/V), independent of donor gender, had developed glomerulosclerosis, tubular atrophy, interstitial fibrosis, inflammatory cellular infiltration, and inti-
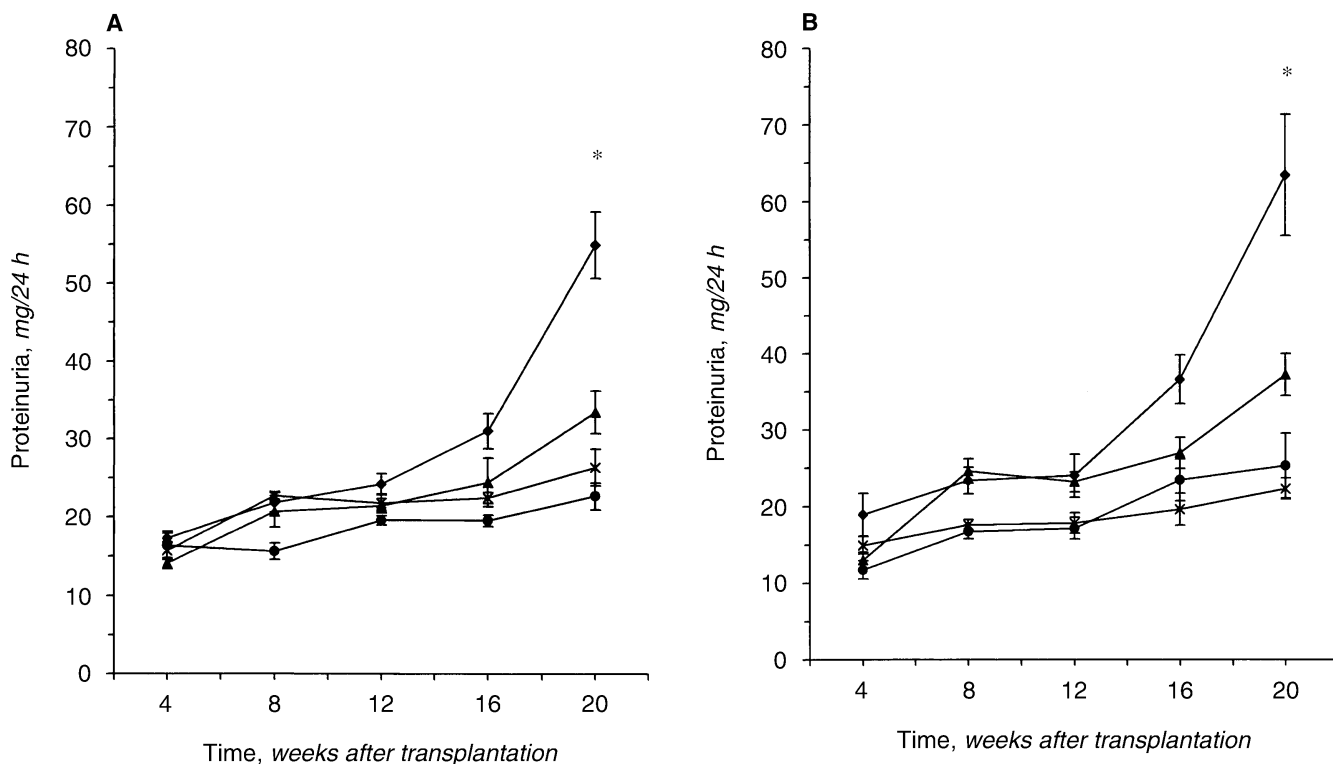
Fig. 1. (*A*) **Changes in 24-hour urinary protein excretion during the course of the experiment in animals that had received a kidney of male origin.** Symbols and abbreviations are: (◆) M/T, male graft and testosterone treatment; (▲) M/V, male graft and vehicle treatment; (●) M/FL, male graft and flutamide treatment; (×) M/FI, male graft and finasteride treatment. *$P < 0.05$ vs. vehicle. (*B*) Changes in 24-hour urinary protein excretion during the course of the experiment in animals that had received a kidney of female origin. Symbols and abbreviations are: (◆) F/T, female graft and testosterone treatment; (▲) F/V, female graft and vehicle treatment; (●) F/FL, female graft and flutamide treatment; (×) F/FI, female graft and finasteride treatment. *$P < 0.05$ vs. vehicle.

Table 1. Mean arterial blood pressure and serum constituents at the time of harvesting

Groups	Mean arterial blood pressure *mm Hg*	Creatinine *mg/dL*	Creatinine clearance *mL/min/100 g*	Testosterone *ng/dL*
Male graft/testosterone (M/T)	90.8 ± 4.7	0.87 ± 0.03	0.33 ± 0.02	23.5 ± 6.8
Male graft/vehicle (M/V)	89.3 ± 11.9	0.82 ± 0.02	0.35 ± 0.01	12.9 ± 2.9
Male graft/flutamide (M/FL)	97.5 ± 6.3	0.80 ± 0.04	0.34 ± 0.02	18.7 ± 4.1
Male graft/finasteride (M/FI)	82.0 ± 9.9	0.84 ± 0.07	0.36 ± 0.03	12.9 ± 3.1
Female graft/testosterone (F/T)	96.7 ± 9.9	0.86 ± 0.04	0.30 ± 0.03	26.0 ± 4.0
Female graft/vehicle (F/V)	114 ± 5.6	0.74 ± 0.04	0.33 ± 0.02	15.7 ± 6.4
Female graft/flutamide (F/FL)	113 ± 5.4	0.69 ± 0.02	0.34 ± 0.04	31.0 ± 6.4[a]
Female graft/finasteride (F/FI)	121 ± 6.3	0.71 ± 0.03	0.34 ± 0.02	13.1 ± 2.3

[a] $P < 0.05$ vs. vehicle treatment in animals with a female graft

mal thickening of graft arteries, as typical signs of chronic allograft nephropathy (Table 3 and Fig. 2).

Administration of testosterone resulted in a markedly elevated degree of glomerular sclerosis (more than 40%) in recipients of a female graft (F/T) as compared with vehicle-treated animals with a kidney of the same origin (F/V). Furthermore, testosterone-treated animals developed a significantly higher degree of interstitial fibrosis, and a moderate tubular atrophy was observed in approximately 25% of the cortical tubuli. In addition, luminal obliteration of graft arteries (up to 25%) and the prolifer-

ation of vascular smooth muscle cells became increasingly obvious. The deleterious effects of testosterone were less pronounced in grafts of male (M/T) origin. Although the glomerulosclerosis index was significantly higher than in vehicle-treated animals (M/V), this was accompanied by a moderate accumulation of matrix proteins in the tubulointerstitium and a slightly elevated degree of intimal proliferation in graft arteries as compared with vehicle treatment (M/V).

The structural changes typically associated with chronic allograft nephropathy remained suppressed, almost to

Table 2. Body weight and donor kidney weight/recipient body weight ratio at the time of the transplantation and harvesting

Groups	N	Prostate	Seminal vesicle	Body weight g		Donor kidney weight/ recipient body weight	
		g		Transplantation	Harvesting	Transplantation	Harvesting
Male graft/testosterone (M/T)	8	0.84	0.89	240 ± 9.1	409 ± 4.9	0.0032	0.0038
Male graft/vehicle (M/V)	7	0.75	0.79	251 ± 5.1	437 ± 6.2	0.0029	0.0035
Male graft/flutamide (M/FL)	6	0.42[a]	0.61	269 ± 12.3	415 ± 14.3	0.0028	0.0034
Male graft/finasteride (M/FI)	7	0.33[a]	0.35[a]	327 ± 6.7	422 ± 7.2	0.0026	0.0034
Female graft/testosterone (F/T)	7	0.66[b]	1.09[b]	280 ± 7.9	398 ± 16.7	0.0021	0.0033
Female graft/vehicle (F/V)	8	0.46	0.72	271 ± 15.2	419 ± 6.6	0.0021	0.0032
Female graft/flutamide (F/FL)	7	0.31[b]	0.46[b]	262 ± 13.8	384 ± 16.5	0.0022	0.0034
Female graft/finasteride (F/FI)	6	0.21[b]	0.26[b]	280 ± 14.7	418 ± 15.6	0.0019	0.0039

Prostate and seminal vesicle weight were measured at harvesting.
[a] $P < 0.05$ vs. vehicle treatment in animals with a male graft
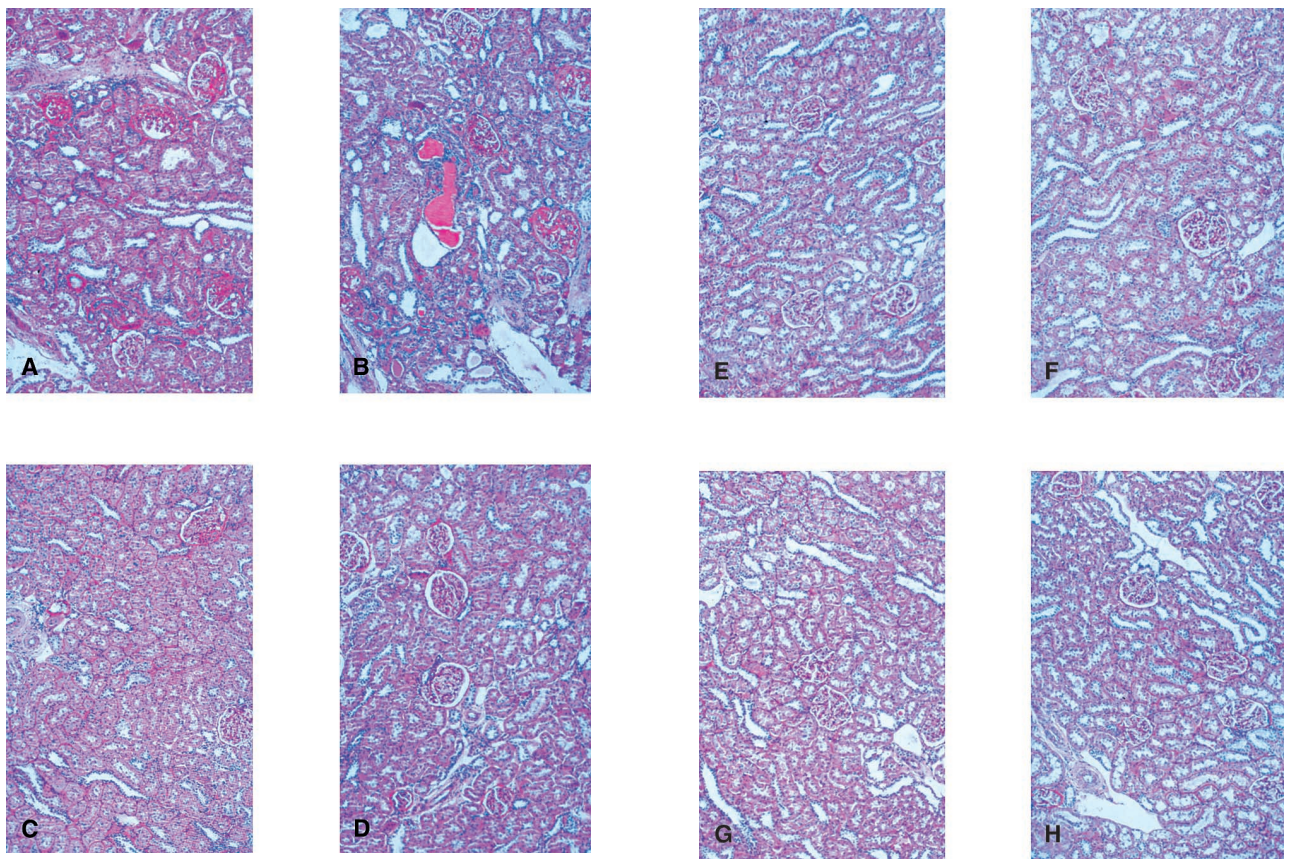[b] $P < 0.05$ vs. vehicle treatment in animals with a female graft

Fig. 2. Periodic acid-Schiff (PAS) staining demonstrated severe glomerulosclerosis and tubulointerstitial fibrosis in testosterone-treated animals that had received a kidney of male (A) or female origin (B) as compared with vehicle-treated rats with a male (C) or female (D) kidney. In contrast, flutamide-treated recipients of a male (E) or female (F) kidney as well as finasteride-treated animals with a graft of male (G) or female (H) origin preserved their renal allograft structure.

the same extent, in animals treated with either flutamide or finasteride as compared with vehicle-treated animals regardless of donor gender. Accordingly, flutamide and finasteride treatment significantly reduced the glomerulosclerosis index, which was accompanied by well-preserved tubulointerstitial architecture and no or only minor intimal thickening of graft arteries.

Immunohistochemical analysis of graft tissues revealed mononuclear cell infiltration in all groups, localizing preferentially in perivascular and periglomerular areas (Table 3). However, while testosterone treatment increased the number of CD5+ T lymphocytes and ED1-positive macrophages, flutamide and finasteride reduced the number of ED1-positive macrophages, particularly

Table 3. Histological and immunohistological analysis of the transplanted kidneys

Groups	Glomerulosclerosis %	Banff score 0–12	Lymphocytes *cells/fv*	Monocytes/ macrophages *cells/fv*
Male graft/testosterone (M/T)	38.9 ± 7.2[a]	4.5 ± 0.6	22.4 ± 3.2	21.5 ± 3.4[a]
Male graft/vehicle (M/V)	25.6 ± 3.8	3.0 ± 0.4	17.6 ± 3.5	13.9 ± 2.8
Male graft/flutamide (M/FL)	9.8 ± 1.1[a]	1.2 ± 0.5[a]	18.1 ± 2.6	5.8 ± 0.7[a]
Male graft/finasteride (M/FI)	10.5 ± 1.8[a]	1.0 ± 0.4[a]	9.8 ± 1.1[a]	7.2 ± 1.1[a]
Female graft/testosterone (F/T)	42.1 ± 9.3[b]	6.3 ± 1.2[b]	25.2 ± 4.7[b]	19.9 ± 3.9[b]
Female graft/vehicle (F/V)	25.9 ± 3.1	2.9 ± 0.4	17.3 ± 1.9	12.7 ± 3.0
Female graft/flutamide (F/FL)	11.1 ± 2.2[b]	1.0 ± 0.2[b]	16.6 ± 2.8	7.9 ± 1.4
Female graft/finasteride (F/FI)	9.4 ± 3.2[b]	1.2 ± 0.3[b]	9.9 ± 1.9[b]	5.9 ± 1.1[b]

Glomerulosclerosis, interstitial fibrosis, tubular atrophy and transplant vasculopathy were evaluated according to the Banff 1997 classification.
[a] $P < 0.05$ vs. vehicle treatment in animals with a male graft
[b] $P < 0.05$ vs. vehicle treatment in animals with a female graft

in perivascular areas. A similar reduction of infiltrating CD5+ T cells was noted in finasteride, but not in flutamide-treated animals.

Molecular analysis

The intragraft mRNA expression of growth factor TGF-β1 and PDGF-A and -B chain paralleled the development of chronic rejection in all groups (Fig. 3 A–C). Accordingly, these levels were most pronounced following testosterone treatment. In contrast, the administration of flutamide and finasteride significantly reduced growth factor levels as compared to vehicle treatment. No donor gender-related differences were noted. The mRNA expression for interleukin (IL)-1, IL-2, IL-5, IL-6, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ) remained below the limits of detection in all groups.

DISCUSSION

According to the hyperfiltration hypothesis, once nephron mass is reduced to a critical level, kidney damage progresses as a result of hypertrophy and subsequent sclerosis of the overworked nephrons [19]. Hyperfiltration may contribute to the progression of chronic allograft nephropathy, as a number of experimental studies demonstrated that reduced transplanted renal mass exaggerates allograft injury, whereas increased functional reserve ameliorates this process [20–22].

For many years, donor gender-related differences in transplantation have been explained by the hyperfiltration theory. As female kidneys are smaller than males, it has been proposed that a mismatch between female donor kidney supply, reflected by donor kidney weight, and male recipient functional demands, reflected by recipient body weight, results in hyperfiltration-induced glomerular injury and that this is responsible for the reduced survival time of female allografts [5, 6, 23]. However, several strains of rats show no gender difference in glomerular numbers [24, 25]. Similarly, preliminary

data by Neugarten et al revealed no difference in humans as well (abstract; Neugarten J et al, *J Am Soc Nephrol* 11:72A, 2000) [26]. In our study, donor gender did not influence long-term allograft outcome to a major degree. Although a decreased kw/bw ratio in recipients of a graft of female origin was noted at the time of transplantation, neither signs of chronic allograft nephropathy nor the kw/bw ratio differed between the sexes at the end of the follow-up. Thus, our data suggest that renal structural differences between the sexes are not responsible for donor gender-related differences: The smaller size of female grafts may represent a reduced tubular mass that has little influence on long-term allograft outcome. Thus, donor gender differences may be attributed to a different sensitivity of male and female grafts to cyclosporine nephrotoxicity or hormones [27].

In contrast to donor gender, modulation of the hormonal milieu did have a significant impact on allograft outcome. We could demonstrate, to our knowledge for the first time, that testosterone receptor blockade with flutamide ameliorates the development of chronic allograft nephropathy. Another novel finding of our study was that the inhibition of the conversion of testosterone to dihydrotestosterone with finasteride had similar beneficial effects. Therefore, as testosterone and dihydrotestosterone are known to bind to the same intracellular receptor, dihydrotestosterone appears to mediate the adverse effects of androgens on chronic allograft nephropathy. In order to get additional proof for the critical role of androgens in the rejection, we further evaluated the effects of testosterone. Indeed, exogenous testosterone aggravated the rejection process, indicating that the elimination of this detrimental hormonal effect accounts for the better allograft outcome in antiandrogen-treated animals. These data further suggest that the inferior long-term outcome in male recipients observed in clinical studies [4] can be explained on the basis of the male hormonal background.
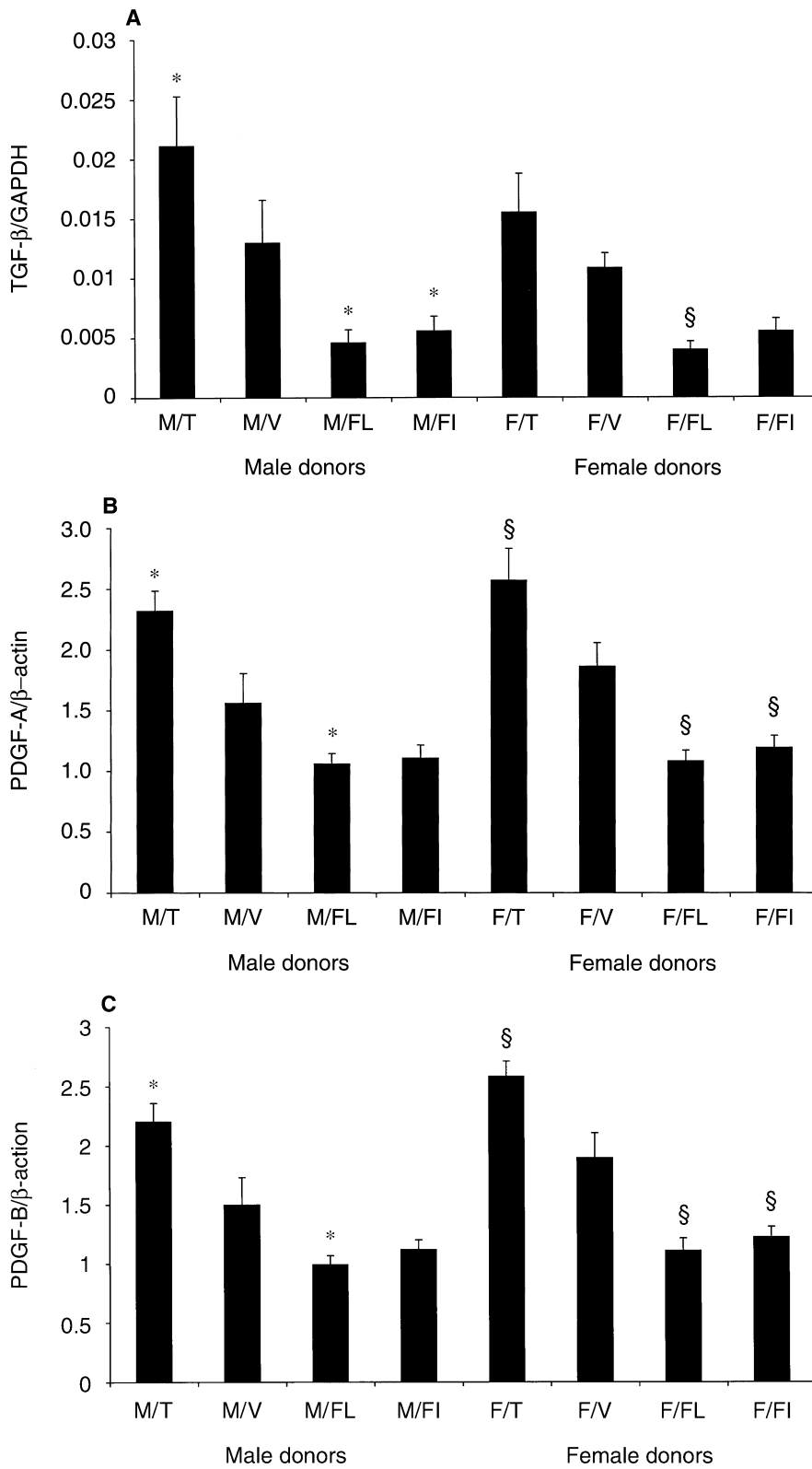
Our results are consistent with the general hypothesis

Fig. 3. (*A*) **Transforming growth factor-β (TGF-β) mRNA expression in renal allografts 20 weeks after transplantation.** *$P < 0.05$ vs. vehicle treatment in animals with a graft of male origin; §$P < 0.05$ vs. vehicle treatment in animals with a graft of female origin. (*B*) PDGF-A chain mRNA expression in renal allografts 20 weeks after transplantation. *$P < 0.05$ vs. vehicle treatment in animals with a graft of male origin; §$P < 0.05$ vs. vehicle treatment in animals with a graft of female origin. (*C*) PDGF-B chain mRNA expression in renal allografts 20 weeks after transplantation. *$P < 0.05$ vs. vehicle treatment in animals with a graft of male origin; §$P < 0.05$ vs. vehicle treatment in animals with a graft of female origin.

that androgens promote renal injury. Male laboratory rats of most strains have a higher susceptibility to the development of proteinuria and glomerulosclerosis, whereas castrated males or female gender seem to be more resistant for these abnormalities [8–10].

It is well established that high dietary protein intake exacerbates renal disease [19]. One may speculate that the anabolic effects of testosterone-induced enhanced protein intake significantly contributed to the development of allograft injury. However, there was no difference in body weight between testosterone- and vehicle-treated animals. Therefore, it is unlikely that an increased protein intake was responsible for the effects of testosterone on the development of allograft lesions.

Testosterone and dihydrotestosterone can have different biological actions. For example, finasteride appears to be beneficial in the treatment of prostatic hypertrophy [28] and male pattern hair loss [29]. In these situations, dihydrotestosterone rather than testosterone mediates androgenic effects. Nonsteroidal antiandrogens such as flutamide exert their antiandrogenic effects by inhibiting androgen uptake and/or nuclear binding of androgens in target tissues [30]. Antiandrogenic compounds are now extensively used in the palliative treatment of patients with advanced prostatic cancer [31]. Furthermore, testosterone—but not dihydrotestosterone—may be involved in the development of increased blood pressure in male animal models of hypertension [32, 33]. In our experiments, testosterone did not increase arterial blood pressure. The reason for this discrepancy is not clear, but it is conceivable that testosterone exacerbates hypertension in a genetically-defective regulation system, but not in an intact one. On the other hand, all animals transplanted with a kidney of female origin developed a slightly elevated systemic blood pressure by week 20. This mild hypertension may have contributed to the more pronounced allograft injury observed in female kidneys as compared with males among the testosterone-treated animals. However, allograft function in female kidneys derived from flutamide- and finasteride-treated rats was well preserved despite an elevated blood pressure. Thus, factors other than hypertension such as the hormonal milieu seem to be more important in determining allograft function.

The mechanisms by which androgens contribute to renal allograft injury remain obscure. Testosterone binding sites have been described in various tissues including the kidney [34]. Furthermore, renal tissues can metabolize testosterone to dihydrotestoterone [35]. Testosterone may be a modest mitogen for mesangial cells, whereas it has no significant effect on collagen synthesis in these cell type [36].

Androgens may modulate the synthesis and/or the release of vasoactive agents, cytokines and growth factors, which in turn are capable of influencing the development of renal disease. Among these factors, TGF-β and PDGF are generally believed to play an important role in the development of chronic allograft nephropathy. TGF-β exerts strong profibrogenic effects, stimulates mesangial cell proliferation and regulates various inflammatory processes [37]. Enhanced TGF-β1 mRNA and/or protein expression has been demonstrated in chronically rejected kidney allografts in animals [38] as well as in humans [39]. PDGF, on the other hand, consists of a disulfide-linked dimer of two polypeptides, the PDGF-A and PDGF-B chain [40]. It stimulates the proliferation of mesangial and vascular smooth muscle cells and stands out as one of the most important agents for the promotion of the migration of smooth muscle cells from the arterial media into the neointima at the site of vascular rejection [41, 42]. Whether androgens stimulate the synthesis of TGF-β and/or PDGF in renal tissues is unclear. However, for example, nonrenal cells such as osteoblasts respond to testosterone treatment with elevated production of TGF-β [43]. In our experiment, the mRNA expression of TGF-β1, PDGF-A and -B chain was profoundly up-regulated in testosterone-treated animals, which may have contributed to the more severe injury in these allografts. In contrast, antiandrogens decreased growth factor mRNA levels in kidneys of both sexes, and this effect was associated with a better long-term outcome of these grafts.

Finally, several lines of evidence indicate that gonadal steroid hormones regulate humoral and cellular immune responses [11]. Testosterone binding sites have been observed in T cells [44] as well as in macrophages [45]. Furthermore, macrophages possess 5α-reductase activity [45]. Thus, direct effects of androgens and their inhibition on immune cells may have contributed to our results. Androgens seem to have immunosuppressive properties, as for example, in the regulation of post-traumatic immunodepression [46]. Moreover, some earlier reports indicate that adult male rats accept allogenic skin grafts for a considerably longer period than females [47]. In our experiment, however, graft function was not improved by the proposed immunosuppressive effects of testosterone.

In conclusion, our data suggest that dihydrotestosterone mediates the adverse effects of androgens on chronic allograft nephropathy. These androgens, antagonized either by receptor blockade or the inhibition of the conversion of testosterone to dihydrotestosterone, could serve as a novel therapeutic approach to prolong kidney graft survival.

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