Kidney International, Vol. 55 (1999), pp. 2011–2020

Sex hormones and gender-related differences: Their influence on chronic renal allograft rejection

Veronika Müller, Attila Szabó, Ondrej Viklicky, Isabell Gaul, Stefan Pörtl, Thomas Philipp, and Uwe W. Heemann

Departments of Nephrology and Endocrinology, University Hospital, Essen, Germany, and Semmelweis Medical University, Budapest, Hungary

Sex hormones and gender-related differences: Their influence on chronic renal allograft rejection.

Background. Renal hemodynamics and immune responses differ between males and females. Thus, sex hormones and genetically determined gender differences may determine the process of chronic rejection to some extent.

Methods. Female (F) or male (M) F344 kidneys were orthotopically transplanted into ovariectomized female Lewis recipients and were treated for 16 weeks with either estradiol, testosterone, or vehicle.

Results. Testosterone treatment resulted in increased urinary protein excretion independently of the donor gender, as well as extended glomerular sclerosis, interstitial fibrosis, and severe vascular lesions. Additionally, mononuclear cell infiltration was most pronounced in these animals, in parallel to an increased expression of intercellular adhesion molecule-1 (ICAM-1), fibronectin, laminin, and transforming growth factor-β (TGF-β) in the grafts. Estradiol treatment resulted in an improved graft function, reduced glomerular sclerosis, and a diminished cellular infiltration, in parallel to a reduced ICAM-1, fibronectin, laminin, and TGF-β expression. In animals treated with vehicle, the gender of the donor influenced the outcome. Grafts of male origin had good graft function and histology, whereas grafts from female donors developed severe proteinuria and glomerular, interstitial, and vascular damage.

Conclusions. These results suggest that a protective effect of estradiol on the progression of chronic rejection exists that is independent of donor gender. Additionally, a male kidney may benefit from the absence of testosterone, whereas the function of a female kidney deteriorates in the absence of estradiol.

Despite the increased short-term success of clinical transplantation during recent years, many renal allografts are lost over the long term because of chronic rejection. Clinically, chronic kidney allograft rejection presents itself as a progressive deterioration of renal function characterized by glomerulosclerosis, tubular atrophy, interstitial fibrosis, vascular obliterative changes, and an intense infiltration of mononuclear cells [1, 2]. Many alloantigen-dependent and -independent factors have been implicated in the development of chronic rejection [3–5].

Are sex hormones also involved in this process? Despite an evident sexual dimorphism in a majority of physiological and pathophysiological conditions, most experimental studies are conducted in male animals only. Genetically determined factors, as well as sex hormones, may contribute to the differences between females and males regarding the incidence and/or progression of several diseases [6].

Observations in both animals and humans indicate an association between male gender and a more rapid progression of renal diseases, independent of blood pressure and serum cholesterol levels [7]. Glomerular hemodynamics, proliferation of mesangial cells, and the extracellular matrix, as well as the synthesis and release of cytokines, vasoactive agents, and growth factors, are involved in the progression of renal diseases. Sex hormones exert a regulatory effect on all of these factors [7].

Sexual dimorphism is the most pronounced before menopause, emphasizing the critical role of estrogens. Estrogens are atheroprotective and vasoprotective and have beneficial effects on serum cholesterol levels [8]. In addition, estrogens may exert potent antioxidant actions, which may contribute to the protective effects of female gender with regards to chronic renal disease [9, 10].

Furthermore, both clinical and experimental observations have substantiated the difference between the two sexes in terms of the immune system. For the regulation of immune response, gonadal steroids seem to be critical, as gonadectomy, sex steroid replacement, and pregnancy profoundly alter its function [11, 12].

We assumed, therefore, that sex hormones are involved in the development of chronic rejection of kidney allografts. In this study, we have investigated the influ-

Key words: chronic rejection, hemodynamics, growth hormones, estradiol, testosterone, transplantation.

Received for publication May 28, 1998
and in revised form December 11, 1998
Accepted for publication December 11, 1998

ence of testosterone and estradiol on the development of chronic rejection in an established rat model. To gain further insights into the role of gender-related differences in renal function and/or structure, donors from both sexes were used.

METHODS

Experimental design

Naive inbred rats (200 to 220 g; Sulzfeld, Germany) were used throughout the experiments. All 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.

To exclude the effect of recipient gender and/or sex hormones on graft function, in all groups, ovariectomized female Lewis rats (LEW, RT1) were used as recipients. Female or male Fisher (F344, RT1v1) animals acted as donors. In order to avoid variations in sex hormone levels throughout estrus cycle, transplantation was performed in recipients and female donors only during the diestrus phase.

The renal vessels of the recipient were isolated and clamped, and the native kidney was removed. The left donor kidney was removed, cooled, and positioned orthotopically into the host. Donor and recipient renal artery, vein, and ureter were then anastomized with 10-0 prolene sutures. The ischemic time ranged from 20 to 25 minutes in all groups. Immediately thereafter, ovariectomy was performed. Transplanted animals were treated with low-dose cyclosporine (1.5 mg/kg/day) for the first 10 days after engraftment to suppress an initial acute rejection episode. The remaining native kidney was excised on day 10.

Animals were divided into six groups according to the gender of the donor and sex hormonal treatment: E/F, estradiol treatment, female graft; V/F, vehicle treatment, female graft; T/F, testosterone treatment, female graft; E/M, estradiol treatment, male graft; V/M, vehicle treatment, male graft; T/M, testosterone treatment, male graft. Following transplantation, either 25 μg/kg β-estradiol 3-benzoate (Sigma, Deisenhote, Germany) or 0.5 mg/kg testosterone propionate (Sigma), both dissolved in sesame oil or sesame oil alone, was given subcutaneously every second day (0.1 ml) until harvesting, according to the methods of Sakemi et al [13, 14]. Although it was in the physiological range, estradiol treatment resulted in slightly higher levels than in naive animals, whereas testosterone treatment induced similar levels (Tables 1 and 2).

Functional measurements

Every four weeks, body weight was measured, and 24-hour urinary protein was determined. At week 16, animals were narcotized. Blood pressure was measured in the aorta (Sirecust 404; Siemens, Germany), and organs were removed for further analysis. Creatinine, total cholesterol, triglycerides, estradiol, and testosterone levels were determined in the serum.

Table 1. Hormone levels and histological results in naive rats

	Estradiol	Testosterone
Rats	*pg/ml*	
Female Lewis	47.2 ± 6.1	< 1.9
Female Fisher	61.5 ± 9.9	< 1.9
Male Lewis	44.2 ± 3.6	10.4 ± 2.4
Male Fisher	40.6 ± 7.6	9.3 ± 4.3

Histological analysis

One sample of the kidney was fixed in 4% neutral-buffered formalin. Paraffin sections were stained with hematoxylin/eosin and periodic acid-Schiff reagent. The extent of glomerular sclerosis was determined in a blinded fashion by light microscopy. Glomerulosclerosis was defined as a collapse of capillaries, adhesion of the obsolescent segment of Bowman's capsule, and entrapment of hyaline. The percentage of sclerotic glomeruli (glomerulosclerosis index) was assessed (more than 200 glomeruli per kidney) [15].

A more sensitive evaluation of transplant rejection is the Banff classification [16]. According to this classification, glomerular, interstitial, tubular, and vascular lesions were defined and scored from 0 to 3+, to produce a numerical coding (0 to 12+) of kidney damage.

Glomerular tuft volume was determined as described by Weibel [17]. Using an image analysis system (Northern Exposure; Bio-Science, Graestad, Denmark), the mean glomerular random cross-sectional areas (Am) were determined in at least 50 glomeruli on histological sections. No glomerulus was counted more than once, and all levels of the cortex were evenly sampled. Mean volume of capillary tuft was calculated using this equation: $V = (\beta/\kappa)(Am)^{3/2}$, where $\kappa = 1.1$ is the size distribution coefficient and $\beta = 1.38$ is the shape coefficient for spheres.

Immunohistological analysis

Monoclonal antibodies against CD5+ T cells (OX19), monocytes/macrophages (ED-1), intercellular adhesion molecule-1 (ICAM-1; CD54), fibronectin, and laminin were obtained from Serotec Camon Labor-service GmbH (Wiesbaden, Germany), secondary (rabbit antimouse) and tertiary antibodies (mouse APAAP) from DAKO A/S (Hamburg, Germany).

Kidney pieces were snap frozen in liquid nitrogen and stored at –80°C. Cryostat sections (4 to 6 μm) were stained individually with monoclonal antibodies (mouse antirat antibodies) from the above panel using the alkaline phosphatase antialkaline phosphatase (APAAP) method. Stained cells were than counted using an ocular