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Differential effects of sex hormones on autoantibody production and proteinuria in chronic graft-*versus*-host disease-induced experimental lupus nephritis

M. VAN GRIENSVEN, E. C. BERGIJK, J. J. BAELDE, E. DE HEER & J. A. BRUIJN
Department of Pathology, University of Leiden, Leiden, The Netherlands

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SUMMARY

In patients with systemic lupus erythematosus, the female-to-male ratio is as high as 10:1. Sex hormones are thought to play a role in this difference in susceptibility. In a previous study, we demonstrated a high susceptibility of female mice to the development of glomerulonephritis after induction of chronic graft-*versus*-host disease (GVHD), compared with male mice. In order to unravel further this gender-related difference (C57Bl/10*DBA/2)F_1 hybrid mice were either castrated or ovariectomized and treated with 17β-ethinyloestradiol or testosterone-decanoate preceding the induction of chronic GVHD. Testosterone-decanoate reduced significantly the development of albuminuria in females. In contrast, proteinuria of 17β-ethinyloestradiol-treated female mice was in the same range as that of sham-operated mice. Autoantibody levels against glomerular basement membrane, renal tubular epithelium, dsDNA and ssDNA, as determined by ELISA, were higher in 17β-ethinyloestradiol-treated female mice than in all other groups. Immunofluorescence studies showed the presence of immunoglobulin and complement deposits in glomeruli of all animals, without significant differences between the experimental groups. Our findings confirm earlier observations, in that testosterone-decanoate is shown to be an inhibitory compound, whereas 17β-ethinyloestradiol has stimulating properties in autoimmunity. Moreover, our results show for the first time differential hormonal effects on autoantibody levels and proteinuria in experimental lupus nephritis.

Keywords autoimmunity glomerulosclerosis graft-*versus*-host disease lupus nephritis sex hormones

INTRODUCTION

Women suffer more often and more severely from dysregulation of the immune system than do men. Higher immunoglobulin levels, increased antibody production after immunization, decreased susceptibility to infections, and decreased graft rejection time are found in females. Among patients with systemic lupus erythematosus (SLE), an autoimmune disease in which multiple organs are affected, the female-to-male ratio is as high as 10:1 [1–3]. In SLE patients the equilibrium between oestrogens and testosterone is aberrant. In women suffering from SLE, increased concentrations of 16α-hydroxy-oestrone and -oestriol are found. This 16α-hydroxylation of oestrogens causes increased oestrogen activity [4]. These hydroxylated metabolites are potent oestrogens, in that they are highly uterotrophic, but they are poorly bound by testosterone–oestrogen-binding protein, and consequently their hormonal effects are maximized. Furthermore, female SLE patients also show abnormally high levels of C17-oxydated testosterone (= androstenedione) [4], a testosterone derivate which is rapidly cleared from the circulation, resulting in decreased amounts of testosterone in the blood [4,5]. The increased activity of oestrogens, together with the decreased amount of testosterone, suggests that the net effect of sex hormones in diseased females is mainly caused by oestrogens. These findings strongly suggest sex hormonal influences in the development of SLE.

Chronic graft-*versus*-host disease (GVHD) in mice is a model for lupus nephritis, a commonly observed renal disorder in SLE patients. Chronic GVHD is induced by intravenous injections of DBA/2 lymphocytes into (C57Bl/10*DBA/2)F_1 hybrid mice. Recipient B cells are polyclonally activated by anti-allo-MHC class II $CD4^+$ donor T cells, which leads to a lupus-like autoimmune syndrome [6]. Previously, we have shown that in this model the serum concentrations of autoantibodies against glomerular basement membrane (GBM), collagen IV, and laminin are significantly higher in female than in male mice 2–4 weeks after induction of the disease [1]. These autoantibodies play a major pathogenic role in the development of immune complex glomerulonephritis in this model [6]. We have also observed increased albuminuria in female mice compared with that in male mice 4–6 weeks after induction of chronic GVHD [1]. These results indicate a higher susceptibility to the development

Correspondence: J. A. Bruijn MD, PhD, Leiden University Hospital, Building 1, L1-Q, Department of Pathology, PO Box 9600, NL-2300 RC Leiden, The Netherlands.

of renal disease in female than in male mice in this experimental model of lupus nephritis.

The current study was designed to investigate the possible accelerating role of oestrogens and the possible inhibiting role of androgens (testosterone) in the development of immune complex glomerulonephritis in chronic GVHD. To achieve this, we treated both ovariectomized female mice and castrated male mice with 17β-ethinyloestradiol or testosterone-decanoate. 17β-ethinyloestradiol is an effective semi-synthetic compound, used in many oral contraceptive preparations. Its effect parallels that of oestrogens [7]. The decanoate-ester of testosterone has been proved to exert an effect similar to that of testosterone itself [8]. We studied the sex hormone-related differences in the development of immune complex glomerulonephritis in this model by examination of albuminuria, autoantibody levels, light microscopy, and by immunohistochemical studies of renal tissue.

MATERIALS AND METHODS

Experimental design

Groups of 35 male and 26 female (C57Bl/10*DBA/2)F_1 hybrid mice were divided into subgroups as indicated in Table 1. Tap water enriched with 17β-ethinyloestradiol (Organon International bv, Oss, The Netherlands) was administered per os in a dose of 50 μg/kg bodyweight per day. The control for 17β-ethinyloestradiol administration was normal tap water. Testosterone-decanoate dissolved in arachis oil (Organon International) was injected subcutaneously in amounts of 10 mg/kg bodyweight in 0·1 ml at fortnightly intervals. As a control, male mice were injected subcutaneously with 0·1 ml arachis oil per injection. Two additional groups of 10 male and six female mice were subjected to sham operations, and did not receive hormonal treatment. In all groups, chronic GVHD was induced by injecting lymphocytes derived from female DBA/2 mice as described elsewhere [9].

Induction of chronic GVHD

Chronic GVHD was induced by methods described in detail elsewhere [10]. Briefly, to obtain donor lymphocytes, spleens, thymi, and cervical, axillary, mesenteric, and inguinal lymph nodes were removed from female DBA/2 donor mice and collected under aseptic conditions. The tissues were separately minced in Hanks' medium and gently pressed through a steel sieve (150 μm pore diameter). The obtained single-cell suspensions were subsequently filtered through a nylon sieve and a sterile pasteur pipette loosely packed with cotton wool. The cell suspensions were washed three times in Hanks' medium, each wash being followed by centrifugation for 10 min at 200 ***g***. The total numbers of lymphocytes in the suspensions were determined using a counting chamber (Bürker) and phase contrast microscopy. At the same time, the percentage viable lymphocytes was determined by trypan blue exclusion. The amount of viable cells was invariably >90%. The suspensions were pooled in Hanks' medium. Approximately 30×10^6 viable DBA/2 cells in 250 μl of Hanks' medium were injected into the tail veins of the mice at days 0, 3, 7 and 10. The ratio splenocytes:thymocytes:lymph node cells was constant, i.e. $\approx$ 6:3:1.

Table 1. Description of the experimental groups

Group	Gender	(*n*)	Operative treatment	Administered chemical
1	Male	(9)	Castration	17β-ethinyloestradiol
2	Female	(7)	Ovariectomy	17β-ethinyloestradiol
3	Male	(7)	Castration	Testosterone-decanoate
4	Female	(7)	Ovariectomy	Testosterone-decanoate
5	Male	(9)	Castration	Arachis oil
6	Female	(6)	Ovariectomy	None
7	Male	(10)	Sham operation	None
8	Female	(6)	Sham operation	None

In all groups chronic graft-*versus*-host disease was induced by injecting female DBA/2 lymphocytes.

Follow up of F_1 mice

To study development of disease, the (C57Bl/10*DBA/2)F_1 hybrid mice were observed for a period of 6 weeks. Earlier studies showed that renal disease develops fully within this period of time [1,6,11]. One week before and 2, 4 and 6 weeks after the first injection of DBA/2 lymphocytes blood was collected from the orbital plexus for preparation of serum. At the same time, the albumin content of the urine of the (C57Bl/10*DBA/2)F_1 hybrid mice was determined. Animals were kept in metabolic cages for 18 h with free access to water and food. Urine albumin levels were assessed by rocket electrophoresis against rabbit anti-mouse albumin, with the use of purified mouse serum albumin (Sigma Chemical Co., St Louis, MO) as standard.

To monitor whether administration of the hormones resulted in physiological serum hormone levels, vaginal smears were taken from all female mice daily during the first 14 days of hormone administration. The smears were stained following Giemsa's method. To evaluate the hormonal effect, the ratio between epithelial cells with nuclei, epithelial cells without nuclei, and neutrophilic granulocytes was scored in each smear (Table 2) [12]. The normal, five daily, oestrus cycle is 2 days di-oestrus (a), followed by 1 day di-oestrus (b), 1 day pro-oestrus (f) and 1 day oestrus (g). In sham-operated female mice (group 8) this cycle was expected. Ovariectomized female mice (group 6) and female mice treated with 17β-ethinyloestradiol (group 2) or testosterone-decanoate (group 4) are thought to remain in oestrus (g). The hormonal effect in male mice was examined by histological analysis of the ventral-prostate after staining with haematoxylin and eosin [13].

ELISA

Serum samples were tested for the presence of autoantibodies against GBM, renal tubular epithelium (RTE), dsDNA, and ssDNA by ELISA, since antibodies with these specificities were shown to be nephritogenic in earlier studies on this model [11,14,15]. The ELISA studies were performed essentially as described earlier [11,16]. RTE was prepared from fresh normal (C57Bl/10*DBA/2)F_1 mouse kidneys and collagenase-digested GBM was prepared from Brown Norway rat kidneys [17]. Purity of all antigens used was verified by absorption studies, gel electrophoresis, ELISA, and immunoblotting, as described earlier [11,17,18]. ELISA results from each sample were corrected for non-specific binding to bovine serum albumin (BSA).

Histological methods

For immunofluorescence studies of the development of renal disease, all mice were killed 6 weeks after induction of chronic GVHD. Kidneys were snap-frozen in CO_2 ice-cooled isopentane

Table 2. Histological changes in vaginal epithelium during a normal oestrus cycle in mice

Cycle stage	Percent granulocytes	Percent epithelial cells with nuclei	Percent epithelial cells without nuclei
Di-oestrus (a)	>66	5	0
Di-oestrus (b)	>5 and <66	>50	0–5
Met-oestrus (c)	>33 and <66	0–5	>50
Met-oestrus (d)	>5 and <33	0–5	>50
Pro-oestrus (e)	<5	>50	0–5
Pro-oestrus (f)	<5	>50	40
Oestrus (g)	<5	<5	> 90

The normal oestrus cycle in mice is: aab(e)fg. See text for detailed explanation.

and stored at −70°C until use. Tissue was processed for immunofluorescence studies as mentioned earlier [9]. The conjugates used for direct immunofluorescence studies to detect immunoglobulin deposits were FITC-labelled goat anti-mouse IgM (dilution 1:400) (Sigma), goat anti-mouse IgG (dilution 1:300), and goat anti-mouse C3 antibodies (dilution 1:300) (both from Nordic Immunology, Tilburg, The Netherlands). Direct immunofluorescence studies were performed on cryostat sections of kidneys from diseased mice. Sections from normal mouse kidneys were used as negative controls.

Statistical analysis

The analysis of statistical significance between the ELISA results and mean urinary albumin contents of separate experimental groups of animals was determined by using Scheffé tests, taking multiple comparisons into account without accumulating the statistical significance value. Statistical significance of one group compared with all other groups was determined by Student's *t*-test. $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

Hormone treatment

The oestrus stage of vaginal epithelium was scored daily by taking vaginal smears. This procedure was continued for 14 days. The group of sham-operated female mice (group 8) continued to have a five-daily oestrus cycle during the entire observation period, indicating that the sham operations did not influence serum levels of sex hormones. All other female groups (groups 2, 4 and 6) went in oestrus (g-phase, see Table 2). However, the morphology of epithelial cells without nuclei showed striking differences between the three last mentioned groups (groups 2, 4 and 6). Epithelial cells without nuclei showed normal cell morphology in 17β-ethinyloestradiol-treated ovariectomized female mice (group 2). The epithelial cells without nuclei in testosterone-decanoate-treated ovariectomized female mice (group 4) and in ovariectomized female mice (group 6) showed signs of collapse in cytoplasmic membranes, indicating a loss of inner cell fluid. Furthermore, the numbers of epithelial cells in the smears of these two groups (groups 4 and 6) were reduced by $\approx 90\%$. These observations confirm that physiological serum levels of oestrogens were present in the 17β-ethinyloestradiol-treated female mice (group 2). The ovariectomies were successful in reducing the concentration of oestrogens (groups 4 and 6).

The ventral prostates of castrated male mice treated with arachis oil (group 5) or with 17β-ethinyloestradiol (group 1) were found to be completely atrophic, and as a result could not be resected. Prostates of castrated male mice treated with testosterone-decanoate (group 3) and those of sham-operated male mice (group 7) showed similar histology, i.e. dilatation of prostatic ducts and a normal secretion function as determined by the number of chief cells (glandulae propriae). These observations implicate physiological levels of androgens in the last two mentioned groups (groups 3 and 7). Castration in the first two mentioned groups (groups 1 and 5) was successful, in that the androgen level had decreased.

Albuminuria

The mean urinary albumin content was calculated for each experimental group (Fig. 1). The highest urinary albumin content was observed in the group of female mice suffering from GVHD which had been subjected to sham operation (group 8) or to ovariectomy (group 6). Castrated male mice treated with 17β-ethinyloestradiol (group 1) or with arachis oil (group 5) did not develop albuminuria upon induction of GVHD. The urinary albumin contents in the two other groups of male mice (groups 3 and 7) were in the same range as those in the group of ovariectomized female mice treated with testosterone-decanoate (group 4; Fig. 1). Six weeks after induction of the disease, the urinary albumin content in the group of ovariectomized female mice treated with testosterone-decanoate (group 4) was significantly lower than that in the group of sham-operated female mice (group 8; $P < 0{\cdot}05$).

Autoantibodies

ELISA studies of autoantibodies against GBM revealed the highest autoantibody levels in 17β-ethinyloestradiol-treated ovariectomized female mice (group 2) 4 weeks after induction of the disease (Fig. 2a). These levels were significantly different from those in all other groups ($P = 0{\cdot}041$). Analysis of data revealed no outlying results. The same ELISA studies revealed lowest autoantibody levels in male mice subjected to sham operations (group 7) at all time points studied. At week 4, autoantibody levels against GBM in sham-operated male mice (group 7) were significantly lower than those in 17β-ethinyloestradiol-treated ovariectomized female mice (group 2; $P < 0{\cdot}001$). At that same time point, autoantibody levels against GBM in arachis oil-treated castrated male mice (group 5) and in 17β-ethinyloestradiol-treated castrated male mice (group 1) were significantly lower than those in 17β-ethinyloestradiol-treated ovariectomized female mice (group 2; $P < 0{\cdot}05$; Fig. 2a). At week 6, autoantibody levels of

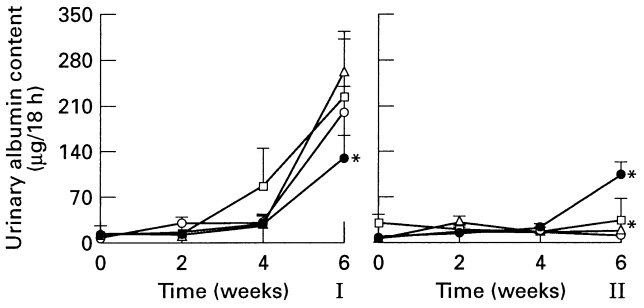
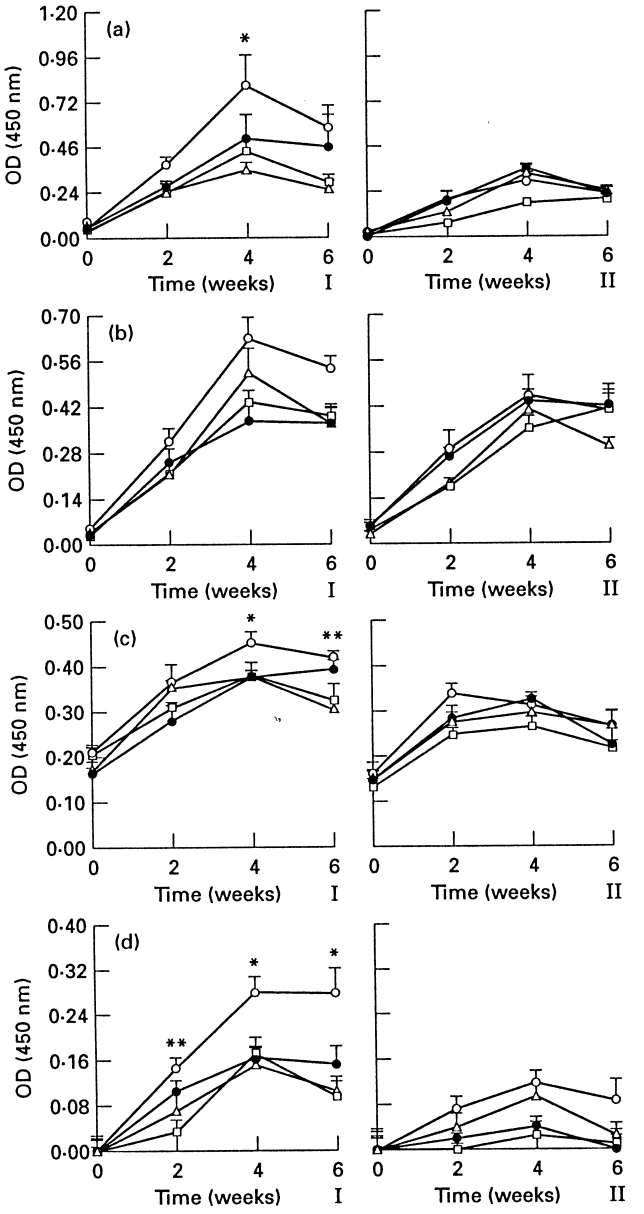


Fig. 1. Albuminuria of (C57Bl/10*DBA/2)F_1 hybrid mice. Per group, the mean urinary albumin content ± s.e.m. is indicated for each time point under investigation. I, Ovariectomized female mice; II, castrated male mice. ○, 17β-ethinyloestradiol-treated (groups 1 and 2; ●, testosterone-decanoate-treated (groups 3 and 4); Δ, operated, but not treated (groups 5 and 6); □, sham-operated (groups 7 and 8). *$P < 0{\cdot}05$ *versus* ovariectomized female mice (IΔ), sham-operated female mice (I□), and 17β-ethinyloestradiol-treated female mice (I○)).



ovariectomized female mice (group 6) were identical to those determined in castrated male mice treated with testosterone-decanoate (group 3).

Autoantibody levels against RTE reached the highest peak levels in all groups at week 4 (Fig. 2b). Again, ovariectomized female mice treated with 17β-ethinyloestradiol (group 2) produced the highest levels of autoantibodies, although no significant differences in anti-RTE levels were measured between any of the experimental groups.

Autoantibodies against both dsDNA and ssDNA in 17β-ethinyloestradiol-treated ovariectomized female mice (group 2) were higher than those in all other groups at all time points in the course of the disease ($P = 0{\cdot}003$ and $P = 0{\cdot}001$, for dsDNA and ssDNA, respectively), with highest peak levels at week 4 (Figs 2c,d). At week 4, 17β-ethinyloestradiol-treated ovariectomized female mice (group 2) displayed significantly higher levels of autoantibodies against dsDNA than sham-operated male mice (group 7; $P < 0{\cdot}001$), arachis oil-treated castrated male mice (group 5; $P < 0{\cdot}01$), and 17β-ethinyloestradiol-treated castrated male mice (group 1; $P < 0{\cdot}05$; Fig. 2c). Significant differences in autoantibody levels against dsDNA were also observed between experimental groups at week 6. Anti-dsDNA autoantibody levels in 17β-ethinyloestradiol-treated ovariectomized female mice (group 2) were significantly higher than those in sham-operated male mice (group 7; $P < 0{\cdot}01$) and in testosterone-decanoate-treated castrated male mice (group 3; $P < 0{\cdot}05$; Fig. 2c). In the ssDNA ELISA as early as 2 weeks after induction of disease, levels of autoantibodies in 17β-ethinyloestradiol-treated ovariectomized female mice (group 2) were significantly higher than those in both sham-operated male mice (group 7; $P < 0{\cdot}01$) and testosterone-decanoate-treated castrated male mice (group 3; $P < 0{\cdot}05$; Fig. 2d). At week 4, the levels of significance between the mentioned groups had risen to 0·000 01 and 0·0001, respectively (Fig. 2d).

Fig. 2. (a) ELISA results showing the presence of anti-glomerular basement membrane (GBM) antibodies in sera of mice suffering from chronic graft-*versus*-host disease (GVHD). Values are mean optical density (OD) at 450 nm ± s.e.m. per group for each time point. I, Ovariectomized female mice; II, castrated male mice. ○, 17β-ethinyloestradiol-treated (groups 1 and 2); ●, testosterone-decanoate-treated (groups 3 and 4); Δ, operated, but not treated (groups 5 and 6); □, sham-operated (groups 7 and 8). *$P < 0{\cdot}05$ *versus* all other groups. (b) Anti-renal tubular epithelium antibodies present in sera of mice at different time points after induction of chronic GVHD. Values are mean OD at 450 nm ± s.d. I, Ovariectomized female mice; II, castrated male mice. ○, 17β-ethinyloestradiol-treated (groups 1 and 2); ●, testosterone-decanoate-treated (groups 3 and 4); Δ, operated, but not treated (groups 5 and 6); □, sham-operated (groups 7 and 8). (c) ELISA results showing the presence of anti-dsDNA antibodies in the sera of (C57Bl/10*DBA/2)F_1 hybrid mice upon induction of chronic GVHD. Values are mean OD at 450 nm ± s.d. I, Ovariectomized female mice; II, castrated male mice. ○, 17β-ethinyloestradiol-treated (groups 1 and 2); ●, testosterone-decanoate-treated (groups 3 and 4); △, operated, but not treated (groups 5 and 6); □, sham-operated (groups 7 and 8). *$P = 0{\cdot}003$ *versus* all other groups; **$P < 0{\cdot}01$ *versus* sham-operated male mice (II□) and $P < 0{\cdot}05$ *versus* testosterone-decanoated-treated male mice (II●). (d) ELISA results showing the presence of anti-ssDNA antibodies in sera of mice suffering from chronic GVHD at all time points in the course of disease. Values are mean OD at 450 nm ± s.d. I, Ovariectomized female mice; II, castrated male mice. ○, 17β-ethinyloestradiol-treated (groups 1 and 2); ●, testosterone-decanoate-treated (groups 3 and 4); △, operated, but not treated (groups 5 and 6); □, sham-operated (groups 7 and 8). *$P < 0{\cdot}01$ *versus* all other groups; **$P < 0{\cdot}05$ *versus* sham-operated male mice (II□) and testosterone-decanoate-treated male mice (II●).

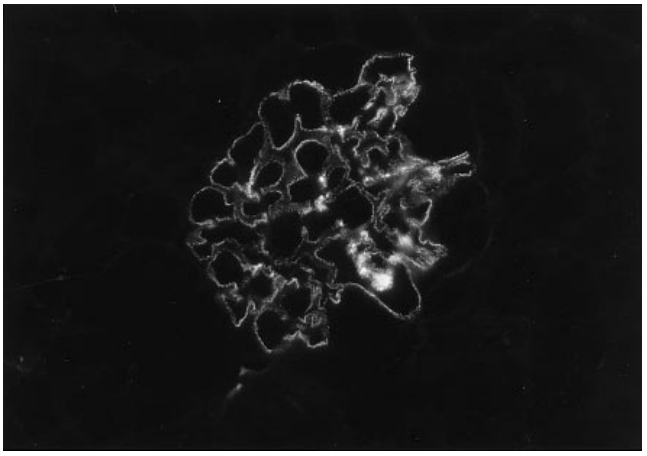


Fig. 3. Immunofluorescence micrograph showing deposition of IgG in glomerulus of (C57Bl/10*DBA/2)F_1 hybrid mouse six weeks after induction of chronic graft-*versus*-host disease. Strong, predominantly granular staining is observed along the glomerular capillary wall, weaker staining is visible in the mesangial area.

Six weeks after the induction of GVHD, autoantibody levels of ovariectomized female mice treated with 17β-ethinyloestradiol (group 2) were significantly higher than those in all other groups, except for testosterone-decanoate-treated female mice (group 4; Fig. 2d).

Immunofluorescence studies

The presence of IgG, IgM, and C3 was determined semi-quantitatively in kidney tissue of all animals at week 6. Strong, predominantly granular staining was observed for IgG along the glomerular capillary walls and for IgM in the mesangial areas. Weaker staining was observed for IgM along the glomerular capillary walls, and for IgG in the mesangial areas (Fig. 3). Granular C3 depositions were observed both along the GBM and in mesangial areas. No significant differences in intensity of staining for mouse immunoglobulin depositions were observed between the experimental groups (data not shown).

DISCUSSION

Sexual dimorphism exists with regard to the immune response between women and men. Women are more often afflicted with autoimmune diseases than their male counterparts [2,3]. Sex hormones are thought to be connected with this difference in susceptibility. In female SLE patients treated with the regular hormonal contraceptive therapies, the clinical manifestations of SLE are more severe than those in untreated female SLE patients [19]. Similar observations were made in experimental animal models. Blank and coworkers described sex hormone involvement in an experimental animal model for SLE induced by administering an anti-DNA idiotype to naive male BALB/c mice. Castration of these mice, followed by administration of oestrogen, resulted in accelerated development of autoantibodies and more severe kidney damage upon induction of the autoimmune disease. Conversely, testosterone treatment led to amelioration of the disease, i.e. partial inhibition of autoantibody development and prevention of renal disease [20]. Similar observations were described for the NZB/NZW model, which is a spontaneous model for lupus nephritis. In this model, testosterone treatment both decreased the anti-DNA autoantibody response [21,22] and showed less evidence of glomerulonephritis [21]. Oestrogens increased autoantibody levels in these mice [21–23]. Androgen treatment of castrated female NZB/NZW mice improved clearance of IgG-sensitized erythrocytes, whereas oestrogen-treated male NZB/NZW mice showed delayed clearance [24]. Increasing concentrations of testosterone (-decanoate) decreased mortality rates in both male and female NZB/NZW mice [8,21]. In addition, exposure to oestrogens rapidly decreased survival of NZB/NZW mice [21] and of NZB/NZW backcross mice [25]. In the same strain, albuminuria and haematuria were significantly increased during oestrogen treatment, while the severity of sialoadenitis was diminished by oestrogens [25], indicating an ameliorating effect of oestrogens on T-helper cell-mediated inflammation. In contrast, in the BXSB mouse model for lupus nephritis, inverse reactions of androgens and oestrogens on the development of autoimmunity have been reported [26] Thus far, however, the relation between gender-related influences on albuminuria on the one hand and autoantibody specificities on the other has not been investigated in detail.

We recently reported significant differences between female and male (C57Bl/10*DBA/2)F_1 hybrid mice with respect to development of albuminuria and autoantibodies against the GBM components laminin and collagen type IV after induction of chronic GVHD [1]. The GVHD model differs from the NZB/NZW model in that it is inducible, and with respect to the specificity of the nephritogenic autoantibodies [6,10,11,27]. In GVHD mice, a nephritogenic role is played by autoantibodies directed against RTE and GBM, as shown extensively in earlier studies involving, amongst others, elution and transfer experiments [11,28–31]. In this model, female mice injected with female DBA/2 lymphocytes developed significantly more albuminuria and higher levels of anti-GBM antibodies. Male mice immunized with female DBA/2 lymphocytes showed the lowest autoantibody levels and proteinuria in all experiments [1]. In the current study, the relationship between these gender-related differences and sex hormones in the GVHD model of experimental lupus nephritis was further investigated. Albuminuria results suggest that testosterone-decanoate may be an inhibitory compound, reducing the development of albuminuria significantly. The absence or presence of oestrogens, from either an autologous or exogenous source, did not seem to correlate with the development of albuminuria (Fig. 1). Differences in albumin levels in urine of both male and female mice which had received sham operations (groups 7 and 8) were comparable to the differences obtained in untreated, unoperated (C57Bl/10*DBA/2)F_1 hybrid mice. This is in concordance with our previous findings [1], in which similar differences between male and female mice were observed after induction of chronic GVHD. Moreover, significantly more sham-operated female mice (group 8) developed proteinuria than sham-operated male mice (group 7).

ELISA studies suggested that 17β-ethinyloestradiol was an accelerating factor in autoantibody production (Figs 2a–d). This was most evident in ovariectomized female mice treated with 17β-ethinyloestradiol (group 2). Autoantibody production was not affected by testosterone-decanoate. Testosterone-decanoate-treated ovariectomized female mice (group 4) produced levels of autoantibodies equal to those measured in sham-operated female mice (group 8). All groups of male mice had low autoantibody levels, and no significant differences were observed between the experimental groups.

Interestingly, although sex hormone therapy modulated antibody levels, females showed consistently higher anti-GBM, -dsDNA and

-ssDNA antibody expression than males in all panels of Fig. 2, regardless of hormone treatment. This may imply the additional possibility of hormone-independent, sex-determined factors, as has been reported in the BXSB model [26,32]. Further studies are necessary to reveal the nature of such factors.

The small effect obtained by 17β-ethinyloestradiol administration to castrated male mice (group 1) could be due to up-regulation of androgen synthesis in the adrenal cortex [7]. Profound production of testosterone in normal, uncastrated male mice is established in testicular tissue. This system was eliminated by castration. Testosterone has been shown to be able to oppose the effects of oestrogens [4,5]. Thus in castrated male mice oestrogens could be opposed by testosterone produced in the adrenal cortex. Similar up-regulatory mechanisms could occur in ovariectomized female mice. In addition, some other tissues, such as liver, muscle, fat, and hair follicles, are able to convert steroid precursors, e.g. cholesterol, into oestrogens [7].

Surprisingly, in our experiments there appeared to be no effect of sex hormones on immune complex formation in the kidneys, since semiquantitative immunohistochemical studies on the presence of immunoglobulins and complement in glomeruli revealed similar results for all experimental groups. IgG was mainly distributed along the GBM, whereas the major immunoglobulin content in mesangial areas consisted of IgM. These semiquantitative results suggest that the two different sex steroids do not modulate immune complex formation in glomeruli, or small differences can not be detected, although they do influence serum autoantibody levels. Hence, another, as yet unknown factor must play a role in the loss of permselectivity in the kidney. Our observations show differential hormonal effects. Autoantibody production was accelerated by 17β-ethinyloestradiol, whereas testosterone-decanoate did not exert any effect. In contrast, testosterone-decanoate partially inhibited proteinuria, which was not influenced by 17β-ethinyloestradiol. Interestingly, this discrepancy points towards a direct hormonal effect on the permeability of the glomerular wall, which may involve alteration of the composition of the glomerular extracellular matrix. This might be established through quantitative and/or qualitative analysis of production of glomerular matrix components in such alterations, which indeed have been observed in this model [9,33–35]. An explanation for this phenomenon may lie in the fact that steroids such as oestrogens and androgens bind to DNA via high-affinity nuclear receptors, and act subsequently as modulators of transcription of genes encoding matrix molecules [36,37]. Of note, DNA binding of sex hormones as observed in mesangial cells regulates, among others, production of collagen type IV, a major component of the GBM determining its permselectivity [38]. The inhibitory effect of testosterone-decanoate on the development of proteinuria possibly results from the stimulatory effect of androgen treatment towards protein reabsorption by renal cells [39]. Furthermore, androgen treatment may cause tubular hypertrophy and an increase in single nephron glomerular filtration rate [40]. In addition, hyperprolactinaemia may also play a role here, since oestrogens are able to up-regulate the synthesis of prolactin, an immunomodulating hormone [41], in the anterior pituitary. We are currently using hormonal manipulation to determine the contribution of sex hormones to glomerular damage in experimental lupus nephritis. In particular, the regulatory role of sex hormones in DNA transcription is under investigation.

In conclusion, our findings confirm earlier observations, in that testosterone-decanoate is shown to be an inhibitory compound, whereas 17β-ethinyloestradiol has stimulating properties in autoimmunity. Moreover, our results show for the first time differential hormonal effects on autoantibody levels and proteinuria in experimental lupus nephritis. The latter finding points towards a novel regulatory pathway of matrix production by glomerular cells, which may eventually be used for therapeutic intervention.

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