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Effects of Castration and Sex Hormones on Immune Clearance and Autoimmune Disease in MRL/Mp-*lpr*/*lpr* and MRL/Mp-+/+ Mice

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The clearance of erythrocytes sensitized with IgG was studied in MRL/Mp-*lpr*/*lpr* (MRL-*lpr*) and MRL/Mp-+/+ (MRL-+/+) mice, which spontaneously develop autoimmune disease. In both strains, an age-dependent decline in clearance of IgG-sensitized erythrocytes was found. Impaired clearance occurred at an earlier age in MRL-*lpr* mice than in MRL-+/+ mice, correlating with the relative severity of autoimmune disease in these strains. Androgen treatment improved clearance in MRL-+/+ mice but not in MRL-*lpr* mice, even though autoantibody levels, renal function, and survival were improved. These results suggest that the beneficial effects of androgen on autoimmune disease are not due solely to improved clearance of immune complexes. Castration followed by estrogen administration did not influence immune clearance or autoimmune disease in MRL-*lpr* mice. These results indicate that impaired immune clearance is a common feature in several autoimmune mouse strains. However, the effects of sex hormones on immune clearance and autoimmune disease may be dissociated in some strains.

INTRODUCTION

Autoimmune disease in general, and systemic lupus erythematosus (SLE)[2] in particular, are much more common in women than in men (1). Recent studies in humans suggest that the female predominance may be due to the influence of sex hormones on a disordered system of immune regulation (2, 3). Support for this concept comes from experiments in NZB/NZW F_1 (B/W mice), which serve as animal models for SLE (4). Female B/W mice are more severely afflicted than males, and the difference in disease expression between the sexes can be attributed to the opposing effects of androgen and estrogen on immunity (5–8).

Recently, we demonstrated that B/W mice have a defect in their ability to clear immunoglobulin-coated erythrocytes (EIgG) (9). This defect, which is more prominent in females than in males, is exacerbated by estrogen administration and alleviated by androgen administration. These observations raise the possibility that sex hormones influence autoimmune disease through their effect on immune

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[2] Abbreviations used: SLE, systemic lupus erythematosus; E, mouse erythrocytes; EIgG, E-sensitized with a 1:1000 dilution of rabbit IgG anti-mouse erythrocytes; MRL-*lpr*, MRL/Mp-*lpr*/*lpr*; MRL-+/+; MRL/Mp-+/+; B/W, NZB/NZW.

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complex clearance. To test this hypothesis, we have studied hormonal influences on immune clearance in two other mouse strains, MRL/Mp-*lpr/lpr* (MRL-*lpr*) and MRL/Mp-+/+ (MRL-+/+), which also spontaneously develop autoimmune disease. MRL-*lpr* and MRL-+/+ are congenic strains which are genetically unrelated to B/W (10). MRL-*lpr* mice are characterized by massive lymphoproliferation, antibodies to nucleic acids, immune complex glomerulonephritis, and death from renal failure at 5–6 months of age in both males and females. MRL-+/+ mice lack the autosomal recessive gene responsible for lymphoproliferation and accelerated autoimmunity in MRL-*lpr* mice. Nonetheless, they develop antinuclear antibodies and immune complex glomerulonephritis at 16–20 months of age.

We found that the ability to clear EIgG is impaired in both MRL-*lpr* and MRL-+/+ mice. Androgen administration improved clearance in MRL-+/+ mice. However, short term androgen administration had no effect on EIgG clearance in MRL-*lpr* mice even though it reduced autoantibody levels, improved renal functions, and prolonged life. These findings demonstrate that impaired clearance of particulate immune complexes is a common feature of several autoimmune mouse strains. They further indicate that androgen therapy may modulate immune complex clearance in some strains but that the beneficial effects of androgen on murine lupus are probably not due solely to this mechanism.

MATERIALS AND METHODS

Reagents and Solutions

Phosphate-buffered saline without Ca^{2+} or Mg^{2+} and Dulbecco's Modified Eagle Medium were obtained from Grand Island Biologicals, Grand Island, New York. Sodium citrate, sodium phosphate (monobasic and dibasic), and glucose were obtained from Sigma Chemical Company, St. Louis, Missouri. Sodium heparin was obtained from Abbott Laboratories, Chicago, Illinois.

Mice

MRL-*lpr* and MRL-+/+ colonies were derived from breeding pairs obtained from The Jackson Laboratory, Bar Harbor, Maine, and maintained at the University of California, San Francisco Vivarium, and the Veterans Administration Medical Center, San Francisco. Young MRL-+/+ mice were obtained directly from The Jackson Laboratory. Castration, sham operation, and hormone replacement were performed as described previously (6). Briefly, a 2-cm Silastic tube containing 6–7 mg of either estradiol-17β or 5-α-dihydrotestosterone powder was implanted subcutaneously, immediately after the castration procedure. This resulted in adequate hormone levels within 12 hr which persisted for at least 3 months. Sham-operated mice received empty implants.

Immune Clearance Assay

Preparation of antiserum: A New Zealand rabbit was immunized by two iv injections of 1.0 ml washed, packed A/J erythrocytes on Days 0 and 3, followed by three intramuscular injections of 1.0 ml packed cells on Days 6, 14, and 21. The serum was collected 4 weeks after the primary immunization. The globulin frac-

tion of the antiserum was obtained by precipitation with 50% NH_4SO_4 and purified by filtration through Sephadex G-200 two times (11).

Labeling with ^{51}chromium: Na ($^{51}CrO_4$), 1 mCi/ml in sterile isotonic saline, was obtained from New England Nuclear (Boston, Mass.). Fresh erythrocytes (E) obtained by bleeding an anesthetized young MRL-*lpr* or MRL-+/+ mouse into 50 units of heparin, were washed in acid citrate–dextrose solution, pH 6.8 (12). Three milliliters of washed E (5.5×10^8 cells/ml) in acid citrate–dextrose solution were incubated with 75 μCi sodium dichromate in a 37°C shaking water bath for 45 min. The labeled E were washed three times in the cold and resuspended to 10^8 E/ml in Dulbecco's medium.

Preparation of EIgG. Equal volumes of a 1:500 dilution of rabbit anti-mouse erythrocyte IgG and suspensions of washed ^{51}Cr-labeled E at 10^8 cells/ml were incubated in a 37°C shaking water bath for 30 min. Erythrocyte suspensions were centrifuged at 500*g*, 4°C for 10 min, the supernatants discarded, and the cells (designated EIgG) washed in Dulbecco's medium.

Erythrocyte clearance and spleen and liver sequestration. Mice were injected iv with 0.2 ml (4×10^7 cells) of ^{51}Cr-labeled nonsensitized or sensitized E. The counts per minute (cpm) in each preparation were determined before each experiment in a gamma scintillation counter (Nuclear-Chicago Corporation, Des Plaines, Ill.). Erythrocyte clearance was determined by serial 25-μl bleedings from the retroorbital plexus with a calibrated, heparinized lambda pipet. The blood samples were suspended in 1.0 ml of cold phosphate-buffered saline, centrifuged at 500*g* for 10 min at 4°C and the supernatants removed from the erythrocyte pellets. The number of cpm in the pellets and the supernatants was determined after subtracting background cpm. The percentage of injected cpm in each pellet was plotted on semilog graphs. The cpm in the supernatants were minimal and consistent throughout the experiment, indicating that little lysis of erythrocytes had occurred.

Some mice were sacrificed 3 hr after injection of EIgG in order to determine the organ localization of the injected erythrocytes. Livers and spleens were removed and the radioactivity present in the whole organ was determined. After subtracting background cpm, the percent retention of erythrocytes was calculated.

Anti-DNA and Anti-Poly A Assays

Antibodies to DNA and to polyriboadenylic acid (poly A) were determined separately using a cellulose ester filter radioimmunoassay. The nucleic acids were double-stranded ^{3}H-DNA (New England Nuclear, Boston, Mass.) and ^{3}H-labeled polyriboadenylic acid (Miles Laboratories, Elkhart, Ind.), a synthetic single-stranded RNA. For this assay, 10 μl of decomplemented whole serum was diluted with 80 μl of borate-buffered saline and incubated with 10 μl of radioactive antigen (1000 cpm/5.5 ng DNA; 1000 cpm/13 ng poly A). After incubation for 30 min at 37°C, followed by overnight incubation at 4°C, the antigen–antibody complexes were collected on cellulose ester filters (Millipore Corp., Bedford, Mass.). The filters were covered with 10 μl of Liquifluortoluene scintillation medium, and radioactivity was measured in a Packard liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.).

Assessment of Renal Disease

Blood urea nitrogen (BUN) was determined using an Abbott Bichromatic Analyzer 100 (Abbott Laboratories Analytical Systems, North Chicago, Ill.). Each serum specimen was run in triplicate.

Statistical Analysis

Clearance data were analyzed using the group t test at each time point. Differences in autoantibody levels and BUN between experimental groups were analyzed by the t test for comparison of two independent means. Differences in mortality were evaluated by χ^2 analysis of 2×2 contingency tables using the Yates correction.

RESULTS

Clearance of EIgG in MRL-lpr Mice

Immune clearance was studied in 14-week-old MRL-*lpr* males that had undergone castration at 8 weeks followed by sustained androgen or estrogen administration by Silastic implant. Sham-operated, untreated, age-matched MRL-*lpr* males served as controls. EIgG clearance was markedly reduced in all three experimental groups compared to 6-week-old MRL-*lpr* mice ($P < 0.01$, from 20 to 180 min) but there were no statistically significant differences among sham-operated, androgen-treated, or estrogen-treated mice (Fig. 1). Comparison of Fig. 1 with Figs. 2 and 3 further demonstrates that EIgG clearance was already impaired in 6-week-old MRL-*lpr* mice relative to MRL-+/+ mice. In addition, MRL-*lpr* males treated at 4 weeks with androgen implants also showed no improvement in immune clearance at 14 weeks (data not shown).

Clearance of EIgG in MRL-+/+ Mice

MRL-+/+ mice were sham-operated or castrated and hormone treated at 5 weeks of age, and their ability to clear EIgG was analyzed at 37–38 weeks of age. Sham-operated adult females (Fig. 2) and males (Fig. 3) cleared significantly less EIgG than young controls from 2 to 60 min ($0.05 > P > 0.01$). In contrast, castrated androgen-treated females and males cleared EIgG as well as young controls and significantly faster than age-matched, sham-operated adults ($0.05 > P > 0.01$) from 2 to 30 min. Estrogen treatment did not affect EIgG clearance in female or male MRL-+/+ mice (Figs. 2, 3).

Clearance in young MRL-+/+ females was mediated mainly by the liver since 64% of injected cpm were present in the liver 3 hr after injection while 3% of cpm were in the spleen. In older MRL-+/+ females there were fewer cpm in the liver (35%) and more cpm in the spleen (10%).

Hormonal Effects on Autoimmune Disease in MRL-lpr Mice

To establish a clinical perspective within which to interpret our immune clearance data, we studied the effect of prepubertal castration and sex hormone administration on autoimmune disease in MRL-*lpr* mice. We found that combined

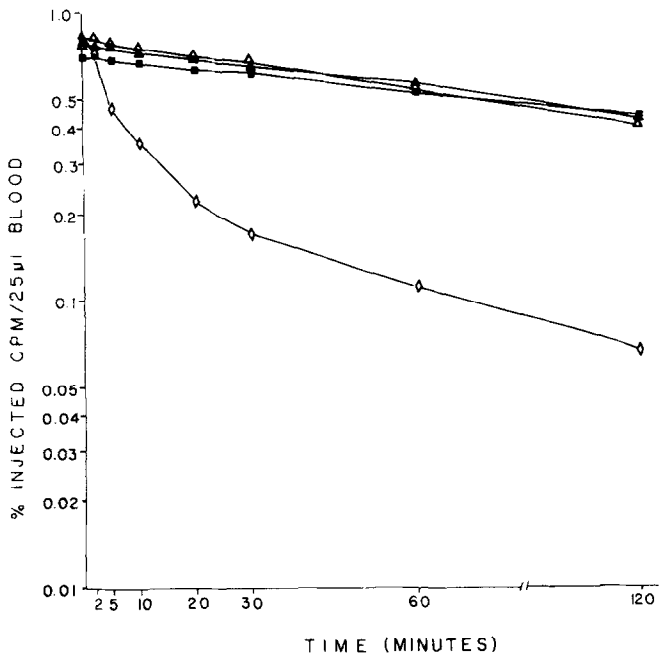

FIG. 1. Clearance of EIgG in male MRL-*lpr* mice. Mice were injected with 4×10^7 ^{51}Cr-labeled EIgG. Clearance was determined with 25-μl blood samples taken at the time points indicated. (◇) 6-week-old mice; (■) sham-operated 14-week-old mice; (▲) castrated, dihydrotestosterone-treated 14-week-old mice; (△) castrated, estrogen-treated 14-week-old mice. Data points represent the mean values of 3–6 mice.

castration and androgen administration at 4 weeks of age significantly improved renal function (Fig. 4) and survival (Fig. 5) in both male and female MRL-*lpr* mice. For example, at 6 months of age, castrated females treated with dihydrotestosterone (DHT) maintained a mean BUN of 20.9 mg/dl compared to 39.2 mg/dl in sham-operated controls ($P < 0.005$); castrated males treated with DHT had a mean BUN of 17.7 mg/dl compared to 37.4 mg/dl in control mice ($P < 0.001$). Castration plus DHT increased 6-month survival from 33 to 80% in female mice and from 32 to 85% in male mice ($P < 0.02$). Castrated, androgen-treated mice also had an early reduction in antibodies to DNA and poly A, but this effect was not sustained beyond 5 months of age (data not shown).

The effects of androgen administration in MRL-*lpr* mice parallel previous observations in B/W mice (5, 6). However, we found that castration alone did not influence the course of autoimmune disease in MRL-*lpr* mice (Figs. 4, 5) as it does in B/W mice (5–7). Furthermore, MRL-*lpr* mice differed from B/W mice in that estrogen administration did not exacerbate autoimmunity. Survival in castrated, estrogen-treated MRL-*lpr* mice of both sexes did not differ significantly from survival in sham-operated controls. In addition, there was no statistically significant effect of combined castration and estrogen therapy on either autoantibody levels or renal function (data not shown).

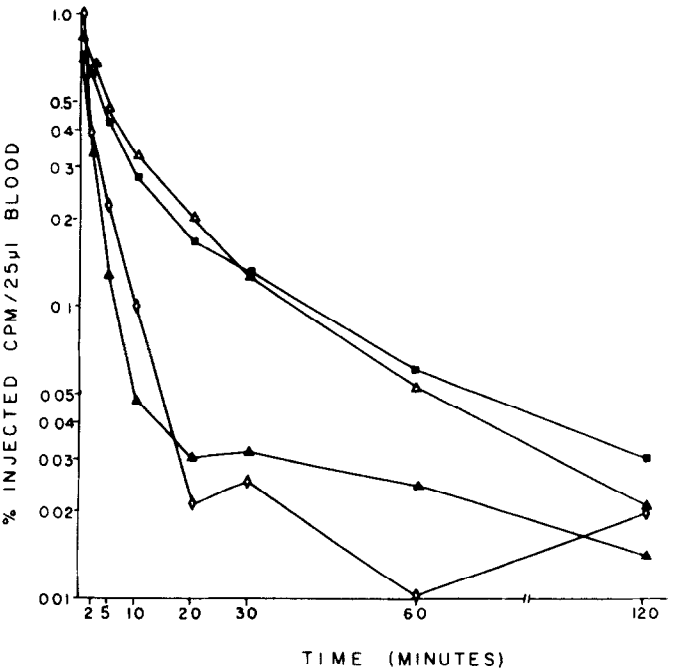

FIG. 2. Clearance of EIgG in female MRL-+/+ mice. Clearance was determined as in Fig. 1. (◇) 3-week-old mice; (■) sham-operated 37-week-old mice; (▲) castrated, dihydrotestosterone-treated 37-week-old mice; (△) castrated, estrogen-treated 37-week-old mice. Data points represents the mean values of 4–9 mice.

DISCUSSION

These experiments demonstrate that the autoimmune mouse strains MRL-*lpr* and MRL-+/+ have an impaired ability to clear particulate immune complexes consisting of autologous erythrocytes coated with IgG antibody. We have previously reported a similar defect in B/W mice (9). The impairment in immune clearance manifests itself at different times in these strains; earlier in female than in male B/W mice (9) and still earlier in MRL-*lpr* mice. Thus, the temporal appearance of this defect correlates with disease activity and not with age per se.

The IgG-sensitized erythrocytes were prepared with a complement-fixing dose of antibody and most of the clearance (64% in young MRL-+/+ female mice) was mediated by the liver. The defect in clearance may therefore be due to depletion of complement which occurs in MRL mice (reviewed in 13). In addition, the clearance defect might be due to saturation of Fc receptors by soluble immune complexes, as suggested in a study of humans with lupus erythematosus (14).

Our data confirm previous reports by Steinberg *et al.* (15), that androgen administration retards autoimmune disease in MRL-*lpr* mice. These findings parallel similar observations in B/W mice (5–8, 15). However, our results also demonstrate several differences between MRL-*lpr* and B/W mice with respect to the effects of sex hormones. First, we have shown that castration alone does not significantly influence the course of autoimmune disease in male MRL-*lpr* mice,

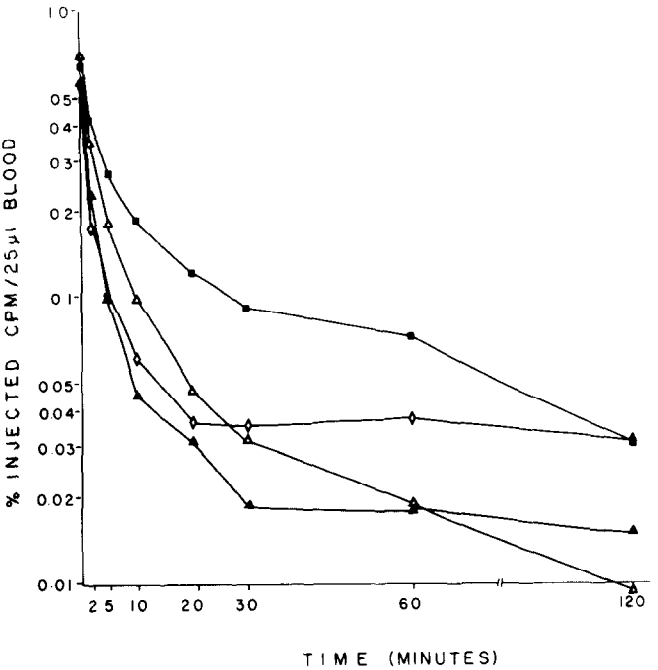

FIG. 3. Clearance of EIgG in male MRL-+/+ mice. Clearance was determined as in Fig. 1. (◇) 8-week-old mice; (■) sham-operated 37-week-old mice; (▲) castrated, dihydrotestosterone-treated 38-week-old mice; (△) castrated, estrogen-treated 38-week-old mice. Data points represent the mean value of 3–7 mice.

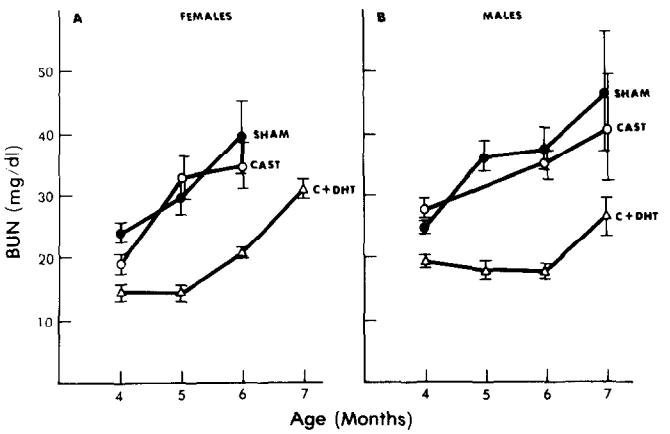

FIG. 4. Effect of prepubertal castration alone on 27 males and 25 females (○); prepubertal castration plus dihydrotestosterone administration of 20 males and 21 females (△); and sham operation of 34 males and 33 females (●) on blood urea nitrogen in (A) female and (B) male MRL-*lpr* mice.

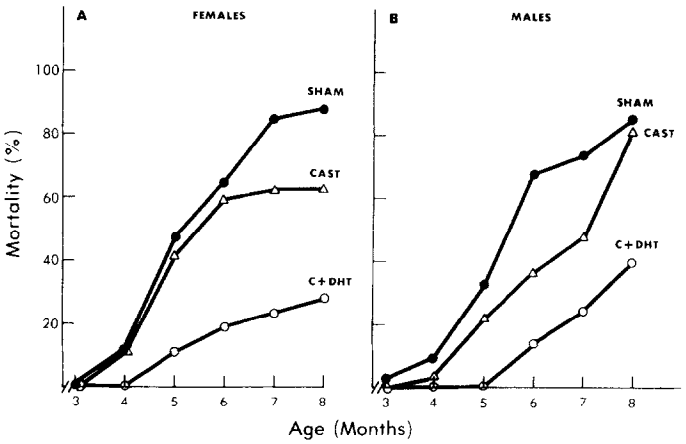

FIG. 5. Effect of prepubertal castration alone and prepubertal castration plus dihydrotestosterone administration on cumulative mortality in (A) female and (B) male MRL-*lpr* mice. Thirty-five females and 27 males underwent castration only (△); 25 females and 20 males underwent castration plus DHT administration (○); 33 females and 20 males underwent sham operations (●).

whereas castration markedly accelerates autoimmune disease in B/W males (5). These findings indicate that physiologic levels of androgen are protective in B/W, but not in MRL-*lpr* mice. Pharmacologic doses of androgen are required to retard autoimmunity in MRL-*lpr* mice. Second, we have shown that neither physiologic nor pharmacologic levels of estrogen influence the course of autoimmune disease in MRL-*lpr* mice, whereas estrogen administration accelerates autoimmunity in B/W mice (6). Finally, we have shown that, in contrast to previous observations in B/W mice (9) androgen therapy does not improve immune clearance in MRL-*lpr* mice.

The lack of improvement in immune clearance in androgen-treated MRL-*lpr* mice suggests that improved clearance is not the sole mechanism through which androgens ameliorate autoimmune disease. The beneficial effects of androgen may be multifactorial, including improved clearance in some strains but not in others. Alternatively, androgens may act by increasing clearance of soluble immune complexes (e.g., DNA-anti-DNA) rather than particulate immune complexes. Androgen did improve clearance in MRL-+/+ mice. However, no data is available on the effect of androgen on the course of autoimmune disease in this strain as yet.

These results and previous studies in humans (14, 16) and mice (9) suggest that impaired clearance is a common feature of several autoimmune diseases. Androgen therapy improves immune clearance in B/W and MRL-+/+ mice but not MRL-*lpr* mice. The reasons for this dissociation warrant further study.

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