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Androgen Suppression of Circulating Immune Complexes and Enhanced Survival in Murine Malaria (41514)

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Abstract. The role of sex hormones as modulators of autoimmune expression, including immune complex deposition and host survival has received considerable attention. In this study BALB/c female mice received 20 mg of depotestosterone cypionate 5 days before and 5 days after infection with the malarial parasite *Plasmodium berghei*. On Day 15 of the infection, surviving mice were monitored for levels of erythrocytes, hematocrits, absolute parasitemias, and circulating immune complexes (CIC). CIC were determined in serum samples by polyethylene glycol insolubilization and direct measurement by helium–neon laser nephelometry. Treated mice showed a 44% reduction in the quantity of CIC, in contrast to infected controls which received cottonseed oil. Mice given the hormone were afforded protection as evidenced by 100% survival on Day 15 of the infection, versus 68% survival for the infected control group. The circulating erythroid and parasitemic levels did not differ significantly between experimental and control groups. We conclude that androgens suppress CIC levels during malaria and further suggest their involvement in the differential host survival noted herein.

Immune complexes have been responsible for a number of immunopathological effects in a variety of diseases, including malaria (1, 2). An immune complex-mediated nephritis may occur in humans (3) and circulating immune complexes (CIC), cryoglobulinemia, and complement consumption have been associated with cerebral malaria (4). In experimental murine malaria, immune complex deposits have been found in the kidneys (5), lungs (6), and choroid plexus (7). The sequential detection of soluble malarial antigens, malarial antibodies, C3 consumption, and CIC have been noted in *Plasmodium berghei* infections (7). Immune complex levels, determined during the course of *P. berghei* infections of several inbred mouse strains, did not relate to numbers of red cells parasitized and observed differences in mortality patterns could not be correlated solely to levels of parasitemia, antibody, or any single serological factor in this study (8). In this regard, CIC and cryoglobulin levels in *Plasmodium falciparum* infections were high in cerebral malaria patients, and low or negligible in uncomplicated or benign malaria (4).

The role of sex hormones in both normal and abnormal immune responses has received considerable attention over the last few years. For some time it has been known that normal pregnant mice may accumulate immune complexes in their renal glomeruli (9) and possible immune complex formation during human pregnancy has recently been reviewed (10). Female B/W (NZB/NZW F_1) mice exhibit an accelerated expression of autoimmune disease, and subsequent development of a fatal immune complex glomerulonephritis (11). It has been further observed that systemic lupus erythematosus occurs about nine times more frequently in women than in men (12). Recently, modulation of the pathological consequences of immune complex disease, including survival, has been demonstrated in a murine lupus model with androgen treatment (13–15) and in experimental autoimmune thyroiditis (16).

This study reports the effect of an androgen primarily on the generation of soluble

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immune complexes, and relates these levels to survival in virulent murine malaria.

Materials and Methods. *Mice.* Twelve-week-old virgin female, inbred BALB/c mice (Charles River Labs, Wilmington, Mass.) were used throughout this investigation. Mice were allowed food and water *ad libitum* until fasted 12 hr before sacrifice.

Malaria infection. Plasmodium berghei berghei, strain NK 65, was maintained by weekly blood passage. Experimental hormone-treated mice, and infected control mice, received an intraperitoneal inoculum containing 5×10^4 parasitized red blood cells. On Day 15 of the infection, mice were tail-bled just prior to exsanguination, and percentage parasitemias, hematocrits, and erythrocyte counts were determined. Serum samples were collected at 4° and normally employed within 24 hr.

Drug treatment. Depotestosterone cypionate (Upjohn, Kalamazoo, Mich.) in cottonseed oil was injected intraperitoneally in a volume of 0.2 ml (20 mg) into mice on two occasions; namely 5 days before and 5 days after infection. Control mice received comparable injections of 0.2 ml cottonseed oil 5 days before and 5 days after infection.

Circulating immune complexes (CIC). The method of Schultz-Ellison *et al.* (17) involving polyethylene glycol insolubilization of CIC and direct measurement by laser nephelometry was performed as described, with the following minor modifications. Twenty-five microliters of a serum sample was combined with 1.0 ml of a pH 8.3 borate buffer containing 3% PEG 6000 (Fisher Scientific Co.) and allowed to incubate for 1 hr at room temperature. A Hyland Laser Nephelometer (Hyland, Costa Mesa, Calif.) incorporating a helium–neon laser light source (6328 Å) was employed to detect the insolubilized complexes. The instrument was used with a photometer blank subtract of medium, and a computing time of 15 sec. A high reference cuvette was used to set the range to 100% relative light scatter (RLS) at a sensitivity of one. The reference cuvette contained a 1:1000 dilution of latex particles, particle size 0.81 μm (Difco Lab., Detroit, Mich.). The percentage relative light scatter (RLS) displayed for each sample was the amount of light scatter produced by the sample, relative to the reference cuvettes. Cohn Fraction II (Sigma Chemical Co., St. Louis, Mo.) was heated to 63° for 20 min, whereupon large aggregates were removed by centrifugation at 2000*g* for 10 min. The concentration of aggregated IgG was determined by absorbance at 280 nm. Twenty-five micrograms of aggregated IgG, combined with 1.0 ml of 3% PEG in borate, generates 12.5% RLS at this setting.

Statistical analyses. Parameters were expressed as the group mean ± one standard error. Based on the nonparametric Mann–Whitney U test, P values <0.05 were considered to be statistically significant.

Results. Mice receiving testosterone exhibited significantly reduced levels of circulating immune complexes on Day 15 of a virulent murine malaria infection as compared to untreated infected control animals (Table I). Specifically, treated mice showed a 44% reduction in the quantity of soluble complexes, relative to infected mice receiving only cottonseed oil. Treated mice were afforded protection by the hormone as evidenced by 100% survival on Day 15 of the infection (Table II). The circulating erythroid and parasitemic levels did not differ significantly between hormone-treated and infected control mice (Table III).

Discussion. Administration of testosterone, before and after infection with *Plasmodium berghei,* reduced levels of cir-

TABLE I. EFFECT OF TESTOSTERONE ON CIRCULATING IMMUNE COMPLEX (CIC) LEVELS ON DAY 15 OF MALARIAL INFECTION

Groups	Nos. of animals	RLS[a]
Uninfected controls	11	7.4 ± 0.36
Infected controls	20	43.4 ± 2.16
Androgen treated	17	27.5 ± 1.26*

[a] Relative light scatter (%) of polyethylene glycol insolubilized CIC in 25 μl serum samples measured by laser nephelometry. Values represent the group mean ± one standard error.

* Significantly different from uninfected and infected control ($P < 0.05$).

TABLE II. EFFECT OF TESTOSTERONE ON HOST SURVIVAL ASSESSED ON DAY 15 OF MALARIAL INFECTION

Groups	Nos. of animals	Animals surviving	Percentage survival
Infected control	38	26	68
Androgen treated	38	38	100

culating immune complexes by 44% on Day 15 of the infection, as compared to non-treated infected control animals (Table I). Day 15 of the infection was monitored in view of the mortality pattern, levels of CIC, degree of parasitemia, and anemia previously observed for this critical period. Reduced levels of CIC are perhaps not too surprising, since there is increasing evidence that androgens may suppress both spontaneous autoantibody production and antibody response to immunization (14). Furthermore, hormone-treated malarial mice, exhibiting reduced levels of soluble complexes, showed enhanced host survival (Table II). Increased survival has been demonstrated in an androgen-treated murine lupus model, along with the reduction of antinuclear and anti-T lymphocyte antibodies, and a decreased deposition of immune complexes in the glomerulus (13–15). Roubinian *et al.* (13) suggest that protection may be due, in part, to androgen promotion of suppressor T-cell function. In this regard, during the course of systemic lupus erythematosus, both immune complexes and lymphocytotoxic autoantibodies may be responsible for the suppressor T-cell defects noted (18). Conceivably autocytotoxins (19) and immune complexes may play a role in the observed T-cell deficits in rodent malaria as well (20).

TABLE III. EFFECT OF TESTOSTERONE ON THE ANEMIA AND PARASITEMIA EVIDENCED ON DAY 15 OF MALARIAL INFECTION

Parameters[a]	
Erythrocytes ($\times 10^6$/mm^3)	
Infected controls	3.93 ± 0.27
Androgen treated	3.24 ± 0.18
Hematocrit (%)	
Infected controls	22.6 ± 3.29
Androgen treated	24.6 ± 1.63
Parasitized erythrocytes (%)	
Infected controls	35.2 ± 4.23
Androgen treated	42.4 ± 1.91
Parasitized erythrocytes ($\times 10^6$/mm^3)	
Infected controls	1.40 ± 0.21
Androgen treated	1.34 ± 0.08

[a] Each value represents the groups mean ± one standard error of 8–10 animals.

We have previously demonstrated that overcoming the anemia associated with murine malaria by red cell hypertransfusion is an effective maneuver to promote recovery (21). However, enhanced survival of androgen-treated malarial infected animals is not effected by a reduced anemia, or parasitemia, as clearly seen in Table III. The erythropoietic capability of mice infected with malaria is impaired (22) and contributes to the severe anemic status. Although androgens have been shown to stimulate erythropoiesis (23), treated infected mice were essentially as anemic as infected control mice. The only parameter monitored, that was significantly different from infected control mice, was the levels of circulating immune complexes. There is obviously a danger in concluding that CIC were subsequently responsible for the variant host survival seen. Nevertheless the varied immunopathological effects of immune complexes on host systems (2) argue for a significant role in host survival patterns observed during this study. In view of the survival benefit associated with CIC reduction, herein described, it might be of interest to determine the effect of CIC removal by *ex vivo* adsorption, as employed in other studies.

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