INFECTION AND IMMUNITY, May 1972, p. 695-698

Vol. 5, No. 5
Printed in U.S.A.

Sex Difference in Antibody Response of CFW Mice to *Candida albicans*

DAVID RIFKIND AND JANET A. FREY

Departments of Microbiology and Medicine, University of Arizona College of Medicine, Tucson, Arizona 85724

Received for publication 12 January 1972

Bilaterally gonadectomized and control unilaterally gonadectomized male and female CFW mice were given a course of *Candida albicans* vaccine, and the agglutinating antibody response was measured. The mean titers in the control females were higher than those in the control males. Bilateral gonadectomy did not affect the mean titer in the females but in the males resulted in a significant increase in the antibody. Similar groups of mice were inoculated intraperitoneally with viable *C. albicans*, and 10 weeks later antibody was measured and the kidneys were cultured. There was no apparent relationship between the degree of the agglutinin response and either gonadectomy or renal candidiasis at autopsy.

Sex differences in resistance to infection and in immune responsiveness, both favoring the female, have been reported in man and in experimental animals (4, 10, 20, 23). In an attempt to define the mechanisms involved in an animal model, *Candida albicans* urinary tract infection in CFW mice was investigated (17). It was found that healing of the infection was more active in the female and that in both sexes resistance was augmented by preinoculation gonadectomy. The present study describes experiments on the antibody response of normal and gonadectomized CFW mice to *C. albicans* vaccination and infection.

MATERIALS AND METHODS

Mice. CFW strain mice of both sexes were obtained from a commercial source (Carworth Farm, New City, N.Y.) and maintained on pelleted food (Purina Mouse Chow, Ralston Purina Co., St. Louis, Mo.) and water ad lib. All mice were 8 to 10 weeks of age at the beginning of inoculation and vaccination experiments. Unilateral and bilateral gonadectomy, when performed, was done at age 3 weeks as described previously (17).

Vaccine. *C. albicans* serotype A was grown in 1% dextrose nutrient broth (Difco Laboratories, Detroit, Mich.) for 2 days at 35 C, washed once in 0.85% saline, resuspended to 1.2×10^8 cells/ml, and heat-killed at 60 C for 1 hr. The cell suspension was sonically disrupted at 100 w for 15 min at 4 C. (Sonifier Cell Disrupter, model W 185D, Ultrasonics, Inc. Plainview, N.Y.). The resulting vaccine was stored in portions at −20 C.

Serologic specimens. Specimens were obtained for serial measurement of antibody by cutting a tail vein and collecting blood in heparinized capillary tubes (75 mm by 1.1 to 1.2 mm inside diameter). The tubes were centrifuged in a capillary tube head at 2,500 × *g* for 20 min at 4 C. A 25-mm length of the plasma-containing end of the capillary tube, equivalent to approximately 0.025 ml, was cut off, and the contents were expelled into 0.175 ml of 0.85% saline. This resulted in a 0.2-ml specimen of plasma diluted 1:8. At the time of sacrifice and autopsy, a final specimen was obtained in a 1-ml syringe from a subclavian vein. Specimens were stored at −20 C for subsequent antibody assay.

Agglutination tests. Agglutinating antibody titers were measured by a modification of the procedure described by Preisler et al. (15). The antigen was prepared from *C. albicans* serotype A grown for 48 hr at 35 C in veal infusion broth (Difco) supplemented with 1% dextrose. The cells were collected by centrifugation, washed twice with 0.85% saline, resuspended to a concentration of 2×10^6/ml, and heat-killed at 60 C for 1 hr.

Antibody titrations were performed in disposable microtiter "U" plates (Cooke Engineering Co., Alexandria, Va.). Serial twofold dilutions of serum or plasma were made in 0.025 ml of 0.85% saline. An equal volume of antigen was added, and the plates were incubated for 2 hr at 47 C followed by refrigeration overnight. The plates were then agitated gently, and the titers were read at 25-fold magnification by using a binocular tissue culture microscope.

All specimens from a single animal were tested simultaneously. Only antibody titer rises of a fourfold dilution or greater were considered to be significant. (In the calculation of geometric mean titers, sera which were negative at the lowest dilution tested, 1:8, were arbitrarily assigned a value of 0.)

Microbiology. The methods of inoculation of mice with viable *C. albicans*, the collections of serial urine specimens for quantitative culture, and autopsy of mice for candida kidney cultures were described previously (17).

TABLE 1. *Effect of gonadectomy on the agglutinating antibody response of CFW mice to candida vaccination*

Length of vaccination[a] (weeks)	No. of mice											
	Male, bilateral gonadectomy[b]			Male, unilateral gonadectomy[b]			Female, bilateral gonadectomy[b]			Female, unilateral gonadectomy[b]		
	No titer[c]	Titer rises[d]	Titer[e]	No titer[c]	Titer rises[d]	Titer[e]	No titer[c]	Titer rises[d]	Titer[e]	No titer[c]	Titer rises[d]	Titer[e]
0	10		1.4 (1.3)	12		0.0 (0)	6		3.2 (1.4)	3		5.0 (1.3)
2	1	4	10 (1.3)	5	0	3.4 (1.4)	4	0	5.0 (1.4)	0	0	9.5 (1.1)
4	0	7	15 (1.1)	4	4	5.3 (1.5)	0	6	18 (1.1)	0	4	16 (1.1)
6	0	10	30 (1.2)	4	6	8.0 (1.6)	1	3	10 (1.4)	0	3	14 (1.2)
10	0	10	17 (1.1)	3	4	6.4 (1.4)	3	2	6.0 (1.4)	3	1	5.7 (1.4)
12	0	11	48 (1.3)	3	9	12 (1.6)	1	8	23 (1.5)	0	6	21 (1.3)

[a] A 0.025 ml amount of 1:2 antigen given intradermally at 0, 2, 4, 10, and 12 weeks.
[b] Number of mice equals 12.
[c] Showed titers of <1.8.
[d] Showed fourfold or greater rise compared to zero week.
[e] Geometric means, reciprocal of dilution and (standard error).

RESULTS

Candida vaccination. Twelve male and 12 female bilaterally gonadectomized mice and an equal number of unilaterally operated controls were each given an intradermal injection of 0.025 ml of a 1:2 dilution of candida vaccine at 0, 2, 4, and 10 weeks. Blood samples were ob-obtained for agglutinating antibody assays just prior to each vaccine dose and again at 6 and 12 weeks.

Preinoculation antibody was detected in 2 of the 24 males and in 15 of the 24 females (Table 1, Fig. 1). However, the titers in these antibody-positive animals were uniformly low. It seems likely that all animals had had prior exposure to candida and that the more frequent detection of preinoculation antibody in the females results from their more vigorous response to antigenic stimulus.

By the end of the immunization period, almost all of the animals had developed a significant agglutinating antibody titer response (Table 1, Fig. 1). The titers were higher in the unilaterally gonadectomized control females than in the comparably operated males. In the females, bilateral gonadectomy did not significantly alter the antibody titers. In contrast, in the males, bilateral gonadectomy resulted in higher antibody titers, and these levels were even greater than in the two groups of females.

The frequency of fourfold or greater antibody titer rises was similar in both groups of females and in the unilaterally gonadectomized males (Table 1, Fig. 1). Such rises, however, were more frequent in the bilaterally gonadectomized males. This difference was most marked at the 10th week of vaccination, 6 weeks after the most recent antigen dose, at which time most of the animals in the groups other than the bilaterally gonadectomized males had lost their fourfold titer increases.

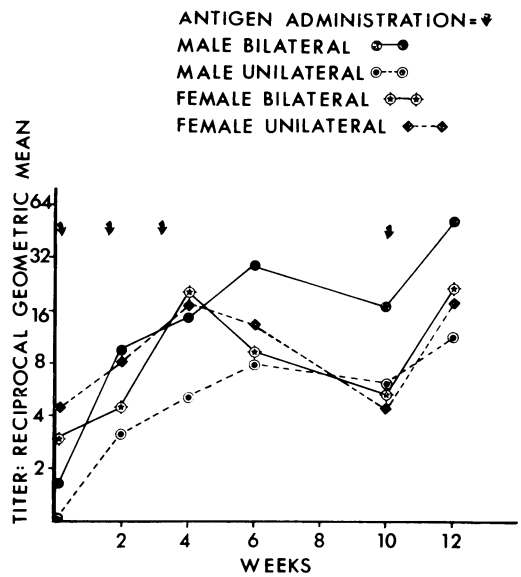

FIG. 1. *Agglutinating antibody response of bilaterally gonadectomized and control unilaterally gonadectomized CFW mice to four injections of sonically disrupted Candida albicans vaccine.*

Viable candida inoculation. Twelve male and 12 female bilaterally gonadectomized mice and an equal number of unilaterally operated controls were each inoculated intraperitoneally with 2×10^7 viable *C. albicans*. Blood samples for agglutinating antibody titrations were ob-

tained preinoculation and again at autopsy 10 weeks later. The kidneys were taken at autopsy and cultured for candida.

Deaths occurred only in the unilaterally operated mice; three in males and two in females.

Preinoculation candida agglutinins were found in 2 of the 48 mice. Fourfold or greater antibody titer rises occurred in 26 of the 43 survivors. There was no correlation between the development of a significant antibody rise and either sex, gonadectomy, or the presence of candida in the kidney at autopsy.

DISCUSSION

The present studies demonstrate that candida vaccination results in higher agglutinating antibody titers in female CFW mice than in males. In the females, the magnitude of this response was not affected by gonadectomy. In contrast, in the males bilateral gonadectomy resulted in higher antibody titers, and the levels achieved were even greater than in the females. In addition, a decline in antibody titer occurred at week 10, 6 weeks after an antigen dose, in most of the animals of all groups with the exception of the bilaterally gonadectomized males.

In contrast to the findings with candida vaccination, antibody titers after viable candida inoculation showed no significant sex difference. This may be related to the fact that the extent of antigenic stimulus in the groups was modified by the mode and rate of clearance of the infecting organisms.

In humans the occurrence of the X-linked agammaglobulinemias indicates some influence of the sex chromosomes on antibody production. Further, X-linked control of the serum concentration of certain immunoglobulin classes has been demonstrated. Rhodes et al. found that the concentration of circulating immunoglobulin M (IgM) paralleled the number of X chromosomes, being highest in patients with an XXX karyotype, intermediate in normal females, and lowest in men (16). The serum concentrations of immunoglobulin G (IgG) and immunoglobulin A (IgA) however, were found not to be similarly X-related. Hughes confirmed the higher serum IgM levels in females, but in addition presented evidence that serum IgA levels are somewhat greater in males (7).

The antibody responses to specific antigens and the classes of immunoglobulins involved in these responses also show a sex difference. In children, the antibody titers to several *Escherichia coli* serotypes (11), to rubella live virus vaccine (11), and to measles infection (6) are higher in females. The fraction of poliovirus type I antibody found in the serum IgA as opposed to IgG immunoglobulin class is greater in females (1). Similarly the antibody response of mice to sheep erythrocytes (8) and to bovine serum albumin (20) is greater in females.

In experimental animals, exogenously administered gonadal hormones have been shown to influence the immune response to nonviable antigens, but the reported direction of the response has varied. Estrogens have been noted to increase (22), to decrease, and not to affect antibody responses (5, 14). Testosterone has been found to increase (5) as well as not to influence antibody responses (14). The progestins have uniformly depressed antibody formation (8). In these studies, the sex of the experimental animal did not appear to be the factor controlling the effect of hormones on the antibody response.

Using the direct Jerne agar plaque method, Kenny and Gray found in weanling mice that the number of spleen cells producing *E. coli* antibody is greater in females than in males (9). In both sexes the number of such spleen cells increases with age, but this increase is more pronounced in the female. Ovariectomy decreased the immune response in the weanling females, but the effect of gonadectomy in males was not reported. However, in the males estradiol treatment increased spleen cell response. It should be noted that the procedure used for detecting antibody-forming spleen cells in their study measures primarily IgM antibody. The results may therefore reflect the specific relationship between serum IgM levels and the number of X chromosomes as observed in humans (16, 7); their applicability to the other immunoglobulin classes would require studies by additional methods.

In contrast to the results of Kenny and Gray (9) and to those reported herein, gonadectomy in adult animals has been reported not to influence antibody responses (8, 22). Whether this discrepancy indicates a significant effect of gonadal hormones only on the development of immune responsiveness or merely a difference in experimental design is unknown.

In 1952, Dougherty suggested that the gonadal hormones may act as important moderators of the immune system throughout life (3). He pointed out that androgens and estrogens in a number of animal systems produce thymic and lymph node atrophy and that gonadectomy at all ages results in an increased amount of lymphoid tissue. In female rats, human chorionic gonadatropin and estrogen in combination produce thymic atrophy, but progesterone is devoid of effect (12). In the chick embryo, testosterone causes atrophy of the bursa of Fabricius, peripheral lymphoid tissue, and spleen; less frequently

the thymus is also depressed by the androgen (19).

In addition to the influence of gonadal hormones on the lymphoid system cited above, the reverse has also been suggested. In neonatally thymectomized mice, ovarian agenesis occurs (13). Testicular development, however, is normal. In ataxia-telangiectasia, a syndrome associated with thymus-dependent cell deficiencies, instances of ovarian agenesis have been reported (2).

It has been suggested that the greater immune responsiveness of the female may result from the additional heterogenity provided by the two X chromosomes (23). The data reported herein rather suggest that the advantage may result from the relative absence of the immunosuppressive androgenic hormones.

Whatever the mechanism, the present information suggests that the gonads exert a significant influence on immunoglobulin production. It appears that a more precise definition of this effect must be made in terms of the individual immunoglobulin classes and the specific gonadal hormones singly and in combination. Such information could provide important insight into the control of immunoglobulin classes as well as suggest additional means of manipulation of the immune response.

Acknowledgment

This study was supported by Public Health Service grant AI08651 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Ainbender, E., R. B. Weisinger, M. Hevizy, and H. L. Hodes. 1968. Difference in the immunoglobulin class of polioantibody in the serum of men and women. J. Immunol. **101**: 92–98.
2. Boder, E., and R. P. Sedgwick. 1958. Ataxia-telangiectasia; a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. Pediatrics **21**:526–554.
3. Dougherty, T. F. 1952. Effect of hormones on lymphatic tissue. Physiol. Rev. **32**:379–401.
4. Editorial. 1969. Oral contraceptives and immune responses. J. Amer. Med. Ass. **209**:410.
5. Healy, J. W., and S. Leeman. 1967. Hemolysin response of rats treated neonatally with steroids. Endocrinology **80**: 762–764.
6. Henson, T. E., J. A. Brody, J. L. Sever, M. L. Dyken, and J. Cannon. 1970. Measles antibody titers in multiple sclerosis patients, siblings, and controls. J. Amer. Med. Ass. **211**: 1985–88.
7. Hughes, N. K. 1971. Serum concentrations of γG, γA and γM immunoglobulins in patients with carcinoma, melanoma, and sarcoma. J. Nat. Cancer Inst. **46**:1015–1027.
8. Hulka, J. F., K. Mohr, and M. W. Lieberman. 1965. Effect of synthetic progestational agents on allograft rejection and circulating antibody production. Endocrinology **77**:897–901.
9. Kenny, J. K., and J. A. Gray. 1971. Sex differences in immunologic response: studies of antibody production by individual spleen cells after stimulus with *Escherichia coli* antigen. Pediat. Res. **5**:246–255.
10. Long, E. R., and A. B. Vogt. 1941. Relation of sex to the course of experimental tuberculosis in mice. Amer. Rev. Tuberc. **44**:196–222.
11. Michaels, R. H., and K. D. Rogers. 1971. A sex difference in immunologic responsiveness. Pediatrics **47**:120–123.
12. Nelson, J. H., Jr., J. E. Hall, G. Manuel Limson, H. Freidberg, and F. J. O'Brien. 1967. Effect of pregnancy on the thymolymphatic system. I. Changes in the intact rat after exogenous HCG, estrogen and progesterone administration. Amer. J. Obstet. Gynecol. **98**.895–899.
13. Nishizuka, Y., and T. Sakakura. 1969. Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. Science **166**:753–755.
14. Oettgen, H. F., R. A. Binaghi, and B. Benacerraf. 1966. The effect of various hormones on the serum level of anaphylactic antibody in the rat. Int. Arch. Allergy Appl. Immunol. **29**:131–139.
15. Preisler, H. D., H. F. Hasenclever, A. A. Levitan, and E. S. Henderson. 1969. Serologic diagnosis of disseminated candidiasis in patients with acute leukemia. Ann. Intern. Med. **70**:19–30.
16. Rhodes, K., R. L. Markham, P. M. Maxwell, and M. E Monk-Jones. 1969. Immunoglobulins and the X-chromosome. Brit. Med. J. **3**:439–441.
17. Rifkind, D. 1972. Influence of gonadectomy on *Candida albicans* urinary tract infection in CFW mice. Infect. Immunity **5**:363–369.
18. Stern, K., and I. Davidsohn. 1955. Effect of estrogen and cortisone on immune hemoantibodies in mice of inbred strains. J. Immunol. **74**:479–484.
19. Szenberg, A., and N. L. Warner. 1962. Dissociation of immunological responsiveness in fowls with a hormonally arrested development of lymphoid tissues. Nature (London) **194**:146–147.
20. Terres, G., S. L. Morrison, and G. S. Habicht. 1968. A quantitative difference in the immune response between male and female mice. Proc. Soc. Exp. Biol. Med. **127**:664–667.
21. Thompson, J. S., M. K. Crawford, R. W. Rielly, and C. D. Severson. 1967. The effect of estrogenic hormones on immune responses in normal and irradiated mice. J. Immunol. **98**:331–335.
22. Von Haam, E., and I. Rosenfeld. 1942. Effect of estrone on antibody-production. J. Immunol. **43**:109–117.
23. Washburn, T. C., D. N. Mediaris, Jr., and B. Childs, 1965. Sex differences in susceptibility to infections. Pediatrics **35**: 57–64.