Sensitivity to androgen. A possible factor in sex differences in the immune response

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SUMMARY

In strains of mice with high or low sensitivity to androgen, previous studies demonstrated that sex differences in the levels of certain Igs occurred only in the high androgen responder (HAR) strain, but not in the low androgen responder (LAR) strain. HAR C57L/J males, whether intact or castrated, had lower levels of IgM and IgG2 than C57L/J females, and lower levels of IgM and IgG2 than LAR A/J mice (Cohn & Hamilton, 1976). The current study indicates that the pattern of low immune response characteristic of HAR males in non-specific responses also occurs in response to three different antigens and is evident in another HAR strain, the RF/J.

Results show that in response to type 3 pneumococcal polysaccharide (SIII), HAR C57L/J males consistently produced lower levels of antibody than C57L/J females, whereas LAR A/J males and females produced similar levels of anti-SIII. HAR C57L/J males also produced significantly lower levels of anti-SIII than LAR A/J males following restricted doses of SIII (50 ng). Altering the concentration of circulating testosterone in HAR C57L/J males had no effect on their anti-SIII responses. In assays of another pair of HAR and LAR strains, the RF/J and 129/J, HAR RF/Js had significantly lower anti-SIII responses than LAR 129/Js. In response to bovine serum albumin (BSA), HAR C57L/J mice showed sex differences in anti-BSA responses both 14 and 21 days after immunization, whereas HAR A/J mice showed sex differences on day 14 only, before peak responses developed. Of all mice assayed, however, HAR C57L/J males had the lowest levels of anti-BSA on both days. When strains differing in sensitivity to androgen and H-2 type were assayed for antibody to bovine gamma-globulin (B-IgG), RF/J females produced comparatively high antibody responses as expected for H-2^k strains, but HAR RF/J males produced the lowest levels of antibody of all mice assayed.

The results of these studies support and extend earlier observations of an apparent correlation in male mice between high sensitivity to androgen and poor immune responsiveness.

INTRODUCTION

It is widely recognized that in most species studied, males have weaker immune responses than females (Batchelor, 1968; Terres, Morrison & Habicht, 1968; Eidinger & Garrett, 1972). Although this condition is thought to contribute to the higher susceptibility to infection (Washburn, Medearis & Childs, 1965; Friedman, Grota & Glasgow, 1972; Goble & Konopka, 1973) and poorer survival of males compared to females (Pease, 1947; Beverton & Holt, 1959; Simms, 1967; Hamilton, Hamilton & Mestler, 1969; Vital Statistics of The US, 1976), mechanisms underlying sex differences in immune responsiveness have remained poorly understood.

Attempts to explain these differences strictly on the basis of differences in the sex chromosomes or

the sex hormones have generally been unsuccessful. In studies of type 3 pneumococcal polysaccharide (SIII), an antigen which elicits responses controlled by the X chromosome, X chromosome inheritance as a sole factor has failed to explain the sex differences in antibody production found among low immune responding mice but not observed in high immune responding strains (Amsbaugh *et al.*, 1972). Direct correlations between levels of IgM and numbers of X chromosomes or regulatory factors on the X chromosome have been demonstrated in some studies of humans (Rhodes *et al.*, 1969; Wood *et al.*, 1969; Grundbacher, 1972) but not in others (Stiehm & Fudenberg, 1966). In mice, Adinolfi *et al.* (1978) have been unable to correlate IgM levels with numbers of X chromosomes in XX, Sxr/+ sex reversed males or in 39, X0 females. Differences in IgG levels between and within the sexes have also been observed. Certain males have lower Ig levels than their respective females (Cohn & Hamilton, 1976) and lower Ig levels than males of other strains or races within the same species (Lichtman, Vaughan & Hames, 1967; Karayalcin, Rosner & Sawitsky, 1973; Cohn & Hamilton, 1976). Additional influences on the immune response other than X chromosome control have been strongly suggested by the work of Amsbaugh *et al.* (1974). In these studies, reciprocal crosses between the F_1 of high and low immune responding strains produced F_2 females which had the same gene dose for the X-linked controlling element, but produced unexpectedly different antibody responses. The results of these experiments, and others using recombinant mice, have led to the conclusion that genetic mechanisms controlling antibody responses to SIII involve genes on the X chromosomes plus other factors that remain to be established (Baker *et al.*, 1976).

With respect to the effects of sex hormones on immune responses, elevated progesterone levels in normal women during pregnancy or the luteal phase have generally been associated with decreased T cell function (Strelkausas, Davies & Dray, 1978; Bulmer & Hancock, 1977). Increased antibodies to *Candida albicans*, but not to SRBC or Herpes virus, have also been observed during periods of progesterone elevation (Mathur *et al.*, 1978). Other types of hormone studies have been more difficult to evaluate because reports have disagreed on the effects of gonadectomy or hormone administration and, in some cases, results for males and females have been indistinguishable (Batchelor, 1968; Eidinger & Garrett, 1972; Friedman *et al.*, 1972; Andersen & Hanson, 1974; Castro, 1974).

It is possible that some of the conflicting data on the mechanisms behind sex differences in immune responses resulted from: (1) not utilizing physiological doses of hormone; and (2) not selecting animal models best suited for investigating this problem, namely groups of males known to have weaker immune responses than other males within the same species. The current studies were designed to overcome these difficulties by: (1) using doses of testosterone which produce known physiological effects; and (2) selecting strains of mice which include males previously shown to differ in plasma Ig levels. C57L/J and RF/J males had been shown to have low plasma levels of IgM and IgG2, whereas A/J and 129/J males have normal or high levels of these Igs (Cohn & Hamilton, 1976; Cohn, 1979). In addition, males of these strains differ in the sensitivity of their target organs to androgen. Prior experiments have shown that it is the males of strains highly responsive to androgen, whether intact or castrated, that have low levels of IgM and IgG2 (Cohn & Hamilton, 1976; Cohn, 1979).

The objectives of the current study were firstly to ascertain whether the correlation between high sensitivity to androgen and poor immune performance suggested by our prior Ig experiments also applied to the specific immune response, and secondly to examine whether specific immune responses of HAR males were influenced by alterations in the level of circulating androgen. Antibody responses to three types of antigens were compared in mice of high (HAR) and low (LAR) androgen responder strains. In one strain which showed marked sex differences in androgen responsiveness, antibody responses were studied further in intact males, males depleted of testosterone by castration and males treated with physiological doses of testosterone.

MATERIALS AND METHODS

Animals. Five-week-old C57L/J, RF/J, A/J and 129/J mice of both sexes were obtained from the Jackson Laboratory, Bar Harbor, Maine. Strains were selected which varied both in target organ responsiveness and in H-2 type. Standard
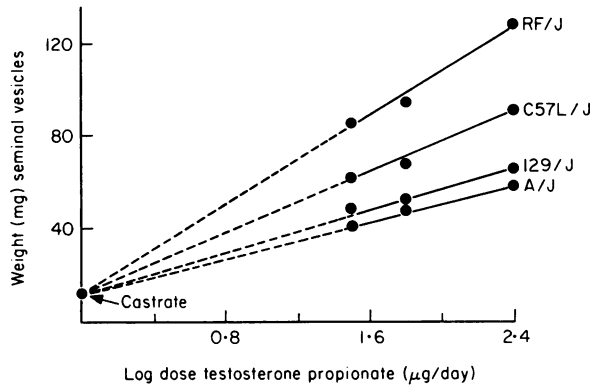
FIG. 1. Increases in seminal vesicle (SV) weight of castrated RF/J, C57L/J, 129/J and A/J males following daily doses of 32, 64, or 256 μg of testosterone propionate for 14 days using a standard SV bioassay (Cohn & Hamilton, 1976). For each dose, differences in SV weight were significant at $P < 0.01$ between the RF/J and 129/J males and the C57L/J and A/J males. Seven to ten mice per point were used.

seminal vesicle bioassays, performed in our laboratory as described previously (Cohn & Hamilton, 1976), established that the C57L/J and RF/J were high androgen responders (HAR) and the A/J and 129/J low androgen responders (LAR) (Fig. 1). H-2 types included: H-2^b in the (HAR) C57L/J and (LAR) 129/J; H-2^k in the (HAR) RF/J; and H-2^a in the (LAR) A/J.

Antigens. Three types of antigens were used: (1) type 3 pneumococcal capsular polysaccharide (SIII), considered to be T cell-independent (Howard *et al.*, 1971); (2) bovine serum albumin (BSA) emulsified with Freund's complete adjuvant (FCA), which is T cell-dependent (Allison & Davies, 1971); and (3) bovine IgG (B-IgG) emulsified with FCA, a T cell-dependent antigen which elicits a response controlled by H-linked Ir genes (Vaz & Levine, 1970).

The antigens were prepared as follows: type 3 pneumococcal polysaccharide (SIII), originally processed by Dr F. Cano of Lederle Laboratories, Pearl River, New York, was obtained from the Pneumococcal Polysaccharide Reference Laboratory, Department of Microbiology and Immunology, Downstate Medical Center through the generosity of Dr G. Schiffman. This preparation was essentially devoid of nitrogen and was of high molecular weight as tested by gel filtration with Sepharose 4B. When tested by quantitative precipitin, radioimmunoassay and inhibition, the preparation was greater than 90% pure immunologically, contained less than 2% of group C carbohydrate, and was pyrogen-free. The stock was diluted in pyrogen-free bacteriostatic sodium chloride (0.9%) (American Quinine Company, Plainview, New York) to a final concentration of 200 ng/ml. Crystalline BSA (lot No. 4300, Nutritional Biochemical Corporation, Cleveland, Ohio), and B-IgB (lot No. 21, Miles Laboratories, Elkhart, Indiana) were each diluted in pyrogen-free bacteriostatic sodium chloride, then emulsified in Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) to yield a final concentration of 100 μg/ml.

Experimental design. All mice were immunized at 14 weeks of age and all assays done individually in duplicate.

Responses to SIII. (1) Forty-three C57L/J and forty-four A/J mice of both sexes were given a single 0.5 ml i.p. injection containing 100 ng of SIII and assayed 9 days later for anti-SIII responses. (2) Another twenty C57L/J males and twenty A/J males were given an i.p. injection of 50 ng or 100 ng of SIII and assayed 5, 9 and 13 days after immunization. (3) Fifty-seven C57L/J males were either gonadectomized or sham-operated at 10 weeks of age and 2 weeks later treated with androgen, the suspending vehicle or left untreated. Two weeks after this, all mice were given an i.p. injection of 100 ng of SIII and assayed 9 days later for antibody responses to SIII. (4) Twenty RF/J and twenty 129/J mice of both sexes were given an i.p. injection of 100 ng of SIII and assayed 9 days later for antibody responses to SIII.

Responses to BSA. Twenty-one C57L/J and twenty-two A/J mice of both sexes were given a single 0.5 ml i.p. injection containing 50 μg of BSA and assayed 14 and 21 days later for anti-BSA responses.

Responses to B-IgG. Ten males and ten females each of the RF/J, A/J and 129/J strains were given an i.p. injection of 50 μg of B-IgG and assayed 21 days later for anti-B-IgG responses.

Antibody determinations. Antibody to SIII was measured by radioimmunoassay (RIA) using the technique developed by Schiffman & Austrian (1971) which utilizes biosynthetically radiolabelled [^{14}C] capsular polysaccharide antigens prepared by Dr Schiffman. Samples of blood from individual mice were collected from the orbital plexus in heparinized microhaematocrit tubes, which were centrifuged and then cut at the interface. In 3 ml centrifuge tubes, 5 μl of plasma were added to 0.5 ml of diluted radiolabelled [^{14}C] capsular polysaccharide antigen containing 10,000 cpm. These were mixed manually, incubated for 15 min at 37°C, and one volume (0.5 ml) of ammonium sulfate, saturated at 37°C, was used to precipitate the antigen–antibody complex. The contents of the tubes were mixed, refrigerated for 15 min and then centrifuged for 15 min at 4°C at 15,000 rpm. The supernatant was discarded and the precipitate dissolved in 50 μl of 10% aqueous Triton X-100 (Rohm and Haas, Philadelphia). Each tube containing the radioactive antigen complexed to antibody was then placed in a counting vial to which 5 ml of scintillant fluor, composed of toluene and Triton X-100 in a 2 : 1 ratio with 4 g/l of 2, 5-diphenyloxazole

(PPO) (Packard Instrument Company, Downers Grove, Illinois) was added. The activity of bound antigen was measured in a liquid scintillation spectrometer. Standards consisted of antisera of known antibody content previously measured by the ninhydrin modification of the quantitative precipitin test (Schiffman, 1966). All unknowns and standards were run in duplicate. Using the reference standards, a curve was constructed by plotting counts per min of precipitate versus amount of added antibody nitrogen (AbN, in ng). The amount of antibody in the unknown plasma samples was read directly from this graph.

Antibodies to BSA and to B-IgG were measured by the Farr technique (Farr, 1958) with the following modifications: antisera and ^{125}I-BSA in normal rabbit serum (1 : 10) were diluted in phosphate-buffered saline (pH 7·5) rather than borate buffer; the incubation of antigen and antibody was done at 37°C rather than 4°C, after which the tubes were refrigerated for 15 min; radioiodination of BSA with ^{125}I (batch 38 BG, New England Nuclear, Boston, Massachusetts) was performed by a modification of the method of Hunter & Greenwood (1962).

Surgical procedures and treatment with androgen. Since levels of circulating androgen are known to fall below the range of detection by RIA within 2 weeks of castration (Bartke, 1974), twenty-nine C57L/J males were gonadectomized in order to deplete their circulating testosterone. Another twenty-eight C57L/J males were sham-operated. Further experiments involving gonadectomy were not performed on A/J males because the A/J strain did not show marked sex differences either in immunoglobulin levels (Cohn & Hamilton, 1976) or in specific immune responses to SIII. Operated mice were anaesthetized by an i.p. injection (0·06 mg/g of body weight) of sodium pentobarbital (Diamond Laboratories, Des Moines, Iowa). For gonadectomy, both testes were excised through a mid-line abdominal incision, the peritoneum closed with one 6-0 silk suture and the skin closed with wound clips (Clay Adams, Parsipany, New Jersey). In sham-operated mice, the same procedures were followed except that instead of excision, the gonads were grasped with forceps and replaced *in situ*. Two weeks after surgery (at 12 weeks of age) gonadectomized and sham-operated mice were divided into three groups. Group I was left untreated; Group II received a subcutaneously implanted pellet containing only the inert suspending vehicle (see below). Group III received a subcutaneously implanted pellet containing 800 µg of testosterone. Prior studies (unpublished) had established: (1) that 800 µg, when pelletized according to our procedure and placed under the skin, slowly released testosterone in physiological amounts sufficient to restore normal weights of seminal vesicles in castrated mice; and (2) that effective amounts of testosterone, as measured by seminal vesicle bioassay, were released from the pellets during the entire experimental period.

Testosterone (4-androsten 17 B-ol-3-one) was obtained from Mann Research Laboratories, formerly of New York. Reagents used in preparing the vehicle were obtained from the Dow Corning Corporation, Midland, Michigan. Pellets were prepared as follows: 21·7 g of silastic, a combination of polydimethylsiloxane polymer and silica filler (No. 382 Medical Grade Elastomer) were mixed with 2·18 g of diluent, a dimethylpolysiloxane fluid (No. 360 Medical Fluid). 161·29 mg of testosterone were added to this and the mixture was stirred for 3 hr. One drop of catalyst (stannous octoate) was mixed in, the mixture quickly drawn into a syringe and then forced into polyethylene tubing (PE 260, Intramedic, Clay Adams, New York). After the contents hardened, the tubing was cut in 1 cm lengths and the contents expelled. For pellets containing the vehicle only, the procedure was the same except that the testosterone step was omitted. All pellets were inserted through a small skin incision in the flank and secured by wound clips. They remained in place throughout immunizations and assays.

Statistics. In responses to 100 ng of SIII, differences between the sexes and strains and between operated and hormonally treated mice were analysed by two-way analyses of variance (ANOVA). Responses to B-IgG were also analysed by a two-way ANOVA for differences between the sexes and strains. Responses to 50 ng and 100 ng of SIII assayed on different days were analysed by a three-way ANOVA for differences in strains, days and dosage. For anti-BSA responses, a three-way ANOVA with repeated measures on days was used to analyse differences between the sexes and strains on days 14 and 21. If the F tests showed statistical significance, multiple comparisons tests were performed to detect specific differences among the groups. Q values at $P = 0.05$ were used in these calculations for computing significant differences (Snedecor, 1958).

RESULTS

In all experiments reported, HAR males were either lower in response than their respective females or lower than all LAR mice.

Responses to SIII

(1) When HAR C57L/J males and females were compared with LAR A/J males and females for antibody responses 9 days after immunization with 100 ng of SIII, sex differences were evident among the HAR mice but not among the LAR mice. C57L/J males had significantly lower levels of antibody to SIII (1059 ng/ml) than C57L/J females (2020 ng/ml) (Table 1), whereas LAR males and females showed no differences in anti-SIII responses. At 100 ng, C57L/J males had lower levels of anti-SIII than A/J males, but the differences were not significant.

(2) To detect whether HAR males were inherently weaker than LAR mice in response to SIII, these experiments were repeated using 100 ng or 50 ng of SIII. Assays were performed 5, 9 and 13 days after

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TABLE 1. Antibody levels to SIII in high androgen responder (HAR) C57L/J mice and low androgen responder (LAR) A/J mice following 100 ng of SIII

Strains	Androgenic response	Antibody levels (arithmetic mean±s.e.m. in ng/ml)	
		Males	Females
C57L/J	High	1059± 94·5 (23)	2020±139* (21)
A/J	Low	1205±101·2† (26)	1409±100·4† (17)

Two-way ANOVA: strains, $F=4·040$ ($P<0·05$); sex, $F=25·668$ ($P<0·01$); interaction $F=10·85$ ($P<0·01$).

Multiple comparisons tests (Q at $P=0·05$):

* Significantly different from C57L/J males.

† Significantly different from C57L/J females.

Numerals in parenthesis represent number of mice per group.

immunization. On all days assayed following 50 ng of SIII, the levels of antibody in HAR C57L/J males were significantly lower (566, 670 and 512 ng/ml) than the values in LAR A/J males (1416 and 1052 ng/ml), 5 and 9 days after immunization (Table 2). By day 13 the response of LAR A/J mice following 50 ng of SIII had tapered off and differences between the strains were not significant. Following 100 ng of SIII, as shown in the previous experiment, HAR C57L/J and LAR A/J mice produced similar levels of antibody with both strains, showing the highest levels on day 5. Except for day 13, the A/J did not show differences in response to the high and low dosages. On all days assayed among the HAR C57L/J, the responses to 50 ng of SIII were significantly lower (566, 670 and 512 ng/ml) than their responses to 100 ng (1464, 1232 and 1054 ng/ml).

(3) The influence of circulating androgen on the antibody response to SIII was investigated in sham-operated mice and in mice depleted of androgen by castration. Mice of both categories were divided into three groups: group I, untreated; group II, given the vehicle, an inert control agent; group III, given

TABLE 2. Antibody levels to SIII in high androgen responder (HAR) C57L/J males and low androgen responder (LAR) A/J males

	Antibody levels (arithmetic mean±s.e.m. in ng/ml)					
	Day 5		Day 9		Day 13	
Strains	50 ng	100 ng	50 ng	100 ng	50 ng	100 ng
C57L/J	566±100	1464±214*	670±132†	1232±182*	512±110†‡	1054±176*†
A/J	1416±284*	1520±155*§	1052±164*†¶**	1158±150* **	834±246†‡¶**	1244±214*††

Ten mice per group, injected with 50 ng or 100 ng SIII. Three-way ANOVA: strains, $F=7·81$ ($P<0·01$); days, $F=3·52$ ($P<0·01$); dosage, $F=18$ ($P<0·01$). Interactions: strains–days, not significant; strains–dosage, $F=4·89$ ($P<0·05$); days–dosage, not significant.

Multiple comparisons tests (Q at $P=0·05$):

* Significantly different from C57L/J males given 50 ng SIII, assayed on days 5, 9 and 13.

† Significantly different from C57L/J males given 100 ng SIII, assayed day 5.

‡ Significantly different from C57L/J males given 100 ng SIII, assayed day 9.

§ Significantly different from C57L/J males given 100 ng SIII, assayed day 13.

¶ Significantly different from A/J males given 50 ng SIII, assayed day 5.

** Significantly different from A/J males given 100 ng SIII, assayed day 5.

†† Significantly different from A/J males given 50 ng SIII, assayed day 13.

TABLE 3. Effects of altering the concentration of circulating androgen on antibody levels to SIII in HAR C57L/J males

Groups	Treatment	Antibody levels (arithmetic mean±s.e.m. in ng/ml)	
		Gonadectomized	Sham-operated
I	Untreated	1275±179·0 (9)	1277± 74·8 (9)
II	Vehicle	786± 76·0 (10)	1148±182·2 (10)
III	Testosterone	1008±192·5 (10)	1195±100·7 (9)

Two-way ANOVA: surgery, n.s., treatment, n.s., interactions, n.s.
Numerals in parentheses represent number of mice per group.

exogenous androgen. Regardless of the treatment, no significant differences were seen among the groups in antibody response to SIII (Table 3). The loss of circulating testosterone following castration did not result in elevated levels of antibody compared to those of sham–operated mice. Conversely, the administration of physiological doses of testosterone did not result in reduced antibody responses to SIII compared to those of untreated or vehicle-treated mice. Vehicle-treated mice appeared to have lower levels of antibody than other groups, but the differences were not significant.

(4) To investigate whether the poor responses of the HAR C57L/J males were unique to the C57L/J strain or associated in general with high sensitivity to androgen, another strain pair, the HAR RF/J and LAR 129/J, were assayed for anti-SIII responses. While HAR RF/J did not show sex differences in response to 100 ng of SIII, as a strain the RF/J were significantly lower (1420 and 1500 ng/ml) than the 129/J (2292 and 2031 ng/ml) in antibody production (Table 4). Differences between the males (1420 and 2292 ng/ml) were significant even after 100 ng of antigen.

Responses to BSA

Among HAR C57L/J mice, males had strikingly lower levels of antibody to BSA than females both 14 and 21 days after immunization (398 *vs* 2850 ng/ml and 3150 *vs* 6177 ng/ml) (Table 5). Among LAR A/J mice, males had lower levels of anti-BSA than females on day 14 only (4627 *vs* 9418 ng/ml), before the peak antibody responses had developed. By day 21, A/J males and females both had high levels of anti-BSA (10,020 and 11,062 ng/ml) and no sex differences were evident. Comparing the strains, all C57L/J mice had consistently lower levels of anti-BSA than all A/J mice. HAR C57L/J males, however, were the lowest of all groups assayed on both days.

TABLE 4. Antibody levels to SIII in high androgen responder (HAR) RF/J and low androgen responder (LAR) 129/J mice following immunization with 100 ng of SIII

Strains	Androgenic response	Antibody levels (arithmetic mean±s.e.m. in ng/ml)	
		Males	Females
RF/J	High	1420±98·6	1500±188
129/J	Low	2292±177*†	2031±183

Ten mice per group.
Two-way ANOVA: strains, F=13·66 ($P<0·01$); sexes, n.s., interaction, n.s.
Multiple comparisons tests (Q at $P=0·05$):
* Significantly different from RF/J males.
† Significantly different from RF/J females.

TABLE 5. Antibody levels to BSA in high androgen responder (HAR) C57L/J and low androgen responder (LAR) A/J mice

| Strain | Androgenic response | Antibody levels (arithmetic mean±s.e.m. in ng/ml) | | | |
| | | 14 days | | 21 days | |
		Males	Females	Males	Females
C57L/J	High	398±55	2850±489*	3150±657	6177±744§
		(11)	(10)	(10)	(9)
A/J	Low	4627±32*	9418±896*†‡	10,020±788§¶	11,062±517§¶
		(11)	(11)	(10)	(8)

Three-way ANOVA with repeated measures on days: strains, $F=19\cdot85$ ($P<0\cdot01$); sexes, $F=4\cdot84$ ($P<0\cdot05$); days, $F=111\cdot50$ ($P<0\cdot01$). Interactions: strains–sexes, n.s.; strains–days, n.s.; sexes–days, $F=9\cdot40$ ($P<0\cdot01$); strains–days–sexes, $F=14\cdot60$ ($P<0\cdot01$).

Multiple comparisons tests for 14 days only:
* Significantly different from C57L/J males.
† Significantly different from C57L/J females.
‡ Significantly different from A/J males.
Multiple comparisons for 21 days only:
§ Significantly different from C57L/J males.
¶ Significantly different from C57L/J females.
Numerals in parentheses represent the number of mice per group.

Responses to B-IgG

Further studies using BSA as an antigen were discontinued because the genetic control of the antibody response to BSA was not well worked out. In order to determine whether differences in sensitivity to androgen played a role in responses controlled by H-linked Ir genes, strains which differ both in sensitivity to androgen and in H-2 type were assayed for responses to B-IgG. H-2^a and H-2^k are associated with high responses and H-2^b with low responses to B-IgG (Vaz & Levine, 1970). Assays were performed 21 days after immunization because this time coincided with an early peak response. As shown in Table 6, sex differences in anti-B-IgG responses only occurred in the HAR RF/J, with males having strikingly lower responses (480 ng/ml) than females (1794 ng/ml). In the LAR A/J and the LAR 129/J, males and females produced similar responses (2798 *vs* 3206 ng/ml and 648 *vs* 780 ng/ml). Except for the HAR RF/J males, the expected strain differences based upon H-2 type were evident. The A/J (H-2^s) were high responders and the 129/J (H-2^b) poor responders. Among the HAR RF/J, the females were comparatively high as was to be expected (1794 ng/ml), but the males, although H-2^k, produced the lowest responses of all groups assayed (480 ng/ml).

TABLE 6. Antibody responses to bovine IgG in strains differing in H-2 type and in androgen sensitivity

| Strains | Androgenic response | H-2 type | Antibody levels (arithmetic mean±s.e.m. in ng/ml) | |
			Males	Females
RF/J	High	k	480±180	1794±325*
A/J	Low	a	2978±127*†	3206±129*†
129/J	Low	b	648±57†‡§	780±172†‡§

Ten mice per group.
Two-way ANOVA: strains, $F=95\cdot11$ ($P<0\cdot01$); sexes, $F=13\cdot42$ ($P<0\cdot01$); interaction, $F=6\cdot56$ ($P<0\cdot01$).
Multiple comparisons tests (Q at $P=0\cdot05$):
* Significantly different from RF/J males.
† Significantly different from RF/J females.
‡ Significantly different from A/J males.
§ Significantly different from A/J females.

DISCUSSION

The results of these experiments indicate that in responses to three types of antigens, males of HAR C57L/J or RF/J strains had consistently lower levels of antibody than females of their respective strains or mice of the LAR A/J or 129/J strains.

With respect to SIII, C57L/J males produced similar antibody responses regardless of whether their plasma testosterone levels were decreased by gonadectomy or increased by the administration of physiological doses of testosterone. This is consistent with the results of our earlier experiments (Cohn & Hamilton, 1976). With respect to B-IgG, the RF/J and A/J were expected to be high antibody responders and the 129/J low antibody responders. LAR A/J and 129/J strains produced the expected immune responses and showed no sex differences in these responses. Among the HAR RF/J mice, the females were comparatively high antibody responders as expected, but some factor interfered with the responses of the males. Although they were H-2^k and should have produced high responses, they produced the lowest responses of all groups assayed.

Vaz & Levine (1970), in their studies of the genetic control of antibody responses to benzylpenicilloyl$_{25}$–conjugated bovine gammaglobulin (BPO$_{25}$BGG), were unable to explain why three strains out of nine (the C57BR/cdj, the MA/J and the RF/J) all bearing the H-2^k haplotype and expected to produce high antibody responses, produced unexpectedly low responses. Since that time, in unrelated studies, we have examined a number of inbred strains, including the three cited in the work of Vaz & Levine, and have found that the C57BR/cdj, the MA/J and the RF/J are high target organ responders to androgen (unpublished observations, courtesy of Dr W. West). Although Vaz & Levine did not recognize this phenomenon, their results suggest that high sensitivity to androgen may have been a factor contributing to the unexpectedly low responses of the C57BR/cdj, MA/J and RF/J.

In the current studies attention has focussed on investigating the influence of sensitivity to androgen as a possible factor contributing to sex differences in immune responsiveness. Emphasis has been placed on the immunobiology of sensitivity to androgen not its genetic inheritance. Several strains of mice which differ in sensitivity to androgen and in H-2 type, have been assayed for Ig levels (Cohn & Hamilton, 1976; Cohn, 1979) and for specific immune responses to different types of antigens. The results indicate that sex differences in immune responsiveness are not uniformly present in all strains or studies, but that in strains with a high sensitivity to androgen, sex differences are evident in Ig levels (Cohn & Hamilton, 1976; Cohn, 1979) and in responses to SIII, BSA and B-IgG. Where sex differences have not occurred in HAR strains, the strains as a whole are low, with females as well as males producing low immune responses.

Differences in sensitivity to androgen can be described as the variations observed in target organ responsiveness or resistance among individuals or groups following uniform doses of hormone. Females of groups highly sensitive to androgen may manifest this trait, as demonstrated by the increased comb growth in white leghorn fowl (Dorfman, 1948) or the high incidence of polycystic ovaries in C57L/J mice (Fekete, 1953). The magnitude of target organ responsiveness to androgen is not necessarily proportional to the concentration of hormone in the circulation, but may vary with it inversely (Bartke, 1974). With respect to genetic inheritance, sensitivity to androgen is thought to be controlled by polygenes acting to produce small additive effects rather than through dominance (Chai, 1960). Suggested gene loci include the Hom-1 locus, near the K end of the H-2 complex (Ivanyi *et al.*, 1973) and the testicular feminization Tfm locus on the X chromosome (Ohno *et al.*, 1973). As shown in the current study sensitivity to androgen is not directly linked to H-2 type. C57L/J (H-2^b) are high and 129/J (H-2^b) low androgen responders. Similarly, RF/J (H-2^k) are high and A/J (H-2^a) are low androgen responders. (H-2^a and H-2^k share the same left part of the H-2 complex where the postulated Hom-1 locus and the Ir genes map (Klein, 1975).)

Our data indicate that males with a high sensitivity to androgen are at a disadvantage immunologically when compared to females of the same strain or to males of LAR strains. The level of circulating androgen in adult mice does not appear to be a major influence in the low immune responses of males since mice deprived of androgen by castration showed no changes either in anti-SIII responses or in levels of

IgM and IgG2 (Cohn & Hamilton, 1976). Similarly, the influence of oestrogen is probably not critical since castrated females showed no differences compared to sham-operated mice either in levels of plasma Igs (Cohn & Hamilton, 1976) or in anti-SIII responses (unpublished observations). High oestrogen responsiveness does not seem to be beneficial either; the only group of females discovered thus far which are significantly lower than their males in levels of IgM and IgG2a are the high oestrogen responder 129/J (Cohn, 1979).

The current data, as well as earlier work (Cohn & Hamilton, 1976), indicate that in adult males it is high responsiveness to androgen, not a high level of circulating androgen, that consistently correlates with poor immune performance. The exact mechanisms underlying the possible relationship between high sensitivity to androgen and poor immune response remain to be clarified. It is known that androgens can ablate post-natal humoral antibody responses by interfering with the growth of the bursa or thymus (Warner, Szenberg & Burnet, 1962) particularly the epithelial component (Meyer, Rao & Aspinall, 1960). Androgens have also been shown to accelerate the differentiation of haemopoietic stem cells by triggering them into cycling, (Byron, 1970). It is well established that androgens are present in the foetus and that they exert a potent influence in organogenesis (Abramovitch & Rowe, 1977). Since the influence of high sensitivity to androgen on immune responses of adults does not seem to be mediated through post-natal androgen levels, it is possible that foetal androgens act on the lymphomyeloid system of animals highly sensitive to this steroid to produce an immune system programmed for low responsiveness. Theoretically, androgens produced by the foetal testes or placenta could influence the microenvironment of the developing thymus in favour of suppressor cell production or could act on haemopoietic stem cells to shift differentiation into a committed line, thereby reducing the pool of uncommitted cells. Although highly speculative, such pre-natal effects exerted by androgens would be non-specific and probably independent of immune response genes or other immunoregulatory factors.

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