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A Quantitative Difference in the Immune Response between Male and Female Mice* (32768)

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In studies using Swiss Albino (Hale-Stoner) mice, a difference in the immune response of male and female mice to bovine serum albumin (BSA) has been frequently noticed. In order to confirm this observation a systematic investigation of the difference was undertaken. The quantities of antibody produced were compared as was the minimum dose required to elicit a primary response.

Materials and Methods. Mice used in these experiments were raised in a closed colony in this laboratory and were from 4 to 8 weeks of age when used. To minimize thyroidal uptake of radioactive iodine in mice injected with ^{125}I labeled antigen, NaI (0.1%) was added to the drinking water (1). In designated experiments, 0.5 mg Thephorin (phenindamine) was injected subcutaneously 15–20 min prior to the test dose of ^{125}I labeled antigen in order to reduce the incidence of fatal anaphylaxis (2).

Antigen. Crystalline bovine serum albumin (BSA) was obtained from Armour Laboratories, Chicago, Illinois. The BSA was labeled with ^{125}I using monoiodochloride (ICl) as described elsewhere (3). In all experiments, the primer and the booster were administered subcutaneously and the challenge was administered intravenously 7 days following the booster. The BSA dilutions were made with a solution containing 1% normal mouse serum.

Antibody. An hyperimmune mouse anti-BSA antiserum was prepared by injecting mice with 16 mg of fluid BSA given in four equal doses followed by 10 mg of BSA in complete Freund's adjuvant. The injections were given on alternate days and the animals were bled 18 days after the final injection.

Immune degradation. The degradation of antigen was followed by whole body measurement of retained radioactivity. The amount of antigen degraded at an accelerated rate (ADAR), which is linearly related to log circulating antibody concentration, was calculated as follows (4):

$$\text{ADAR} = (1-\frac{S}{N})_t 100.$$

S = percent of antigen retained in experimental mice at time (t); N = percent of antigen retained in control mice at time (t); t = time of determination. Mice which were fatally shocked when tested were assigned an ADAR of 85.

Farr Technique. The primary interaction of iodine labeled antigen with antibody was measured by ammonium sulfate precipitation as previously described (5). The antibody concentrations are expressed as the amount of antigen that was 10% precipitated by 0.1 ml of the antiserum. This quantity has been found to be linearly related to the concentration of antibody (6) such that 0.66 log ratio of the amounts of BSA that are 10% precipitated equals log ratio of the antibody

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TABLE I. Mice Primed with Either 2 or 4 mg of BSA and Tested 61 Days Later with 0.005 mg of BSA-^{125}I.

Group	Primer (BSA) (mg)	No. of mice per sex	Av ADAR	
			Female	Male
1	2	40	84.9 ± 3.1[a]	47.8 ± 4.6
2	4	42	81.2 ± 3.7	52.7 ± 4.3

[a] SE of the sample mean.

titers, i.e., $0.66 \log\left(\frac{AgA}{AgB}\right) = \log\left(\frac{AbA}{AbB}\right)$.

Experimental procedures and results. Primary response. Mice were injected subcutaneously with either 2 or 4 mg of BSA, and 61 days later 0.005 mg of BSA-^{125}I was injected intravenously as a test dose. The results are summarized by Table I. The immune response as judged by the average ADAR was significantly greater in female than male mice. The probability that such a distribution arose by chance is small ($p<0.001$).

Amount of BSA required to prime mice for a secondary response. Mice were injected subcutaneously (primer) with amounts of BSA that ranged from 1×10^{-6} to 1 mg of antigen, and 50 days later they were reinjected with 4 mg of BSA (booster). Seven days after the booster the mice were tested with 0.005 mg of BSA-^{125}I.

The individual ADAR's for the animals which were given a primer of 1×10^{-6} mg of BSA are presented in Table II. Based on the average ADAR, the female mice gave a stronger immune response than did the male mice. Statistically, these results could have been obtained by chance alone once in approximately 1000 trials ($p = 0.001$). The groups which received a primary dose of 1×10^{-4}, 1×10^{-2} or 1 mg of BSA did not give a statistically significant difference because with a test dose of 0.005 mg of BSA-^{125}I all the ADAR's were near the maximum and differences were not resolvable even if a difference existed. Control mice given 4 mg of BSA and challenged 7 days later did not respond.

Temporal development of the immune response. The purpose of the following experiment was to determine whether the difference in the immune response might be explained on a temporal basis, *i.e.*, the immune response in female mice might begin earlier or last longer than that in males. All mice were given a primer consisting of 2 mg of BSA. At various times (28–77 days) after the primer the immune response was measured by injecting intravenously either 0.005 (group 1) or 0.01 mg BSA-^{125}I. The results are summarized in Table III. They indicate that in females the immune response is initially stronger and lasts longer than in males.

Immune degradation. The following experiment was performed in order to determine whether female mice were more efficient in degrading antigen–antibody complexes than males and to establish the quantitative relationship between the ADAR and the amount of circulating antibody in mice of both sexes. Serial dilutions of mouse anti-BSA antiserum were added to equal volumes of BSA-^{125}I solution containing 0.05 mg of protein/ml. The mixtures were stored for at least 30 min at room temperature. Normal mice were pretreated with Thephorin and then injected intravenously with 0.2 ml of the BSA-^{125}I antiserum combinations. Thus, each mouse received an injection equivalent to 0.1 ml of

TABLE II. ADAR of Individual Mice Given 1×10^{-6} mg of BSA as a Primer and 4 mg as a Booster.[a]

Mouse	ADAR	
	Female	Male
1	81	32
2	77	19
3	39	17
4	38	14
5	28	5
6	24	0
7	20	0
8	20	0
9	18	0
10	14	0
11	10	0
12	8	0
13	—	0
Average	31.4 ± 7[b]	6.7 ± 2.9

[a] The ADAR measurement was made 7 days after the booster.

[b] SE of the sample mean.

TABLE III. Development and Duration of the Immune Response in Female and Male Mice.

Group	No. of mice (F/M)	Tested (days after primer)	Av ADAR		p^a
			Female	Male	
1	12/11	28[b]	65.9 ± 9.1[c]	35.8 ± 9.9	0.026
2	12/11	42	28.8 ± 6.2	24.6 ± 6.0	0.638
3	12/11	56	38.3 ± 3.3	10.4 ± 4.1	<0.001
4	12/9	63	43.3 ±10.3	12.2 ± 5.7	0.008
5	13/9	77	32.1 ± 8.3	4.2 ± 2.6	0.001

[a] Probability that the observed distribution occurred by chance alone.
[b] Test dose was 0.005 mg of BSA-^{125}I; remaining groups tested with 0.01 mg of BSA-^{125}I.
[c] SE of the sample mean.

TABLE IV. *In Vitro* Determination of Circulating Anti-BSA in Female and Male Mice.

Group[a]	Time of booster (days after primer)	Amount of BSA resulting in 10% precipitation of the BSA (μg)		Difference in antibody titer
		Female	Male	
1	56	3.450	0.106	10×
2	70	3.450	0.063	14×
3	—[b]	7.000	0.006	159×

[a] 12 mice per sex per group.
[b] No booster; bled on day 77.

diluted antiserum plus 0.005 mg of I^{125}-BSA-^{125}I. When the average ADAR's obtained at 26 hours were plotted *vs* log antiserum dilution a straight line was obtained. There was no significant difference between the ability of male and female mice to degrade antigen–antibody complexes. The slope of the straight line was such that doubling the antibody concentration raised the ADAR 17.5 units.

In vitro *quantitation of the antibody response.* Normal adult mice of both sexes were injected with 2 mg of BSA and 56 or 70 days later they were reinjected with 1×10^{-5} mg of BSA. Seven days following the booster injection the mice were bled and the sera within each group were pooled. The control mice were injected with 2 mg of BSA and were bled 77 days later. The antisera were titered by the Farr technique and the results are presented in Table IV. In both experimental and control mice the females gave a stronger response.

Discussion. The results presented above clearly indicate that female mice develop a stronger and longer lasting immune response to BSA than do male mice. The magnitude of the difference was dependent upon the conditions of sensitization, the time of measurement, and the method of measurement of the immune response.

Female mice were more responsive to BSA as a primer than male mice. The amount of BSA required for a primer (Table II) in female mice was equal to or less than 1×10^{-6} mg; in males to obtain a uniform response the amount required was greater than 1×10^{-6} mg. As the amount of antigen used as primer was increased the immune response in both sexes increased, but the difference in the immune response between female or male remained constant (e.g., Table I) with about 2–4 times more antibody being produced by females. The results presented in Table III show that this difference in response is not merely a function of the time of measurement but that the immune response in females has both a greater magnitude and a longer duration.

When the circulating antibody titers are compared it can be seen that female mice pro-

duce 10–85 times more antibody than males. The difference as a result of the primary exposure to the antigen is greater than that of the secondary response. That the magnitude of the difference in circulating antibody titers is greater than that as measured by the ADAR may be due to tissue fixed antibody present in greater proportion in males than in females. There is no reason to postulate that circulating antibody in females is unable to participate in the degradation of antigen.

The difference in the immune response could be due to a difference in the number of cells synthesizing antibody or the rate of antibody synthesis per cell. No evidence is presented to suggest that the rates of synthesis differ. Numerous investigators (7–11) have shown that phagocytosis is greater in female than in male animals and that this difference is under estrogen control. Greater phagocytic efficiency would explain the greater sensitivity of the female to small doses of antigen assuming that phagocytosis of antigen is a necessary step in the immune response. A difference in the number of circulating lymphocytes (12) in male and female mice has been reported. Neonatal thymectomy (13) which would affect the number of circulating lymphocytes has been shown to abolish the sex differences in the immune response in mice of the Hale-Stoner strain. Human postpubertal females (14) show a significantly higher circulating concentration of macroglobulins (IgM) although the IgG and IgA levels do not differ between males and females. Such a difference may reflect the difference in immunological responsiveness rather than explain it.

Greater immunological responsiveness in female animals has been shown using cellular antigens (sheep red blood cells) (15), viable and nonviable tumor cells (16). These instances do not eliminate the possibility that the increased response in females is due to an antigen resulting from the Y chromosome and hence one more immunogenic in females than in males. The use of the protein antigen BSA eliminates this criticism.

Summary. The immune response to a protein antigen (BSA, bovine serum albumin) was compared in male and female mice. It was found that female mice developed a stronger and longer lasting immune response and that female mice were more responsive to small doses of antigen. An explanation based on the effect estrogens have on phagocytosis is discussed.

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