GENETIC CONTROL OF THE ANTIBODY RESPONSE TO TYPE III PNEUMOCOCCAL POLYSACCHARIDE IN MICE

I. Evidence That an X-Linked Gene Plays a Decisive Role in Determining Responsiveness

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Thymic-derived cells (T cells)[1] and bone marrow-derived precursors of antibody-forming cells (B cells) have been shown to cooperate in the antibody response to several antigens (1–3). Thus far, most well-described forms of genetic control for the immune response appear to involve genes associated with T cell rather than B cell function (4). In contrast to B cells, there is no evidence to indicate that T cells secrete antibody upon stimulation by antigen, and there are conflicting reports concerning whether T cells have immunoglobulin on their surface (5–8). Studies on the genetic control of the antibody response by B cells, therefore, would provide information on those genetic factors that (*a*) regulate the formation of antigen-specific immunoglobulin receptors, and (*b*) influence the rate of antibody synthesis. The antibody response of inbred mice to Type III pneumococcal polysaccharide (SSS-III) provides an ideal experimental model system for obtaining such information for a number of reasons. First, cooperation between helper T cells and B cells is not a requisite for the antibody response to this antigen (9–11); consequently, the antibody response to SSS-III is considered to be largely a B cell response. Second, previous studies have shown that mice immunized with SSS-III produce mostly antibody of the IgM class (12–14); the avidity of this antibody for SSS-III remains essentially constant over a 10,000-fold range of immunizing doses, and upon reimmunization with high or low doses of antigen (15). Such homogeneity suggests that a restricted population of B cells participates in the antibody response to SSS-III; thus, complexities associated with an antigen-mediated cell selection process (16) are greatly minimized.

The present work describes the results obtained in preliminary studies on the genetic control of the antibody response to SSS-III. They show that an X-linked gene plays a decisive role in determining responsiveness to this anti-

[1] *Abbreviations used in this paper*: ATS, anti-thymocyte serum; B cells, bone marrow-derived precursors of antibody-forming cells; NIH, National Institutes of Health; PFC, plaque-forming cells; Pnc III, Type III pneumococci; SRBC, sheep erythrocytes; SSS-III, Type III pneumococcal polysaccharide; T cells, thymic-derived cells.

gen, and that other factors influence the magnitude of the antibody response produced.

Materials and Methods

Animals.—BALB/cAnN, C3H/HeN, C57BL/6N, NZB/BLN, DBA/2N, AKR/N, and CBA/HN mice, as well as progeny derived from crosses involving BALB/cAnN and CBA/HN mice, were obtained from the Rodent and Rabbit Production Section of the National Institutes of Health (NIH), Bethesda, Md. CBA/J mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. CBA/H and CBA/H·T6T6 mice were generously provided by Doctors Lloyd Law and Michael Potter, National Cancer Institute, NIH, from a colony maintained in their laboratory. All mice were 8–12 wk of age at the time of immunization.

CBA/HN mice constitute a genetically distinct subline of CBA mice; they were established in the Genetics Unit of the Rodent and Rabbit Production Section, NIH, in 1966 from a pen-bred strain of CBA mice carrying the gene for foam cell reticulosis (*fm*). Since the *fm* gene is lethal (17), the initial stock of mice consisted of about six to eight carriers, i.e., mice heterozygous for *fm*; the *fm* gene arose as a spontaneous mutation in the strain of CBA mice maintained at the Radiological Research Unit, Harwell, England. After a series of test matings were conducted to determine the genotype of individuals, homozygous normal mice were used to reestablish the inbred strain by brother-sister mating. The mice used in the present work were derived from parents between the 15th and 25th generation of brother-sister mating. However, during the initial stages of reestablishing an inbred strain, a severe reproductive crisis occurred at about the seventh generation. As a result, the present colony of CBA/HN mice is derived from a single female who, fortunately, produced a litter composed of six females and one male. Presently, CBA/HN mice reproduce about as well as other inbred strains maintained at NIH.

The notation of Gill et al. (18) was used to designate parental strains of mice, as well as F_1, F_2, and backcross generations. BALB/cAnN and CBA/HN mice were referred to as B mice and C mice, respectively. The first symbol used to describe a hybrid identifies the maternal member of a cross, e.g., CB mice are F_1 mice derived from a cross between female C mice and male B mice. For progeny derived from backcrosses, the symbol preceding the slash mark denotes the maternal member of the cross, e.g., CB/B mice are progeny derived from a backcross between female CB (F_1 mice) and male B mice. F_2 mice are referred to as CB/CB mice.

Antigens and Immunization Procedures.—The immunological properties of the SSS-III used, as well as the method by which it was prepared, have been described (12, 13, 15, 19); SSS-I and SSS-II were made by the same method. Unless indicated otherwise, mice were given a single intraperitoneal injection (0.5 μg) of these antigens in 0.5 ml of saline. The antibody response was assessed 5 days later.

Mice immunized with sheep erythrocytes (SRBC) received a single intraperitoneal injection of known numbers of washed cells in 0.2 ml of saline. The antibody response was assessed 4 days after immunization.

Escherichia coli 0127 lipopolysaccharide B, Lot 478203, was purchased from Difco Laboratories, Detroit, Mich. Mice were given a single intraperitoneal injection of 10 μg of this antigen in 0.5 ml of saline. Serum hemolytic antibody titers were determined 5 days later.

Immunological Methods.—Serum antibody and splenic antibody-forming cells specific for SSS-III were detected by the specific hemolysis of SSS-III-coated SRBC, and by a slide version of the technique of localized hemolysis-in-gel, respectively (12, 13). SRBC were coated with SSS-III by the chromium chloride method (20). In studies on the antibody response to SRBC, SSS-I, and SSS-II, native SRBC or SRBC coated with SSS-I or SSS-II by the chromium chloride method were used in the above serological tests. SRBC sensitized with alkali-treated antigen (21) were used for the detection of serum hemolytic antibody specific for *E. coli* lipopolysaccharide.

Serum antibody titers were expressed as $1/\log_2$ of the highest dilution of antiserum producing complete hemolysis of a standard suspension of antigen-coated or native SRBC. Values for numbers of antibody-producing or plaque-forming cells (PFC) were given as $\log_{10}$ PFC/spleen and $\log_{10}$ PFC/10^6 spleen cells. For mice immunized with SSS-III, a correction was made for the small number of SRBC-specific background PFC present (200–400/spleen), so that all values cited represent numbers of SSS-III-specific PFC detected (12, 13).

Classification of Mice with Respect to Their Ability to Respond to SSS-III.—In the genetic studies to be described, mice were classified as low, intermediate, or high responders to SSS-III. C mice regularly give an extremely low PFC response to SSS-III; no serum hemolytic antibody can be detected in any of these mice after immunization with an optimally immunogenic dose of antigen. All mice giving a C type of antibody response (Table III) were classified as low responders. B mice produced the highest PFC and serum antibody response observed thus far; all mice giving a B type response (Table II) were classified as high responders. Other mice giving a response greater than that of C mice, but less than that of B mice, were considered to be intermediate responders. In crosses between B and C mice yielding low and either intermediate or high responders, mice having <1000 PFC/spleen were classified as low responders; only 2 of the 151 intermediate or high responders obtained in such cases, i.e. mice having ≥ 1000 PFC/spleen, had serum antibody titers $<1/\log_2 1$.

Statistical Procedures.—The results of this study were analyzed by three different statistical procedures. (*a*) The χ^2 test (22) was used to determine whether the number of low responders derived from various crosses differed significantly from that expected if responsiveness to SSS-III were transmitted as a single X-linked genetic trait. (*b*) A two-tailed *t* test (22) was used to detect significant differences in the magnitude of the antibody response produced by intermediate and/or high responding progeny derived from different crosses. For crosses yielding low as well as intermediate or high responders, all low responders were excluded from such comparisons. (*c*) The Kolmogorov-Smirnov two-sample test (23) was used to determine whether cumulative frequency distributions for the antibody response produced by only the intermediate and/or high responders derived from two different crosses differed significantly with respect to central tendency, dispersion, and skewness. Significant differences between two sample distributions occur when the largest difference noted at any 0.2 log test interval (D_{max}) exceeds that expected for random variations between two sample distributions drawn from the same population.

It should be emphasized that the same data were subjected to analysis by both the two-tailed *t* test and the Kolmogorov-Smirnov two-sample test and that values for low responders were excluded from such analyses. This was done to permit analysis of additional genetic factors which influence the magnitude of the antibody response produced by intermediate and high responders. In all of the above statistical procedures, differences were considered to be significant when probability values <0.05 were obtained.

RESULTS

Selection of High and Low Responding Strains of Mice.—Several strains of inbred mice were immunized with from 0.005 to 50 μg of SSS-III. With the exception of C3H/HeN mice, a maximal antibody response was obtained 5 days after immunization with 0.5 μg of antigen for all strains tested. Consequently, this dose was used to assess the ability of various strains of mice to respond to SSS-III. C3H/HeN mice gave an erratic response; in some experiments (data not shown), an antibody response equal to, or greater than, that obtained with 0.5 μg of SSS-III was produced after immunization with greater or lesser amounts of antigen.

The data of Table I show that the strains of mice examined differed markedly in their ability to respond to SSS-III. The average PFC response ranged from a low of 1880 PFC/spleen (CBA/J) to a high of 14,800 PFC/spleen (B mice); serum antibody titers from $1/\log_2 2.05 \pm 0.28$ (DBA/2N) to $1/\log_2 7.37 \pm 0.17$

TABLE I

*Magnitude of the PFC and Serum Antibody Response to 0.5 μg of SSS-III for Various Strains of Inbred Mice**

Strain	*H-2* type	Sex	PFC/spleen $\log_{10} \pm S\bar{x}$	Serum titer $1/\log_2 \pm S\bar{x}$	No. of mice
B mice (BALB/cAnN)	*d*	♂	4.116 ± 0.02 (12,900)	7.37 ± 0.17	67
		♀	4.170 ± 0.02 (14,800)	7.31 ± 0.13	148 (133)‡
C3H/HeN	*k*	♀	4.048 ± 0.098 (11,200)	6.20 ± 1.32	5
CBA/H·T_6T_6	—	♀	3.743 ± 0.108 (5530)	3.75 ± 0.25	4
C57BL/6N	*b*	♀	3.725 ± 0.045 (5300)	4.00 ± 0.00	5
NZB/BLN	*d*	♀	3.611 ± 0.094 (4080)	7.80 ± 0.20	5
DBA/2N	*d*	♂	3.568 ± 0.093 (3700)	4.28 ± 0.27	25
		♀	3.324 ± 0.079 (2110)	2.05 ± 0.28	40
CBA/H	*k*	♂	3.553 ± 0.045 (3570)	3.71 ± 0.29	58
		♀	3.546 ± 0.055 (3560)	4.80 ± 0.17	50
AKR/N	*k*	♀	3.513 ± 0.078 (3260)	6.80 ± 0.27	25
CBA/J	*k*	♂	3.276 ± 0.066 (1890)	5.78 ± 0.34	22
		♀	3.274 ± 0.092 (1880)	4.47 ± 0.37	19

* Geometric means are shown in parentheses.

‡ No. of mice used for serum titer determinations.

(B mice) were obtained. C3H/HeN, CBA/H.T6T6, C57BL/6N, NZB/BLN, CBA/H, and AKR/N mice gave intermediate responses.

No direct relationship between *H-2* type and the magnitude of the antibody response to SSS-III was evident. Several strains of $H\text{-}2^k$ mice (CBA/H, AKR/N, and CBA/J) responded poorly; yet, C3H/HeN mice ($H\text{-}2^k$) responded almost as well as B mice ($H\text{-}2^d$), the highest responding strain. The response of other strains of $H\text{-}2^d$ mice was more like that of low responding CBA/H, AKR/N, and CBA/J mice ($H\text{-}2^k$) than of B mice ($H\text{-}2^d$).

Other strains of inbred mice were also tested for their ability to respond to SSS-III (24); in no case was a response greater than that produced by B mice obtained. Since the response produced by B mice may be typical of the upper limit of responsiveness to SSS-III in mice, and since the antibody response to SSS-III in B mice has been well characterized (12, 13, 15, 19), these mice were used as high responders in the present work. Values for $\log_{10}$ PFC/spleen, $\log_{10}$ PFC/10^6 spleen cells, and $1/\log_2$ serum antibody titer for B mice immunized with 0.5 μg of SSS-III are given in Table II; male and female B mice did not differ significantly in their response to antigen ($P \geq 0.05$, two-tailed t test).

CBA/HN or C mice produced an extremely low antibody response to SSS-III (Table III); no serum antibody could be detected in any of these mice immu-

TABLE II

*Magnitude of the PFC and Serum Antibody Response of Male and Female B Mice Immunized with 0.5 μg of SSS-III**

Sex	PFC/spleen		PFC/10^6 spleen cells		1/$\log_2$ serum antibody titer	
	Range and No. of mice	Mean $\log_{10} \pm S\bar{x}$	Range and No. of mice	Mean $\log_{10} \pm S\bar{x}$	Range and No. of mice	Mean $\pm S\bar{x}$
♂	3650–31,250	4.11 ± 0.02	18–170	1.81 ± 0.04	4–11	7.37 ± 0.17
	67	(12,900)	37	(65)	67	
♀	2350–50,100	4.17 ± 0.02	12–234	1.86 ± 0.02	4–10	7.31 ± 0.13
	148	(14,800)	115	(73)	133	

* Geometric means are shown in parentheses.

nized with 0.5 μg of antigen. In 55 of 64 male, and in 20 of 36 female C mice, no SSS-III-specific PFC could be detected (values listed as <100 PFC/spleen). Since values of 100–200 PFC/spleen are of questionable significance, i.e. they represent only 1–2 PFC more than background values for SRBC-specific PFC, one may conclude that most of these mice failed to give an unequivocal antibody response to SSS-III. The response produced by remaining C mice was considerably below that for B mice (Table II), and it was easy to distinguish mice giving a C-type response from those giving a B-type response. Female C mice tended to respond slightly better than males.

Attempts were made to increase the magnitude of the antibody response to SSS-III in C mice under a variety of experimental conditions. These included (*a*) immunization with different doses (0.005–50 μg) of SSS-III, (*b*) immunization with formalin-treated Type III pneumococci (Pnc III), and (*c*) treatment with from 0.003 to 0.5 ml of anti-thymocyte serum (ATS).[2] None of these procedures elevated the magnitude of the response produced. In other studies, class-specific facilitating antisera (12) failed to reveal the presence of SSS-III-

[2] Treatment with ATS has been shown to produce a significant increase in the magnitude of the antibody response to SSS-III in B mice and in other strains of mice tested (24–26).

specific PFC making antibody of the IgM, IgG, or IgA class in C mice given either SSS-III or Pnc III. Also, C mice did not appear to be cross-tolerant to SSS-III since Type III-specific antisera, obtained from either immunized rabbits or B mice, did not agglutinate erythrocytes or spleen cells from C mice. The addition of spleen cells (or serum) from C mice to spleen cells (or serum) from immunized B mice provided no evidence to indicate that C mice produce a substance that interferes with the detection of SSS-III-specific PFC or serum antibody. In view of the above findings, C mice were used as low responders in the genetic studies to be described.

Incidence of Low Responders among Progeny Derived from Various Crosses.— The antibody response produced by CB mice illustrates that major differences

TABLE III

Magnitude of the PFC and Serum Antibody Response to 0.5 μg of SSS-III for Male and Female C Mice

Immunological test	Range of values*	No. of mice giving a stated response/ total No. of mice examined	
		♂	♀
PFC/spleen	<100	55/64	20/36
	100–200	5/64	3/36
	200–500	4/64	6/36
	500–1000	0/64	7/36
	>1000	0/64	0/36
PFC/10^6 spleen cells	<1	11/15	12/21
	1–10	4/15	7/21
	10–12	0/15	2/21
$1/\log_2$ serum antibody titer	<1	21/21	36/36

* Numbers of SSS-III-specific PFC or $1/\log_2$ serum antibody titer.

in the ability of B and C mice to respond to SSS-III are not governed by an autosomal dominant gene. All male CB mice gave a low response to SSS-III (Table IV). In contrast, female CB mice gave an intermediate, rather than a high, response to antigen; the magnitude of the mean serum antibody and PFC response was about one-half that obtained for B mice (Table II). These findings suggest that a major component involved in the antibody response to SSS-III is X-linked, i.e., carried on the X chromosome. This component appears to be present on the X chromosome of high responding B mice (X^+X^+ or X^+Y), but not of low-responding C mice (X^-X^- or X^-Y);[3] according to Lyon (27), CB females (X^+X^-) would be expected to give an intermediate response, whereas X^+Y or X^-Y mice should be high responders and low responders, respectively. To test this hypothesis, the number of low responders

[3] The symbols X^+ and X^- are used only to indicate the presence or absence of a hypothetical X-linked component that we assume exerts a positive influence on the antibody response to SSS-III.

among progeny derived from several crosses was determined; these values were then compared with those expected if responsiveness to SSS-III was transmitted as a single X-linked trait. The χ^2 test was used to detect significant differences between the observed values and those expected if the hypothesis is

TABLE IV

Magnitude of the PFC and Serum Antibody Response Produced by Male and Female Progeny from Various Crosses

Mice	No. of mice*	Hypothetical genotype	PFC/spleen		PFC/10^6 spleen cells		$1/\log_2$ serum antibody titer	
			Range	Mean $\log_{10} \pm S\bar{x}$‡	Range	Mean $\log_{10} \pm S\bar{x}$‡	Range	Mean $\pm S\bar{x}$
CB	83/83	X^-Y	<100–100	N. C.§	<0.5–1	N. C.	<1‖	N. C.
	63/63	X^+X^-	2200–20,450	3.90 ± 0.03 (7940)	6–152	1.62 ± 0.03 (42)	5–11¶	6.75 ± 0.27
CB/CB	44/95	X^-Y	<100–550	N. C.	<0.5–8	N. C.	<1‖	N. C.
	51/95	X^+Y	1650–25,650	4.05 ± 0.03 (11,200)	15–176	1.79 ± 0.03 (62)	3–9**	5.69 ± 0.25
	51/94	X^-X^-	<100–550	N. C.	<0.5–3	N. C.	<1‖	N. C.
	43/94	X^+X^-	2000–19,850	3.86 ± 0.04 (7240)	10–143	1.67 ± 0.04 (47)	4–9	5.79 ± 0.20
B/CB	36/36	X^+Y	3300–45,000	4.16 ± 0.04 (14,500)	14–213	1.88 ± 0.04 (76)	5–10	6.89 ± 0.22
	34/34	X^+X^-	1300–27,700	3.94 ± 0.05 (8710)	10–166	1.67 ± 0.05 (47)	5–9	6.59 ± 0.22
CB/B	7/11	X^-Y	<100–100	N. C.	<0.5–0.5	N. C.	<1‖	N. C.
	4/11	X^+Y	4750–20,200	—	30–89	—	6–8	—
	28/28	(X^+X^+, X^+X^-)	650–24,450	3.91 ± 0.07 (8130)	3–143	1.64 ± 0.07 (44)	4–10	6.68 ± 0.27
C/CB	36/36	X^-Y	<100–400	N. C.	<0.5–3	N. C.	<1‖	N. C.
	29/29	X^-X^-	<100–200	N. C.	<0.5–3	N. C.	<1‖	N. C.
CB/C	28/54	X^-Y	<100–100	N. C.	<0.5–0.8	N. C.	<1‖	N. C.
	26/54	X^+Y	4000–24,600	4.03 ± 0.03 (10,700)	23–182	1.72 ± 0.04 (53)	5–9	6.38 ± 0.19
	18/45	X^-X^-	<100–200	N. C.	<0.5–3	N. C.	<1‖	N. C.
	27/45	X^+X^-	1500–16,000	3.76 ± 0.05 (5750)	10–123	1.59 ± 0.05 (39)	4–9	5.56 ± 0.25

* No. of mice giving a stated response/total No. of mice examined.
‡ Geometric means are shown in parenthesis.
§ Not calculable, since no PFC or serum antibody could be detected in all but a few of the mice examined.
‖ No serum antibody detected in any of the mice examined.
¶ Does not include three values of <1.
** Does not include two values of <1.

valid. The data of Table V clearly show that in all of the crosses examined, the number of low responders observed agreed with that expected for an X-linked trait ($P \geq 0.05$ using a χ^2 test for all comparisons). In view of these findings, it is reasonable to assume that the hypothesis advanced is valid. The symbols X^+ and X^- will, therefore, be used in subsequent sections of this report to classify progeny derived from various crosses with respect to gene dose for the X-linked gene, and to facilitate analysis of autosomal factors involved in the antibody response to SSS-III.

Analysis of Cumulative Frequency Distributions.—If the above X-linked component is the only factor which accounts for differences in the ability of C and B mice to respond to SSS-III, then all mice having the same hypothetical genotype, with respect to the X-linked component, should respond similarly to antigen. The data of Tables VI–X, which permit one to compare the response produced by one group of mice with that of any other group listed, indicate that this is not the case.

Cumulative frequency distributions for only the intermediate and high responders derived from various crosses were compared; the results of such com-

TABLE V

*Incidence of Low Responders in Progeny Derived from Various Crosses**

Mice	Sex	Observed	Expected
CB	♂	83/83	83/83
	♀	0/63	0/63
CB/CB	♂	44/95	47.5/95
	♀	51/94	47/94
B/CB	♂	0/36	0/36
	♀	0/34	0/34
CB/B	♂	7/11	5.5/11
	♀	0/28	0/28
C/CB	♂	36/36	36/36
	♀	29/29	29/29
CB/C	♂	28/54	27/54
	♀	18/45	22.5/45

* Values listed represent No. of low responders observed or expected/total No. of mice examined.

parisons are summarized in Tables VI and VII. When distributions for all male responders (X^+Y mice) were compared (Table VI), four significant differences were found for the serum antibody response; these were not accompanied by significant differences in distribution for the PFC response. For CB/C and B/CB males, the distributions for PFC per 10^6 spleen cells, but not for the serum antibody response, differed significantly. With respect to PFC per spleen, no significant differences were noted among all distributions considered.

Except for B/CB females, the cumulative frequency distributions for all females giving an intermediate response (X^+X^- mice) differed significantly from those giving a high response, i.e., X^+X^+ or B mice (Table VII); however, B/CB females did not differ from B females with respect to two of three immunological parameters (PFC per 10^6 spleen cells and serum antibody). Comparisons involving CB/B females presented a problem; here, both intermediate (X^+X^-) and high (X^+X^+) responders were present within the same population. Because of overlap in the distributions of the responses produced by both types of mice, one could not classify individuals as either intermediate or high

responders. Thus, the distributions for CB/B females are not typical of populations composed exclusively of intermediate or high responders. If one excludes all comparisons made involving B and CB/B females, no significant differences were noted with respect to the PFC response for remaining groups of mice; however, three significant differences were noted in distributions for the serum antibody response.

TABLE VI

*Comparison of Maximal Differences between Cumulative Frequency Distributions for Three Immunological Parameters among Various Groups of Male Intermediate and High Responders**

No. of mice	Hypothetical genotype	Mice	Mice			
			B	B/CB	CB/CB	CB/C
				log_{10} PFC/spleen		
67	X^+Y	B	—	0.11	0.09	0.12
36	X^+Y	B/CB	0.11	—	0.17	0.23
51	X^+Y	CB/CB	0.09	0.17	—	0.06
26	X^+Y	CB/C	0.12	0.23	0.06	—
				log_{10} PFC/10^6 spleen cells		
36	X^+Y	B	—	0.13	0.04	0.27
36	X^+Y	B/CB	0.13	—	0.12	0.40*
51	X^+Y	CB/CB	0.04	0.12	—	0.28
26	X^+Y	CB/C	0.27	0.40*	0.28	—
				$1/log_2$ serum antibody titer		
67	X^+Y	B	—	0.31*	0.47*	0.42*
36	X^+Y	B/CB	0.31*	—	0.37*	0.16
51	X^+Y	CB/CB	0.47*	0.37*	—	0.30
26	X^+Y	CB/C	0.42*	0.16	0.30	—

* Cumulative frequency distributions were constructed from the data of Table IV. The figures listed above represent D_{max} values for comparisons made between two groups of mice. Significant differences are indicated by an asterisk. Except for CB/C mice, the Y chromosome came from B mice in all cases.

Analysis of the Magnitude of the Antibody Response.—When the magnitudes of the antibody response produced by all high responding males (X^+Y mice) were compared, three, one, and three significant differences were noted for PFC per spleen, PFC per 10^6 spleen cells, and the serum antibody response, respectively (Table VIII); in no comparisons were significant differences among males demonstrable for all three immunological parameters. The magnitude of the antibody response for high responding B females (X^+X^+ mice) differed significantly from that for all intermediate responders (X^+X^- mice) with respect to all three immunological parameters (Table IX). If one excludes all comparisons made involving B females, two and six additional significant differences were noted with respect to PFC per spleen and the serum antibody response, respectively. When one considers all comparisons cited in Tables

VI–IX, there is only one case (the serum antibody response for B/CB vs. B males) in which a significant difference in distribution was not accompanied by a significant difference in the magnitude of the antibody response. However, significant differences in the magnitude of the response were not accompanied by significant differences in the distribution in several cases. More significant

TABLE VII

*Comparison of Maximal Differences between Cumulative Frequency Distributions for Three Immunological Parameters among Various Groups of Female Intermediate and High Responders**

No. of mice	Hypothetical genotype	Mice	Mice					
			B	CB/B	B/CB	CB/CB	CB	CB/C
			log_{10} PFC/spleen					
148	X^+X^+	B	—	0.36*	0.35*	0.50*	0.48*	0.61*
28	(X^+X^+, X^+X^-)	CB/B	0.36*	—	0.09	0.24	0.22	0.35
34	X^+X^-	B/CB	0.35*	0.09	—	0.20	0.18	0.31
43	X^+X^-	CB/CB	0.50*	0.24	0.20	—	0.13	0.19
63	X^+X^-	CB	0.48*	0.22	0.18	0.13	—	0.23
27	X^+X^-	CB/C	0.61*	0.35	0.31	0.19	0.23	—
			log_{10} PFC/10^6 spleen cells					
115	X^+X^+	B	—	0.30*	0.22	0.29*	0.39*	0.44*
28	(X^+X^+, X^+X^-)	CB/B	0.30*	—	0.08	0.09	0.12	0.14
34	X^+X^-	B/CB	0.22	0.08	—	0.07	0.17	0.22
43	X^+X^-	CB/CB	0.29*	0.09	0.07	—	0.13	0.15
63	X^+X^-	CB	0.39*	0.12	0.17	0.13	—	0.05
27	X^+X^-	CB/C	0.44*	0.14	0.22	0.15	0.05	—
			$1/log_2$ serum antibody titer					
133	X^+X^+	B	—	0.21	0.23	0.48*	0.21*	0.57*
28	(X^+X^+, X^+X^-)	CB/B	0.21	—	0.04	0.34*	0.14	0.42*
34	X^+X^-	B/CB	0.23	0.04	—	0.30	0.15	0.38*
43	X^+X^-	CB/CB	0.48*	0.34*	0.30	—	0.31*	0.09
63	X^+X^-	CB	0.21*	0.14	0.15	0.37*	—	0.41*
27	X^+X^-	CB/C	0.57*	0.42*	0.38*	0.09	0.41*	—

* Cumulative frequency distributions were constructed from the data of Table IV. The figures listed above represent D_{max} values for comparisons made between two groups of mice. Significant differences are indicated by an asterisk.

differences were noted with comparisons based on the serum antibody than on the PFC response.

Significant differences were noted for most comparisons made for the PFC and serum antibody response of X^+X^- and X^+Y mice; these mice were identical for gene dose with respect to the X-linked component (Table X). In contrast to the data of Tables VI–IX, more significant differences were detected for comparisons based on the PFC than on the serum antibody responses. In several cases, significant differences were noted for all three immunological parameters considered.

Magnitude of the Antibody Response to Other Antigens.—The data of Table XI show that the direct PFC and serum hemolytic antibody responses to SRBC were lower in C than in either B or CBA/J mice; as was the case for the antibody response to SSS-III, CB mice gave an intermediate response. B mice responded well to SSS-I, SSS-II, and *E. coli* 0127 lipopolysaccharide; however, no serum hemolytic antibody could be detected in C mice immunized with these antigens. CBA/J mice gave a good response to *E. coli* lipopolysaccharide.

TABLE VIII

Comparison of the magnitudes of the PFC and Serum Antibody Response to 0.5 μg of SSS-III among Various Groups of Male Intermediate and High Responders

No. of mice	Mean ± $S\bar{x}$*	Hypothetical genotype	Mice‡	Mice			
				B	B/CB	CB/CB	CB/C
					log_{10} PFC/spleen		
67	4.11 ± 0.02	X^+Y	B	—	NS§	NS	$P < 0.05$
36	4.16 ± 0.04	X^+Y	B/CB	NS	—	$P < 0.05$	$P < 0.05$
51	4.05 ± 0.03	X^+Y	CB/CB	NS	$P < 0.05$	—	NS
26	4.03 ± 0.03	X^+Y	CB/C	$P < 0.05$	$P < 0.05$	NS	—
					log_{10} PFC/10^6 spleen cells		
37	1.81 ± 0.04	X^+Y	B	—	NS	NS	NS
36	1.88 ± 0.04	X^+Y	B/CB	NS	—	NS	$P < 0.05$
51	1.79 ± 0.03	X^+Y	CB/CB	NS	NS	—	NS
26	1.72 ± 0.04	X^+Y	CB/C	NS	$P < 0.05$	NS	—
					$1/log_2$ serum antibody titer		
67	7.37 ± 0.17	X^+Y	B	—	NS	$P < 0.05$	$P < 0.05$
36	6.89 ± 0.22	X^+Y	B/CB	NS	—	$P < 0.05$	NS
51	5.69 ± 0.25	X^+Y	CB/CB	$P < 0.05$	$P < 0.05$	—	NS
26	6.38 ± 0.19	X^+Y	CB/C	$P < 0.05$	NS	NS	—

* Geometric means for PFC values are listed in Table IV.
‡ Except for CB/C mice, the Y chromosome comes from B mice in all cases.
§ No significant difference.

DISCUSSION

Although several strains of inbred mice were found to differ widely in their ability to respond to SSS-III, all strains examined, with the exception of C3H/HeN mice, showed a single optimal dose of antigen for immunization; there was no relationship between *H-2* type and the magnitude of the PFC and serum antibody responses produced (Table I). Both of these findings are in agreement with the work of Braley and Freeman (28), who obtained antibody responses of similar magnitude using related strains of mice and another preparation of SSS-III. The antibody response of BALB/cAnN or B mice has been well characterized and appears to be representative of the upper limit of responsiveness to SSS-III in mice (12, 13, 15, 19). Consequently, B mice were used as high responders in the present work.

Several strains of mice responded poorly to SSS-III; however, the antibody response of CBA/HN or C mice was extremely low (Table III). Unlike other strains of mice examined, no serum antibody could be detected in any of these

mice after immunization with an optimally immunogenic dose of antigen. Immunization with various doses of either SSS-III or Type III pneumococci, as well as treatment with ATS, failed to increase the magnitude of the response produced. No evidence was obtained to indicate that C mice either are cross-tolerant to SSS-III, or produce a substance that interferes with the detection of SSS-III-specific antibody or PFC. Therefore, C mice were considered to be

TABLE IX

Comparison of the Magnitudes of the PFC and Serum Antibody Response to 0.5 μg of SSS-III among Various Groups of Female Intermediate and High Responders

No. of mice	Mean ± $S\bar{x}$*	Hypothetical genotype	Mice	Mice					
				B	CB/B	B/CB	CB/CB	CB	CB/C
				$\log_{10}$ PFC/spleen					
148	4.17 ± 0.02	X^+X^+	B	—	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
28	3.91 ± 0.07	(X^+X^+, X^+X^-)	CB/B	$P < 0.05$	—	NS‡	NS	NS	NS
34	3.94 ± 0.05	X^+X^-	B/CB	$P < 0.05$	NS	—	NS	NS	$P < 0.05$
43	3.86 ± 0.04	X^+X^-	CB/CB	$P < 0.05$	NS	NS	—	NS	NS
63	3.90 ± 0.03	X^+X^-	CB	$P < 0.05$	NS	NS	NS	—	$P < 0.05$
27	3.76 ± 0.05	X^+X^-	CB/C	$P < 0.05$	NS	$P < 0.05$	NS	$P < 0.05$	—
				$\log_{10}$ PFC/10^6 spleen cells					
115	1.86 ± 0.02	X^+X^+	B	—	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
28	1.64 ± 0.07	(X^+X^+, X^+X^-)	CB/B	$P < 0.05$	—	NS	NS	NS	NS
34	1.67 ± 0.05	X^+X^-	B/CB	$P < 0.05$	NS	—	NS	NS	NS
43	1.67 ± 0.04	X^+X^-	CB/CB	$P < 0.05$	NS	NS	—	NS	NS
63	1.62 ± 0.03	X^+X^-	CB	$P < 0.05$	NS	NS	NS	—	NS
27	1.59 ± 0.05	X^+X^-	CB/C	$P < 0.05$	NS	NS	NS	NS	—
				1/$\log_2$ serum antibody titer					
133	7.31 ± 0.13	X^+X^+	B	—	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
28	6.68 ± 0.27	(X^+X^+, X^+X^-)	CB/B	$P < 0.05$	—	NS	$P < 0.05$	NS	$P < 0.05$
34	6.59 ± 0.22	X^+X^-	B/CB	$P < 0.05$	NS	—	$P < 0.05$	NS	$P > 0.05$
43	5.79 ± 0.20	X^+X^-	CB/CB	$P < 0.05$	$P < 0.05$	$P < 0.05$	—	$P < 0.05$	NS
63	6.75 ± 0.27	X^+X^-	CB	$P < 0.05$	NS	NS	$P < 0.05$	—	$P < 0.05$
27	5.56 ± 0.25	X^+X^-	CB/C	$P < 0.05$	$P < 0.05$	$P < 0.05$	SN	$P < 0.05$	—

* Geometric means for PFC values are listed in Table IV.

‡ No significant difference.

low responders, and were used in conjunction with high responding B mice in the genetic studies described.

The results of this work clearly show that the antibody response to SSS-III in B and C mice is governed by at least two distinct types of genetic control. The first type influences the ability to respond to SSS-III in an almost quantal or "all-or-none" manner; the incidence of low responders among progeny derived from various crosses indicates that responsiveness to SSS-III is controlled largely by a gene present on the X chromosome (Table V). The second type appears to regulate the magnitude of the antibody response produced by both male and female mice possessing the X-linked gene. The data of Tables VI–X

illustrate that for mice identical with respect to gene dose for the X-linked component, significant differences in the magnitude of the PFC response were not always accompanied by significant differences in the magnitude of the serum antibody response, and conversely. Such quantitative effects may be mediated by autosomal genes that act independently to regulate (*a*) the number of antibody-forming cells found after immunization with SSS-III, and (*b*) the amount of antibody made by such cells.

TABLE X

*Comparison of the Magnitudes of the PFC and Serum Antibody Response to 0.5 μg of SSS-III among X^+Y and X^+X^- Mice**

X^+X^- mice	X^+Y mice			
	B	B/CB	CB/CB	CB/C
		$\log_{10}$ PFC/spleen		
CB/B‡	<0.05	<0.05	<0.05	NS§
B/CB	<0.05	<0.05	<0.05	NS
CB/CB	<0.05	<0.05	<0.05	<0.05
CB	<0.05	<0.05	<0.05	<0.05
CB/C	<0.05	<0.05	<0.05	<0.05
		$\log_{10}$ PFC/10^6 spleen cells		
CB/B‡	<0.05	<0.05	<0.05	NS
B/CB	<0.05	<0.05	<0.05	NS
CB/CB	<0.05	<0.05	<0.05	NS
CB	<0.05	<0.05	<0.05	NS
CB/C	<0.05	<0.05	<0.05	<0.05
		$1/\log_2$ serum antibody titer		
CB/B‡	<0.05	NS	<0.05	NS
B/CB	<0.05	NS	<0.05	NS
CB/CB	<0.05	<0.05	NS	NS
CB	NS	NS	<0.05	NS
CB/C	<0.05	<0.05	NS	<0.05

* Mean ± $S\bar{x}$ values of Table IV were used to obtain the probability values shown.
‡ Both X^+X^+ and X^+X^- were present in this group.
§ No significant difference.

It is tempting at this point to propose that genes governing the structure and synthesis by B cells of antibody specific for SSS-III are X-linked. However, several observations tend to weaken, but not necessarily exclude, such an argument. First, Braley and Freeman (28) reported that the ability of strains of inbred mice to respond well to SSS-III is transmitted as an autosomal dominant trait; the quantitative effects observed in their work are similar to those described in the present study. It remains to be established whether these effects can be ascribed solely to differences with respect to gene dose for autosomal dominant genes that govern the ability of B cells to produce antibody specific for SSS-III, or to the action of a separate homeostatic control mech-

anism in which thymus cells appear to exert a negative, rather than a positive, influence on the expression of such genes (25); such a control mechanism will be considered later. Second, C mice give an extremely low, or undetectable, IgM antibody response, not only to SSS-III, but also to SRBC and to several

TABLE XI

*Magnitude of the PFC and Serum Antibody Responses Produced by B, C, CB, and CBA/J Mice to Other Antigens**

Antigen and dose	Time of assay‡	Mice	PFC/spleen $\log_{10} \pm S\bar{x}$	PFC/10^6 spleen cells $\log_2 \pm S\bar{x}$	$1/\log_2$ serum antibody $\pm S\bar{x}$
	days				
SRBC, 2×10^9	4	B	5.395 ± 0.038 (248,000)§	2.958 ± 0.033 (907)	10.00 ± 0.33 —
		C	3.625 ± 0.182 (4400)	2.160 ± 0.124 (145)	4.00 ± 0.27 —
		CB	5.181 ± 0.052 (152,000)	2.891 ± 0.062 (779)	7.80 ± 0.37 —
		CBA/J	5.287 ± 0.062 (194,000)	— —	11.50 ± 0.29 —
SRBC, 5×10^9	4	B	5.432 ± 0.047 (270,000)	2.987 ± 0.079 (970)	9.50 ± 0.43 —
		C	3.744 ± 0.162 (5550)	1.912 ± 0.091 (81)	4.00 ± 0.22 —
SSS-I, 0.5 μg	5	B	—	—	7.20 ± 0.37
		C	—	—	None detected
SSS-II, 0.5 μg	5	B	—	—	5.75 ± 0.63
		C	—	—	None detected
E. coli lipopoly-saccharide, 10 μg	5	B	—	—	6.20 ± 0.58
		C	—	—	None detected
		CBA/J	—	—	7.83 ± 0.17

* Five to eight mice were used to obtain all values listed.

‡ Days after immunization.

§ Geometric means are shown in parentheses.

polysaccharide antigens (Table XI). While it is possible that the ability to respond to several polysaccharide antigens is governed by an array of X-linked genes, not present in C mice, the results of preliminary studies reveal that the IgM levels of C mice, as well as other low responders used in this work, are significantly lower than those of intermediate and high responders. This suggests that the above X-linked gene may constitute (*a*) a controller gene for

IgM synthesis, or (*b*) govern the formation of a product, perhaps a hormone, that regulates the expression of a controller gene located on another chromosome. In this context, it has been reported recently that the X chromosome of man carries a gene that markedly influences serum IgM levels (29). Since mostly antibody of the IgM class is produced in mice after immunization with SSS-III (12–14), such a gene could exert a secondary yet decisive influence in determining responsiveness not only to SSS-III, but also to other antigens that elicit primarily an IgM antibody response. At present we are attempting to define the precise nature of the genetic defect observed in C mice. Clearly, studies on the antibody response of C mice to other antigens would enable one to determine whether this genetic defect affects all IgM antibody responses. If this is the case, then C mice may provide a suitable experimental model system for the study of certain primary immune deficiency diseases of man, several of which have been reported to be X-linked (30–34).

If the above X-linked gene acts primarily to regulate IgM formation, then the differences noted in the magnitude of the antibody response of mice having the X-linked gene may be attributed to differences in gene dose with respect to autosomal dominant genes that govern, in a positive manner, the ability of B cells to synthesize antibody specific for SSS-III; such genes would, of course, be cryptic in C mice lacking the X-linked gene, but expressed in progeny derived from several of the crosses considered in this work. If such is the case, then one might assume that high responding B mice are dominant for one or more of these genes, and that low responding C mice are recessive for any or all of them. Accordingly, B/CB and intermediate and high responding CB/B mice would be expected to give a similar antibody response to SSS-III and be among the highest responders; in contrast, intermediate and high responding CB/C mice should give the lowest response produced among all mice having the X-linked gene; the data of Tables IV–IX are consistent with such a view. Alternatively, C mice and B mice may be identical in gene dose for such autosomal dominant genes, and the quantitative differences observed among mice having the X-linked gene may be the result of factors that exert a negative rather than a positive influence on their expression. Previous studies have shown that treatment with ATS results in an 8–10-fold increase in the magnitude of the antibody response to SSS-III (24–26); such increases are considerably greater than the quantitative differences noted in this work. While the infusion of syngeneic thymus cells abrogated the enhancement produced after treatment with ATS, the infusion of syngeneic peripheral white blood cells further increases the magnitude of the response (25). On the basis of these findings, we proposed that two types of presumably thymic-derived cells (a suppressor cell and an amplifier cell) act in an opposing manner to regulate the magnitude of the antibody response to SSS-III. The ability of ATS to increase the magnitude of the antibody response to SSS-III is apparently the result of the inactivation of a cell type that normally serves to suppress the antibody response produced after immunization. There is increasing evidence that such a homeostatic control

mechanism plays an important role in immune responses to several antigens (35–42); the results of preliminary studies reveal that treatment with ATS results in a significant increase in both the number of PFC produced in the antibody response to SSS-III and the amount of antibody made by such cells (24, 43). One must, therefore, consider the possibility that the quantitative differences observed in this work may reflect differences between C and B mice in the numbers, proportions, or specific activities of suppressor and amplifier cells. If such processes are under separate genetic control, this would add an additional complexity and introduce a new dimension to studies on the genetic control of the immune response. We are currently attempting to assess the influence of such factors in the genetic studies described.

SUMMARY

The IgM antibody response to Type III pneumococcal polysaccharide (SSS-III) was assessed in F_1, F_2, and backcross progeny derived from high (BALB/cAnN) and extremely low (CBA/HN) responding parental strains of inbred mice. The results of these studies indicated that a major component involved in the antibody response is X-linked, i.e., carried on the X chromosome; this component determines responsiveness to SSS-III in an almost quantal or "all-or-none" manner. Other factors, presumably autosomal genes, regulate the magnitude of the antibody response produced by mice possessing the X-linked gene; these appear to influence independently the number of antibody-producing cells found after immunization and the amount of antibody made by such cells. Strains of inbred mice varied widely in their ability to respond to SSS-III. Responsiveness was not associated with *H-2* histocompatibility type. The implications of these findings with respect to the genetic control of the antibody response to SSS-III are discussed.

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