GENETIC CONTROL OF RESPONSES TO BACTERIAL LIPOPOLYSACCHARIDES IN MICE

I. Evidence for a Single Gene that Influences Mitogenic and Immunogenic Respones to Lipopolysaccharides*

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Bacterial lipopolysaccharides (LPS)[1] elicit a variety of physiological responses in mice. LPS are extremely antigenic substances, small doses are capable of stimulating large immune responses in both normal (1) and thymus (T) cell-depleted mice (2). Mice are highly susceptible to toxic effects of high concentrations of LPS (3). In murine spleen cultures LPS has been shown to exert three effects. First, LPS induces DNA synthesis in most bone marrow-derived (B) lymphocytes (4–6). Second, high concentrations of LPS increase background immune responses to a wide variety of determinants (5, 7, 8). This maturation of antibody-forming cells is independent of added antigen and has been termed the polyclonal response (5, 7). Third, low concentrations of LPS stimulate immune responses to antigenic determinants presented in nonimmunogenic forms. This can be done by using heterologous erythrocyte antigens in T-cell-depleted cultures (5–8), or by using determinants which because of their size or structure cannot participate in the cell cooperative events that are required for the induction of antibody synthesis (8, 9). Whereas immune responses to LPS are restricted to only those B lymphocytes that possess surface immunoglobulin receptors capable of binding antigenic determinants on LPS, the mitogenic responses to LPS appear to involve most B lymphocytes requiring the binding of LPS at nonimmunoglobulin receptor sites (4–8). To analyze the genetic control of these two LPS responses we have utilized the finding that there exists a strain of mice, C3H/HeJ, which does not support in vitro mitogenic responses to LPS (reference 10 and footnote 2), and which is resistant to the toxic effects of high concentrations of LPS (3). In this paper we show that C3H/HeJ mice support small in vivo immune responses to LPS in a restricted dose range.

*Supported by a Leukemia Society of America Special Fellowship and grants AI-11092, AI-05875, and AI-00430 from the National Institutes of Health.

[1] *Abbreviations used in this paper:* LPS, lipopolysaccharides; PFC, plaque-forming cells.

[2] Skidmore, B. J., J. M. Chiller, D. C. Morrison, and W. O. Weigle. 1974. Immunological properties of bacterial lipopolysaccharides (LPS). Correlation between the mitogenic, adjuvant, and immunogenic activities. Manuscript submitted for publication.

Since C3H/HeJ mice support small immune responses to LPS and no mitogenic responses to LPS, we refer to these mice as low LPS responders. In contrast, other similar strains of C3H mice we have tested are high LPS responders. Such high LPS responder C3H strains produce large immune responses to LPS over a wide dose range in vivo, and spleen cultures prepared from these high LPS responder mice all support mitogenic responses to LPS. LPS prepared from a variety of strains of *Escherichia coli* or *Salmonella* fail to elicit in vivo immune responses or in vitro mitogenic responses in C3H/HeJ mice but elicit both responses in high LPS responder C3H strains. The data from backcross experiments are presented which reveal that the failure of C3H/HeJ mice to support in vitro mitogenic responses and in vivo immune responses to LPS seems to be due to a single gene defect. This autosomal gene is not linked to the histocompatibility or to heavy-chain allotype loci. These findings imply that the immune response and mitogenic response induced by LPS share a common regulatory mechanism.

Materials and Methods

Mice. The following strains of mice were obtained from Jackson Laboratories, Bar Harbor, Maine: C3H/HeJ, C3HeB/FeJ, C3H/DiSn (all *H-2*^{k}), DBA/2J (*H-2*^{d}), and C3D2F$_1$ (C3H/HeJ × DBA/2J). C3H/Str mice were obtained from Strong Laboratories, Del Mar, Calif. The breeding nuclei of C3H.SW (CSW) and C3H.SW-Ig-1^b (CWB/13) strains were obtained from Dr. Leonard Herzenberg, Stanford University, Stanford, Calif. C3H.SW (*H-2*^{b}) is congenic with C3H/DiSn; C3H.SW-Ig-1^b differs in IgG2a immunoglobulin allotype from the other C3H strains. (CWB × C3H/HeJ)F$_1$ and (F$_1$ × C3H/HeJ) backcross mice were bred at the Salk Institute, San Diego, Calif. The following strains were obtained from Dr. Donald Schreffler, University of Michigan, Ann Arbor, Mich., C3H/Sf, C3H.OL, C3H.OH, C3H.Q, and C3H.B10. Mice were between 6 and 12 wk of age at the beginning of immunization.

Lipopolysaccharides. The following bacterial lipopolysaccharides, prepared by the Westphal procedure, were obtained from Difco Laboratories, Detroit, Mich.: *E. coli* 0127:B8, *E. coli* 0111:B4, *E. coli* 0113, *E. coli* 026:B6, *E. coli* 0128:B12, and *E. coli* 055:B5; and *Salmonella abortis equi*, *S. flexerni*, *S. marcescens*, *S. typhosa*, *S. typhimurium*, and *S. enteriditis*. *S. paratyphi C* was a gift from Dr. S. Sarkar, Salk Institute. Each LPS preparation was resuspended in a phosphate-buffered saline (pH 7.2) at a concentration of 1 mg/ml and boiled for 60 min before use. Lipid A was prepared from *E. coli* 0127:B8 LPS as described elsewhere (8).

Immune Responses to LPS. Mice were injected intraperitoneally with 0.1 ml of LPS solutions as described in the text. At day 7, animals were bled to provide serum samples for hemagglutination assays, or animals were sacrificed and their spleens removed to determine the number of LPS-specific antibody-forming cells. LPS-coated sheep erythrocytes (SRBC) were used as indicator cells for both hemagglutination and plaque assays. An aliquot containing 0.1 ml packed SRBC was incubated with 0.5 ml LPS (1 mg/ml) at 37°C for 60 min, and cells were then washed five times with a balanced salt solution (11). The LPS-coated erythrocytes were resuspended at a dilution of 1:15 for the hemolytic plaque assay or 1:100 for the hemagglutination assay. The number of antibody-forming cells was determined using a microscope slide modification of the Jerne plaque assay (12). Each spleen cell preparation was assayed on SRBC and LPS-coated SRBC, the difference being the LPS-specific plaque-forming cells (PFC). The hemagglutination assay used is described elsewhere.[3] The hemagglutination data is presented as a reciprocal of the end point dilution at which hemagglutination was observed, the initial serum dilution was always 1:10.

Mitogenic Responses to LPS. Spleen cultures were prepared in RPMI-1640 medium (Microbiological Associates, Inc., Bethesda, Md.) supplemented with 5% fetal bovine serum (lot no. 728E309, International Scientific Industries, Cary, Ill.) as detailed elsewhere (8). Each culture contained 1 ml

[3]Riblet, R., and M. Weigert. 1974. Manuscript in preparation.

medium, 5×10^6 spleen cells, and the amount of LPS described in the text. Cultures were rocked at 5–7 cycles/min (12) at 37°C for 66 h and then incubated for 6 h with 0.5 μCi [^{3}H]thymidine (52 Ci/mmol, New England Nuclear, Boston, Mass.), 10^{-5} M thymidine, and 10^{-7} M fluorodeoxyuridine. The cells from each culture were then collected by filtration through glass fiber membranes (Whatman GF/C). The filters were then washed successively with 5% TCA and 95% ethanol, dried, and radioactive measurements made using a terphenyl-toluene scintillation fluid (8).

Allotype Determination. The backcross mice were tested for the presence of the Ig-1^b allotype of the CWB grandparent by using Ouchterlony double-diffusion testing with a BALB/c anti-BAB/14 (Ig-1^a anti-Ig-1^b) antiallotype serum (13).

H-2 Typing. The backcross mice were tested for the presence of the *H-2^b* allele from the CWB grandparent by a modification of the polyvinylpyrrolidone technique of Rubinstein and Kaliss (14). The agglutination reactions were performed in 10 × 75-mm glass tubes. Rather than reading the reactions microscopically (14) the tubes were simply inverted, drained, and leaned against a nearly vertical light box. The cells were observed as they drained slowly down the tube. Positive agglutination was easily seen as the cells drained as large islands with a rather oily appearance as compared to the finely granular aspect of the nonagglutinated cells. The testing was done with a single dilution, 1:500, of the anti-*H-2* serum and compared with a parallel reaction with a normal serum at 1:500. The typing serum was a pool of hyperimmune sera taken after eight weekly intraperitoneal injections of C3H/HeJ mice with spleen, thymus, and mesenteric lymph node cells from CSW mice, the cells from 1 donor were divided among 10 recipients each week. This yielded a strong congenic *H-2^k* anti-*H-2^b* typing serum.

Results

Mitogenic Responses to LPS. The data presented in Fig. 1 show the effects of different concentrations of *E. coli* 0127:B8 LPS (0.1–25 μg) on mitogenic responses in spleen cultures prepared from various C3H mouse strains. At no concentration of LPS is a significant mitogenic response observed in C3H/HeJ

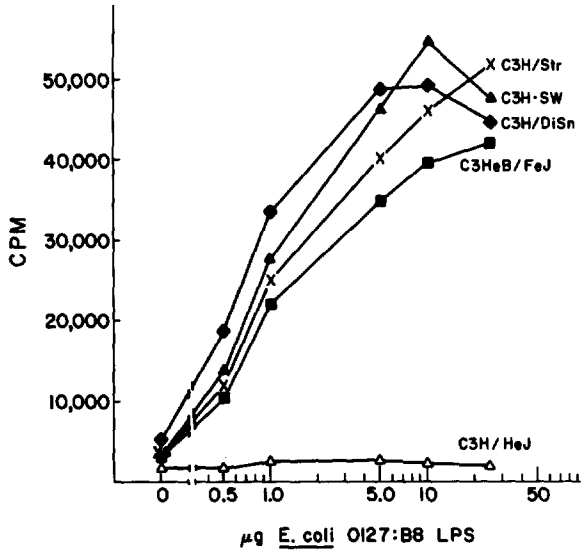

FIG. 1. Mitogenic responses to *E. coli* 0127:B8 LPS in strains of C3H mice. Spleen cultures were prepared from C3H/Str, C3H3B/FeJ, C3H/HeJ, and C3H/DiSn and its congenic C3H/SW. Cultures were assayed for DNA synthesis at day 3. Each point represents the mean of duplicate cultures.

spleen cultures. In contrast, spleen cultures prepared from C3HeB/FeJ, C3H/Str, C3H/DiSn, and its congenic C3H.SW, all support mitogenic responses in the presence of LPS. Optimum mitogenic responses are observed generally in the range of 10–25 μg LPS. In addition, C3H/Sf mice and the *H-2* congenic strains derived from C3H/Sf, C3H.OL, C3H.OH, C3H.Q, and C3H.B10 mice kindly provided by Dr. D. Schreffler, all supported mitogenic responses in spleen cultures (data not shown).

The data presented in Fig. 2 compare the mitogenic responses to LPS in spleen cultures prepared from F_1 hybrid mice to those prepared from the parental strains. C3H/HeJ × DBA/2J ($C3D2F_1$) and (C3H/HeJ × CWB)F_1 hybrid mice both support mitogenic responses to LPS that are similar to those observed in cultures from parental CWB or DBA/2J mice. Therefore, mitogenic responsiveness to LPS is a dominant trait (Fig. 2).

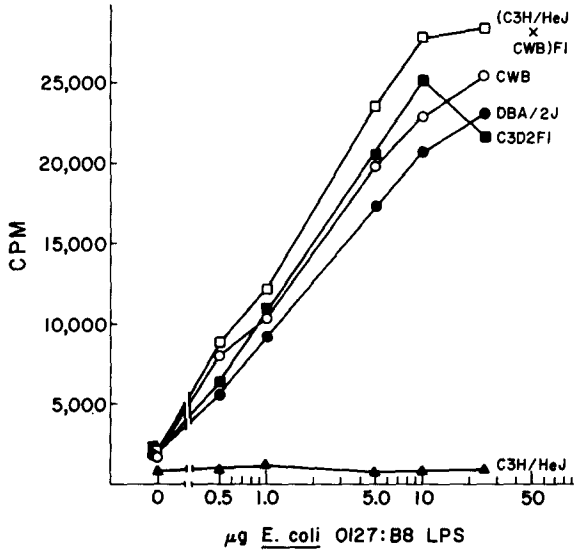

FIG. 2. Mitogenic responses to *E. coli* 0127:B8 LPS in F_1 hybrid mice. Each point represents the mean of duplicate cultures.

The mitogenic response of C3H/HeJ and C3HeB/FeJ spleen cells to a variety of different types of LPS was analyzed. Six types of *E. coli* LPS and seven types of *Salmonella* LPS were compared (Table I). All LPS preparations stimulated mitogenic responses in C3HeB/FeJ spleen cultures. In comparison, all LPS preparations were only weakly or nonmitogenic in spleen cultures prepared from C3H/HeJ mice (Table I). Weak mitogenic responses observed in C3H/HeJ spleen cultures were always less than fourfold above background. Similar concentrations of LPS in C3HeB/FeJ spleen cultures resulted in stimulations generally greater than 10-fold (Table I).

The data presented in Fig. 3 compare mitogenic responses to lipid A prepared from *E. coli* 0127:B8 LPS. Lipid A is nonmitogenic in C3H/HeJ spleen cultures but is mitogenic in C3HeB/FeJ spleen cultures.

Immune responses to LPS. The data presented in Fig. 4 compare the in vivo

TABLE I

Mitogenic Responses to Various LPS Strains in Spleen Cultures Prepared from C3H/HeJ and C3HeB/FeJ Mice

LPS strain	C3H/HeJ cultures				C3HeB/FeJ cultures			
	No LPS	1μg	10μg	25μg	No LPS	1μg	10μg	25μg
	cpm × 10^{-3}				*cpm* × 10^{-3}			
S. abortus equi	1.56	2.24	2.19	2.22	2.08	21.69	33.32	43.09
S. flexerni	2.19	2.10	3.40	3.43	2.34	13.92	24.80	27.23
S. marcescens	1.30	1.65	4.38	4.48	2.29	4.52	14.91	16.25
S. typhimurium	1.79	3.01	3.16	3.87	2.60	8.85	13.71	15.47
S. typhosa	1.21	2.61	2.14	2.08	2.14	10.40	26.40	22.51
S. paratyphi C	1.60	2.40	2.73	3.14	3.51	14.99	28.49	33.25
E. coli 026:B6	1.29	1.41	3.42	3.07	2.64	7.92	18.49	30.30
E. coli 0128:B12	1.84	2.28	4.65	2.50	2.65	18.94	30.42	41.58
E. coli 055:B5	2.18	2.14	2.56	2.81	2.34	16.74	27.43	47.60
E. coli 0127:B8	1.64	2.55	2.31	2.31	2.16	20.78	39.20	31.43
E. coli 0111:B4	1.76	2.43	1.84	2.64	2.02	14.20	36.50	30.40

Spleen cells were cultured at an initial density of 10^7 cells/ml with LPS and radioactively labeled after 66 h as described in the Materials Methods. Each figure represents the mean counts per minute of duplicate cultures.

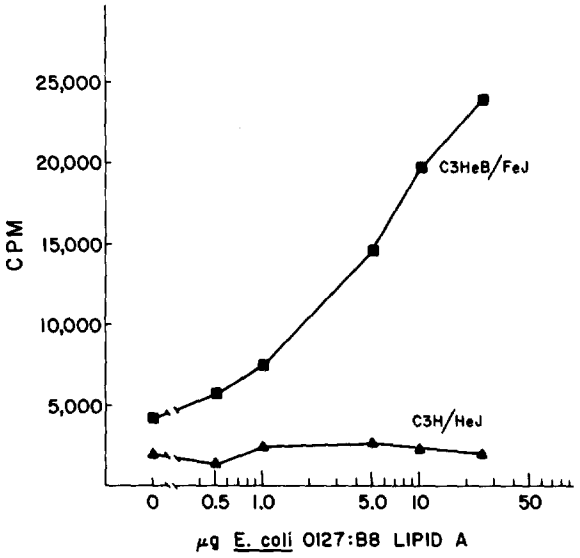

FIG. 3. Mitogenic responses to *E. coli* 0127:B8 lipid A. Each point represents the mean of duplicate cultures.

immune response to *E. coli* 0127:B8 LPS in C3H/HeJ and C3HeB/FeJ mice. Groups of mice were immunized with 0.1, 1.0, 10, or 100 μg LPS intraperitoneally. After 7 days the immune response to LPS in individual mice was analyzed using LPS-coated SRBC to determine the number of LPS-specific PFC in the spleen. Background PFC against SRBC were subtracted. At all dose ranges of

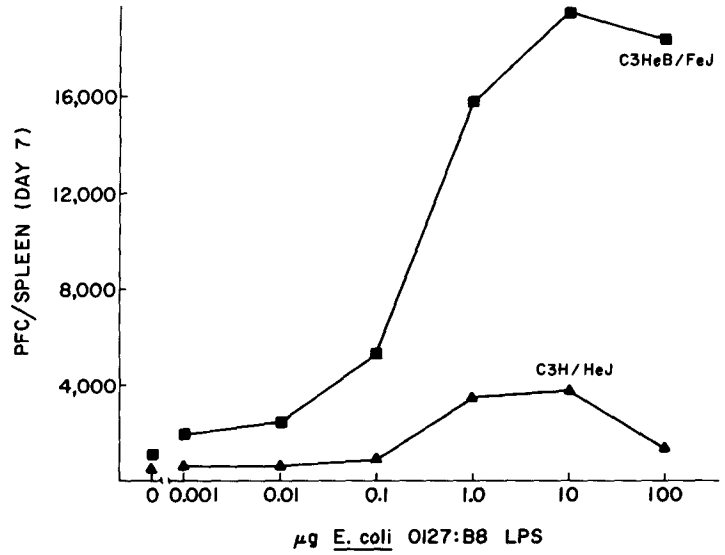

FIG. 4. PFC response to *E. coli* 0127:B8 LPS when injected into C3H/HeJ and C3HeB/FeJ mice. Groups of five mice were injected with the concentrations of LPS indicated. At day 7 the LPS-specific PFC were determined. Each point represents the mean PFC/spleen from the five mice. There was no significant difference in the cell numbers recovered from C3H/HeJ and C3HeB/FeJ spleens.

LPS tested, the immune response as determined by antibody-forming cells in the spleen, was greater in C3HeB/FeJ than C3H/HeJ mice. Only in the range of 1–10 μg LPS were immune responses to LPS observed in C3H/HeJ mice. Only IgM responses to LPS were observed (data not shown) consistent with reports of others (11). In Fig. 5 the serum titers to *E. coli* 0127:B8 LPS in C3H/HeJ and C3HeB/FeJ mice on day 7 are described. As observed for the spleen antibody-forming cells, the serum titers in C3H/HeJ mice in the range of 1.0–10 μg consistently show significant increases above the background titers. However, serum titers to LPS in C3HeB/FeJ mice show two very consistent differences. In the range of 1.0–10 μg LPS titers are generally fourfold higher than observed in C3H/HeJ mice. Also, lower (0.1 μg) and higher (10–200 μg) concentrations of LPS elicit significant antibody responses, where as small or no immune responses are seen in C3H/HeJ mice (Fig. 5).

The categorization of C3H strains as either high or low LPS responders is most easily accomplished when LPS is administered at low (<1 μg) and high (>10 μg) doses. In all subsequent experiments reported here we have administered 100 μg LPS intraperitoneally to C3H mice to reveal the maximum immune response differences between the low and high LPS responder strains as determined by serum hemagglutination titers and spleen PFC numbers.

The data presented in Fig. 6 show immune responses to 100 μg *E. coli* 0127:B8 LPS in C3H/HeJ, CWB, (C3H/HeJ $\times$ CWB)F_1, C3H/Str, and C3H/DiSn mice. No immune response to LPS was seen in C3H/HeJ mice, while responses were elicited in all other strains. Repeated injections of 100 μg LPS to these mice show no significant increase in serum titers (data not presented). As in the mitogenic

response, F_1 hybrid mice of a high and low responder strain are also high LPS responders (Fig. 6).

The response of C3H strains to different types of *E. coli* and *Salmonella* LPS preparations is similar to that described for *E. coli* 0127:B8 LPS. When C3H/HeJ

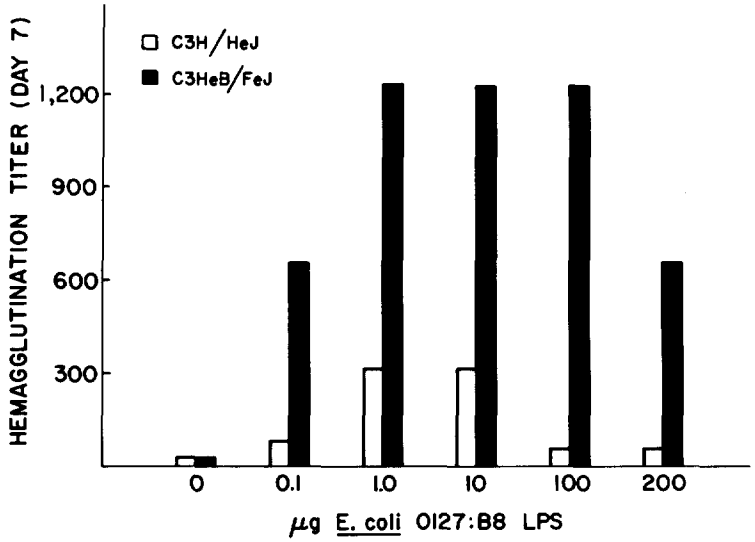

FIG. 5. Hemagglutination titers of C3H/HeJ and C3HeB/FeJ mice responding to *E. coli* 0127:B8 LPS. Groups of three mice were injected with the concentrations of LPS indicated. At day 7 serum samples from each mouse were taken and individually titered. The data represents the mean titer from the three mice in each group.

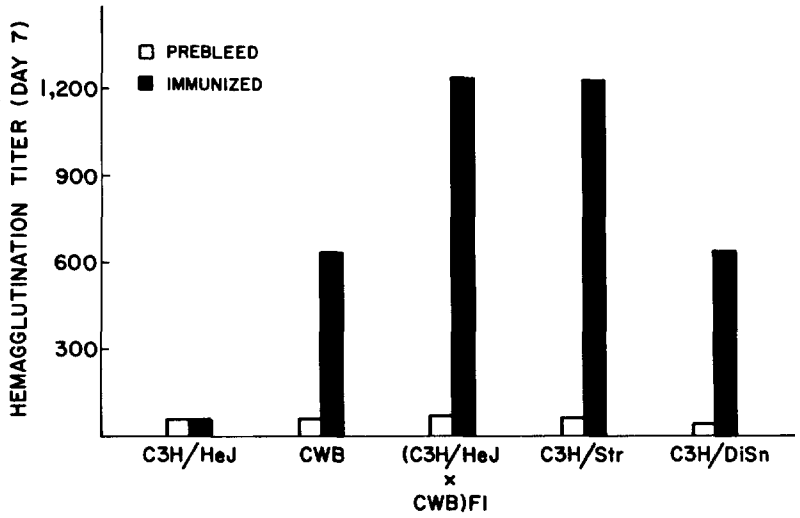

FIG. 6. In vivo immune responses to *E. coli* 0127:B8 LPS in C3H strains of mice. Each group contained three mice. Each mouse was bled before immunization to determine the background titers. 7 days after immunization serum titers were again determined for each animal. The data is the mean titer from each group.

and C3HeB/FeJ mice were immunized with 100 μg *E. coli* 0111:B4, *E. coli* 0127:B8, *E. coli* 0113, *S. paratyphi C*, and *S. marcescens* LPS, immune responses to these LPS preparations were always higher in C3HeB/FeJ mice than C3H/HeJ mice (Fig. 7).

Backcross Linkage Analysis. The backcross mice from (C3H/HeJ × CWB)F_1 × C3H/HeJ mice were examined for the ability to elicit in vivo immune responses and in vitro mitogenic responses to *E. coli* 0127:B8 LPS. Each mouse was prebled to determine the background serum titer to LPS, and then immunized with 100 μg LPS. After 7 days the immune sera were analyzed for an increase in titer to LPS. Each backcross animal was also typed for the Ig-1^b

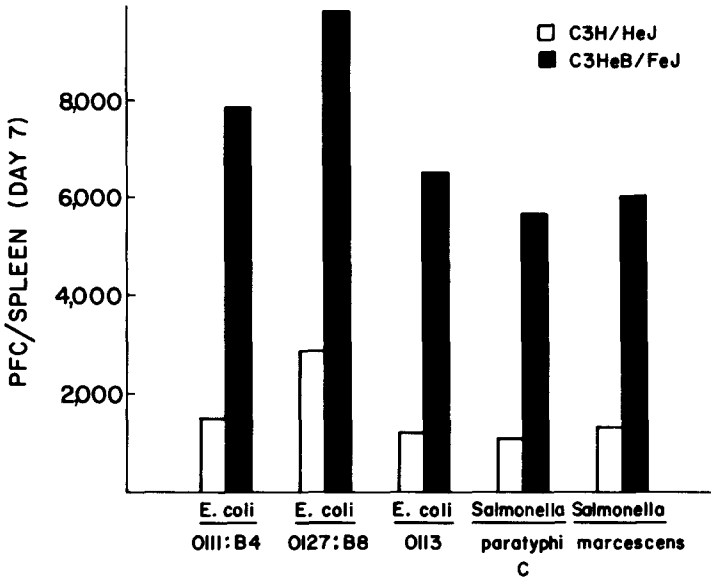

FIG. 7. In vivo immune responses to various *E. coli* and *Salmonella* LPS in C3H/HeJ and C3HeB/FeJ mice. Groups of three mice were injected with 100 μg of the LPS preparation described and after 7 days LPS-specific PFC in the spleens determined. There was no significant difference in the cell numbers from C3H/HeJ and C3HeB/FeJ spleens. The mean PFF/spleen from each group is presented. The background response (unimmunized) of both mouse strains to these LPS preparations is in the range of 500 PFC/spleen.

allotype and *H-2*^{b} characteristic of CWB mice. A total of 44 backcross mice have been individually assayed in this manner and the data obtained from each mouse is presented in Table II. In control C3H/HeJ mice which were not immunized with LPS, no immune or mitogenic responses to LPS were observed. However in immunized C3H/HeJ mice slight increases in serum titers to LPS and in the mitogenic response occur (Table II). Of the 44 backcross mice tested, 24 mice were categorized as high LPS responders on the basis that immunization with LPS resulted in significant increases in serum titers to LPS, and in subsequent spleen assays, mitogenic responses to LPS were always elicited (Table II). The remaining 20 mice were all classed as low LPS responders. While none of these low LPS responders showed significant in vivo immune responses to LPS, spleen

cultures prepared from four of these animals (A1, E1, F1, and F7; Table II) supported small mitogenic responses to LPS. Similar increases in mitogenic responsiveness are sometimes seen in C3H/HeJ mice which have been preimmunized with LPS (Table II). Since such mitogenic responses generally never exceed a fourfold stimulation, and are much smaller than observed in high LPS responder mice, the responses have been designated as low. These backcross data clearly show that the ability to support immune and mitogenic responses to LPS segregate together, and since there are similar numbers of high and low LPS responder mice the defect in C3H/HeJ mice may be due to a single gene.

There is no correlation in backcross mice between *H-2*, allotype, and LPS responsiveness. The loci governing the expression of each of these traits segregate independently. Therefore the classification of C3H mice as high and low LPS responders is not a distinction that is linked to the expression of *H-2* or heavy-chain allotype loci, and is not sex-linked (Table III).

Discussion

The experiments reported in this paper demonstrate that strains of C3H mice can be classified as high or low responders to bacterial LPS. Responsiveness is defined in two ways: the ability to elicit in vivo immune responses to LPS and the ability to support in vitro mitogenic responses to LPS. When compared in these two LPS response systems, C3H/HeJ mice are low LPS responders whereas C3HeB/FeJ, C3H/Str, C3H/DiSn, C3H/Sf, C3H.SW (CSW), and C3H.SW-Ig-1^b (CWB) are high LPS responders. In the mitogenic assay, C3H/HeJ spleen cells generally support no response to LPS (Fig. 1). However, the in vivo immune response difference to LPS between C3H/HeJ mice and other C3H strains is a quantitative difference (Figs. 4 and 5). Doses of 1.0–10 μg LPS elicit small IgM responses in C3H/HeJ mice. Lower or higher doses of LPS fail to elicit significant immune responses. In contrast, other C3H strains elicit a much greater immune response to LPS over a much wider dose range (0.1–200 μg) (Fig. 5). We have used an immunizing dose of 100 μg LPS to obtain the maximum immune response difference between C3H/HeJ and other C3H strains. The ability to respond well to LPS in C3H mice is dominant, as shown by the response of the F_1 hybrid of a high responder (CWB) and a low responder (C3H/HeJ) strain (Fig. 2).

Responsiveness to LPS has several interesting characteristics. Spleen cultures from C3H/HeJ mice do not support mitogenic responses to a variety of noncross-reacting LPS preparations from *E. coli* or *Salmonella* (Table I). However, the active region for the mitogenic activity of LPS is lipid A which has a common structure (15). In vivo, C3H/HeJ mice also fail to support an immune response to high doses of these LPS preparations (Fig. 7). The antigenic determinants are found mainly in the polysaccharide moiety of LPS and are very different in *E. coli* and *Salmonella* preparations. Mitogenic responses elicited by LPS require the interaction of lipid A with the surface of most B lymphocytes (7, 8, 15). In contrast, the induction of immune responses to LPS results from the interaction of antigenic determinants in the polysaccharide region with immunoglobulin receptors on B lymphocytes (1, 2, 16). Whereas most B lymphocytes ap-

TABLE II

Backcross Linkage Analysis

Animal	Sex	Ig-1	*H-2*	Serum titer	Mitogenic response			Responder (low/high)
					No LPS	5 μg	10 μg	
					cpm × 10^{-3}			
Unprimed C3H/HeJ	♀	—	—	<10/<10	2.72	4.75	5.87	L
Primed C3H/HeJ	♀	—	—	10/20	3.04	7.75	10.08	L
Unprimed (C3H/HeJ × CWB)F_1	♀	b	b	10/320	4.60	63.61	49.29	H
A1	♀	—	b	10/20	2.98	13.11	12.26	L*
A2	♀	b	b	20/320	3.05	36.09	39.20	H
A3	♀	—	b	<10/160	4.95	66.15	58.48	H
A4	♀	b	b	10/20	3.81	5.83	6.86	L
A5	♀	—	b	<10/320	1.79	35.62	35.34	H
A6	♀	—	—	10/320	2.78	34.15	39.77	H
A7	♀	b	b	10/320	3.01	45.52	42.26	H
A8	♀	—	b	10/10	3.51	10.66	10.77	L
B1	♂	—	b	ND/10	2.18	6.74	9.87	L
B2	♂	b	b	ND/160	3.45	62.53	34.45	H
B3	♂	—	—	ND/10	1.17	2.11	1.89	L
B4	♂	b	—	ND/20	1.96	4.81	5.05	L
B5	♂	b	—	ND/10	0.67	1.55	1.68	L
B6	♂	b	—	ND/40	1.48	0.69	1.01	L
B7	♂	—	b	ND/40	3.52	53.93	61.30	H
B8	♂	b	b	ND/10	1.61	5.51	4.94	L
D1	♂	b	b	20/20	0.34	1.31	1.07	L
D2	♂	b	b	10/160	0.21	16.03	12.18	H
D3	♂	—	—	<10/160	1.93	17.28	33.64	H
D4	♂	b	b	10/160	1.49	42.90	47.31	H
D5	♀	b	b	10/10	3.19	8.14	8.78	L
D6	♀	b	b	10/160	2.85	15.32	11.64	H
D7	♀	—	b	10/320	1.87	37.90	38.91	H
D8	♀	b	b	10/10	ND	ND	ND	L
E1	♀	—	—	10/10	3.80	12.67	13.05	L*
E2	♀	b	—	80/80	5.30	13.86	10.68	L
E3	♀	—	b	20/320	4.85	53.57	55.10	H
E4	♀	b	b	20/640	3.80	38.96	53.96	H
E5	♀	b	b	10/1280	4.61	25.16	31.39	H
E6	♀	—	—	10/10	3.87	11.10	12.65	L*
E7	♀	b	—	40/1280	5.03	63.78	68.48	H
E8	♀	—	b	10/10	4.24	12.67	10.48	L
E9	♀	—	—	10/10	6.49	15.40	16.57	L
E10	♀	b	b	40/1280	5.43	47.96	44.30	H
E11	♀	b	—	20/640	8.26	59.60	62.06	H
E12	♀	b	—	10/1280	6.39	67.16	84.14	H

TABLE II—*Continued*

Animal	Sex	Ig-1	*H-2*	Serum titer	Mitogenic response			Responder (low/high)
					No LPS	5 μg	10 μg	
					cpm × 10^{-3}			
F1	♂	b	b	10/10	2.50	8.82	11.00	L*
F2	♂	b	b	20/160	2.41	37.59	39.55	H
F3	♂	b	—	20/1280	3.14	44.80	42.38	H
F4	♂	—	—	<10/80	3.02	37.66	46.09	H
F5	♂	—	b	10/320	4.07	52.56	59.83	H
F6	♂	ND	b	10/10	1.82	8.44	8.51	L
F7	♂	b	—	10/20	3.93	13.80	17.49	L
F8	♂	—	—	10/160	4.67	61.73	75.85	H

(C3H/HeJ × CWB)F_1 mice were backcrossed to C3H/HeJ and the progeny tested for Ig-1 allotypes, *H-2*, and for response to LPS. Ig-1 is the allotype locus for IgG2a immunoglobulins. CWB is Ig-1^b, C3H is Ig-1^a; phenotype b = b/a, — = a/a. All animals with the exception of the unprimed C3H/HeJ were immunized with 100 μg *E. coli* 0127:B8. Two figures are presented for the serum titers. The first represents the titer of prebled serum, and the second represents the titer 7 days after immunization with LPS. The serum titers are presented as a reciprocal of the endpoint dilution. Mitogenic assays were performed in spleen cultures 2-4 wk after serum titers were determined in individual animals. The mice have been categorized as high or low responders on the basis of their in vivo immune and in vitro mitogenic responses to LPS. D8 has been designated as a low LPS responder on the basis of serum titers, the animal did not survive for subsequent mitogenic assays. F6 was not allotyped. ND = not done. B7 was designated as a high LPS responder because of its high mitogenic response. The primed C3H/HeJ mice used as controls received 100 μg *E. coli* 0127:B8 LPS intraperitoneally 3 wk before use.

*The mice marked above are designated low responders because the serum titer to LPS was very low although slight mitogenic responses were observed.

TABLE III

Summary of Backcross Linkage Analysis

Genotype	Number of high responder mice	Number of low responder mice*
Ig-$I^{b/a}$	13	11
Ig-$I^{a/a}$	11	8
H-2$^{b/k}$	16	11
H-2$^{k/k}$	8	9
♀	14	10
♂	10	10

*One mouse was not tested for the presence of the Ig-1^b allotype. The data summarizes the segregation of allotype and *H-2* with high and low LPS responder male and female backcross mice from Table II.

pear to respond mitogenically to LPS (7), very few respond by synthesizing specific antibody to LPS. The analysis of backcross mice revealed that immune and mitogenic responsiveness segregated together and that the defect in C3H/HeJ mice that limits mitogenic and immune responses to LPS appears to be due to a single locus (Table II). The *H-2* and allotype characteristics of the high and low LPS responder backcross mice were also analyzed (Tables II and III). These data show that the defective locus in C3H/HeJ mice is not associated with the major histocompatibility complex (*H-2*) or to the heavy-chain allotype region. This is consistent with the fact that we have seen no response difference with any of the *H-2* or allotype congenic strains.

There have been a number of reports concerning the response of C3H/HeJ mice to LPS or lipid A. When immunized with high concentrations of lipid A, C3H/HeJ mice unlike other strains, fail to show an increase in PFC directed against normal SRBC (17). C3H/HeJ are resistant to the lethal toxic effects of LPS and increase their mononuclear cells in the peritoneal cavity after LPS stimulation (3, 18). The toxic and mononuclear cell response in C3H/HeJ mice appears to be under polygenic control (18) unlike control of immune and mitogenic responses to LPS described here. There are several genetic analyses of immune responses to LPS in other strains of mice. Di Pauli (19) has shown differences in the response of mice to *Salmonella* LPS. BALB/c and DBA/2An differ in the antibody specificity produced against the same LPS. This strain difference resides in the process that recognizes the antigen and is not due to a lack of antibody-producing precursor cells in BALB/c and DBA/2An mice (19). Backcross analyses show that this immune response difference to LPS may be controlled by a single gēne (20).

A number of loci have been identified in mice that influence immune responses to different antigens: one maps within the *H-2*-gene complex (21–23), another group is linked to heavy-chain allotype loci (24–28), and a third is sex-linked (29, 30). It is unlikely that the defect in C3H/HeJ mice that limits LPS responsiveness is associated with any of these loci. The immune responsiveness to LPS is not sex-linked (Tables II and III). Therefore the defect in C3H/HeJ mice is unrelated to the defect in CBA/HN mice which limits their immune responses to a variety of antigens (29, 30). CBA/HN mice lack an X-linked gene that governs the level of the IgM response.

The defect in C3H/HeJ mice which limits the level of the IgM response to bacterial LPS appears different from other immune response defects detected in mice. In view of the finding that C3H/HeJ mice are low responders to a variety of unrelated LPS, and do not respond mitogenically to either LPS or lipid A, it is puzzling that the backcross animals revealed that both LPS responses are governed by a single locus. LPS has a variety of physiological effects on mice. It has immunogenic, mitogenic, pyrogenic, and toxic properties and affects the distribution of neutrophils and monocytes (references 3, 10, 17, and 18, and footnote 2). Since C3H/HeJ mice have been reported to show defects in many such LPS-induced responses (3, 9, 17, 18), it is necessary to analyze the linkage of immune and mitogenic responses in backcross animals to other LPS-induced responses to determine if other responses are also regulated by the locus we have

detected. There may be a single mutation affecting the surface structure of many kinds of cells in C3H/HeJ mice that alters the interaction of cells with LPS.

Summary

In vivo immune responses and in vitro mitogenic responses to bacterial lipopolysaccharides (LPS) have been compared in strains of C3H mice. C3H/HeJ spleen cultures did not support mitogenic responses to LPS and in vivo these mice produce low IgM responses to LPS. On the basis of these two responses, C3H/HeJ mice have been termed low LPS responders. All other strains of C3H mice tested (C3HeB/FeJ, C3H/DiSn, C3H/Str, CWB, CSW, and C3H/Sf and its *H-2* congenics) are high LPS responders supporting large in vitro mitogenic and in vivo immune responses to LPS. The immune response difference between low and high LPS responders is a quantitative one. IgM responses are observed in C3H/HeJ mice in the range of 1.0–10 μg LPS. At lower and higher LPS concentrations, immune responses are not observed. In contrast, high LPS responders elicit LPS immune responses over a much wider dose range (0.1–200 μg). The ability to respond well to LPS is dominant as shown by the response of F_1 hybrid mice of low responder and high responder strains. The linkage relationships of mitogenic and immune responsiveness to LPS have been investigated in backcross (C3H/HeJ × CWB)F_1 × CWB mice. All mice that gave in vivo immune responses to LPS also supported mitogenic responses to LPS. The defect in C3H/HeJ mice that limits mitogenic and immune responsiveness to be due to a single autosomal gene which is not linked to the *H-2* histocompatibility or heavy-chain allotype loci.

We wish to express our appreciation to Mr. B. Skidmore and Dr. J. Chiller for their constant and generous discussion of the details of their own unpublished work with these strains of mice during the course of this study.

Received for publication 11 July 1974.

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