GENETIC CONTROL OF ENDOTOXIC RESPONSES IN MICE*

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Endotoxin, the lipopolysaccharide (LPS)[1] obtained from the cell wall of gram-negative bacteria, elicits many physiological responses in mammals (1). The large number and diversity of these endotoxic reactions to LPS have made it difficult to analyze the control mechanisms involved. In particular, it has been impossible to determine whether most or all of these diverse responses to LPS are regulated by the expression of common genetic loci. In this paper we compare the genetic control of a number of endotoxic responses which appear to result from the interaction of LPS with B lymphocytes in mice, to responses involving different cell types.

The C3H/HeJ mouse strain is unique in its resistance to many of the effects of LPS observed in other mouse strains (2–9).[2] These include resistance to the mitogenic, polyclonal, and adjuvant effects of LPS (4–9),[2] which all appear to result from the interaction of LPS with bone marrow-derived (B) lymphocytes (4, 5), as well as to a variety of other effects such as toxicity (3), resistance to bacterial infections (10), macrophage activation (10, 11), and increases in levels of various serum components such as colony-stimulating factor (CSF) (12), and the acute phase serum amyloid protein, SAA (13). LPS elicits several different types of responses in murine B lymphocytes. LPS induces the nonproliferative expression of cell surface antigens in more immature B-cell types (14, 15), and a mitogenic response in more mature B-cell types (2–9).[2] B lymphocytes from C3H/HeJ mice are unresponsive in both of these LPS response assays (16). The implication from all of these observations is that the defective gene in C3H/HeJ mice that limits LPS responsiveness in these various assay systems is involved in the expression of a receptor found in many different cell types.[2] However, there are no genetic studies that directly show that the loci responsible for the defective LPS responses of different cell types in C3H/HeJ, are in fact identical.

The low responsiveness to LPS of C3H/HeJ mice is determined by a single gene linked to *Mup-1* on chromosome 4 in mice.[2] We have proposed the locus symbol *Lps* with the mutant allele of C3H/HeJ designated Lps^d (defective response) and the normal allele of other strains designated Lps^n (normal response). We have used the locus *Ps* as a marker to determine the location of *Lps* on chromosome 4. The segregation of

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[1] *Abbreviations used in this paper:* BC, backcross; CSF, colony-stimulating factor; *Lps*, LPS response locus; LPS, lipopolysaccharide; *Mup-1*, major urinary protein locus; RI, recombinant inbred; *SAA*-serum amyloid A protein.

[2] Watson, J., K. Kelly, M. Largen, and B. A. Taylor. 1978. The genetic mapping of a defective LPS response gene in C3H/HeJ Mice. *J. Immunol.* In press.

phenotypes for *Mup-1*, *Lps*, and *Ps* markers in a backcross showed *Lps* is located between the *Mup-1* and *Ps* loci. Our estimate of the distance between *Mup-1* and *Lps* is a value of 0.06 ± 0.02 recombination units.[2]

Thus far, these genetic studies have dealt with the expression of *Lps* in responses involving B lymphocytes (9).[2] The major problem in comparing the genetic control of LPS responses in B lymphocytes to responses resulting from the interaction of LPS with other cell types, involves performing more than one type of LPS response in individual animals.

In this paper we utilize another genetic marker, *Mup-1*, closely linked to *Lps* on chromosome 4, to analyze the expression of the *Lps* locus. We examine three responses induced by LPS in 12 recombinant inbred strains of mice derived from C3H/HeJ and C57BL/6J parental strains (9),[2] and in a backcross linkage analysis. The first response is the fluctuation in body temperature. A hypothermia is induced by LPS when mice are placed in an environment below 25°C, which is in contrast to the hyperthermia observed when the temperature is greater than 30°C (17). The second response involves a serum CSF which stimulates granulocyte and macrophage colony formation by mouse bone marrow cells in agar cultures (18). LPS does not elevate serum CSF levels in C3H/HeJ mice as in other strains of mice (12, 18). The third LPS response examined is the production of the serum precursor SAA (19) of the secondary amyloid protein AA (20). In contrast to what is observed in other strains, LPS does not elevate SAA in C3H/HeJ mice (13). We show that the expression of *Mup-1* is a useful marker for analyzing the *Lps* locus when multiple LPS response assays are performed by using single mice. The data show that the expression of *Lps* in different cell types regulates the initiation of LPS responses.

Materials and Methods

Mice. C3H/HeJ and C57BL/6J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. The backcross mice used in this study were obtained from female (C57BL/6J × C3H/HeJ) F_1 × C3H/HeJ mice. Recombinant inbred (RI) strains derived from the C3H/HeJ and C57BL/6J progenitor strains are described elsewhere (9). The strains used were BXH-2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 14, and 19. C57BL/6J mice have been designated Lps^n, and C3H/HeJ as Lps^d.[2]

Major Urinary Protein. Urine samples from individual mice were examined by polyacrylamide gel electrophoresis as detailed elsewhere.[2] C3H/HeJ carry the $Mup\text{-}1^a$ allele whereas C57BL/6J mice possess the $Mup\text{-}1^b$ allele (9).[2]

Mitogenic Responses. Escherichia coli K235 LPS prepared by a phenol water extraction procedure (21) was generously provided by Abbott Laboratories, N. Chicago, Ill. Spleen cells were cultured with 10 μg/ml LPS, and the incorporation of radioactive thymidine was measured after 48 h, as detailed elsewhere (9).

Hypothermal Responses. Mice were kept in an environment at 22°C. Rectal temperatures were recorded by using a microthermometer (°F). Temperatures were determined in individual mice before injection of 50 μg *E. coli* K235 LPS intraperitoneally. After 1 and 2 h, rectal temperatures were recorded. The rectal probe was inserted at a distance of approximately 1 cm, and the temperature recorded 20–30 s later.

Colony-Forming Cells. Colonies containing granulocytes and macrophages were grown by using mouse bone marrow cells cultured in a semisolid agar medium (22). Female C57BL/6J mice were used as the source of bone marrow cells. Femora were removed and the marrow cells flushed from the medullary cavity with Eagle's medium containing 10% fetal calf serum. The semisolid medium was prepared at 45°C and contained 80% Eagle's medium, 15% fetal bovine serum, and 5% trypticase soy broth (Difco Laboratories, Detroit, Mich.), and 0.3% washed agar

which had been boiled for 10 min to give a solution. 1 ml of this cell mixture was layered into 35 mm × 12-mm plastic dishes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) containing a sample of mouse serum, and allowed to solidify. This solid basal layer was then layered with 1 ml of the above agar mixture containing 10^5 nucleated bone marrow cells. The cultures were incubated at 37°C in a humidified incubator. After 8 days, macrocolonies were counted (22).

To determine CSF activity, individual mice were injected intraperitoneally with 10 μg *E. coli* K235 LPS. After 6 h, serum samples were prepared from mice (18). These serum samples were individually assayed for their colony-stimulating activity at a final concentration of 2% in the assay mixture.

Radioimmunoassay for SAA. Mice were immunized with 50 μg *E. coli* K235 LPS intraperitoneally and bled after 18 h from the orbital sinus. Serum aliquots (10 μl) were incubated with formic acid at 37°C or 24 h, diluted with distilled water, frozen, and lyophilized before being assayed in duplicate on plastic microtitration plates coated with affinity purified antibodies to mouse amyloid A protein (13).

Results

Hypothermia in C3H/HeJ Mice. The data summarized in Table I compare the expression of the *Lps* and *Mup-1* loci of C3H/HeJ mice and 12 BXH strains, to changes in body temperature observed after injection of LPS. Individual mice were given a single injection of 50 μg *E. coli* K235 LPS, and the rectal temperature recorded 2 h later. C3H/HeJ mice, when kept in an environment of 22°C, have a body temperature of 99 ± 1°F. Little change in this temperature is observed after injection of 50 μg LPS (Table I). The data in Table I also show the expression of *Mup-1* in these various strains. There is complete concordance in the expression of *Mup-1* and *Lps* in these strains. All LPS responder strains express the alleles $Mup\text{-}1^b$ and Lps^n, whereas all LPS nonresponder strains express the alleles $Mup\text{-}1^a$ and Lps^d (Table I).

The data obtained by using these RI strains indicate that the expression of *Lps* is required for the hypothermia induced by LPS in mice, and that *Mup-1* may be useful as a marker for the *Lps* locus. This is confirmed in a backcross linkage analysis, where we have examined the correlation between the expression of *Mup-1* and LPS-induced hypothermia. We have first typed 20 backcross (C3H/HeJ × C57BL/6J) F_1 × C3H/HeJ mice for *Mup-1*, and then assayed the hypothermal response of these mice to a single injection of 50 μg K235 LPS. These data are shown in Fig. 1. 11 of the 20 backcross mice were $Mup\text{-}1^a/Mup\text{-}1^a$ homozygotes and did not show significant fluctuations in body temperature in response to an injection of 50 μg LPS. Eight backcross mice were $Mup\text{-}1^a/Mup\text{-}1^b$ heterozygotes and exhibited a hypothermal response, after injection of 50 μg LPS. One mouse, a $Mup\text{-}1^a/Mup\text{-}1^b$ heterozygote, did not show a hypothermal response to LPS and we presume it is a recombinant phenotype (Fig. 1). In this hypothermal response assay, the expression of $Mup\text{-}1^a$ correlates well with LPS unresponsiveness, and $Mup\text{-}1^b$ with LPS responsiveness.

Serum Levels of CSF. It has been shown that the injection of LPS into mice causes an elevation in the capacity of the serum (CSF) to stimulate granulocyte and macrophage colony formation by mouse bone marrow cells in agar culture (18). It has also been shown that LPS does not elicit changes in CSF levels in C3H/HeJ mice, due to a defective autosomal gene (12). The serum levels of CSF in mice from 12 BXH strains have been assayed 6 h after a single injection of LPS (Table I). The Lps^d strains showed low CSF activity, similar to what is

TABLE I
Responses to LPS in RI Strains

Strain	*Lps*	*Mup-1*	Temperature	CSF
BXH-2	d	a	98.2	22 ± 6
BXH-3	d	a	98.4	38 ± 12
BXH-4	d	a	99.4	44 ± 10
BXH-5	n	b	95.6	138 ± 32
BXH-6	d	a	99.4	18 ± 6
BXH-7	d	a	99.6	42 ± 8
BXH-8	d	a	99.0	36 ± 9
BXH-9	d	a	98.8	28 ± 6
BXH-11	n	b	94.2	90 ± 22
BXH-12	d	a	99.4	42 ± 12
BXH-14	n	b	94.8	174 ± 44
BXH-19	n	b	95.2	122 ± 26
C3H/HeJ	d	a	99.8	30 ± 10
C57BL/6J	n	b	94.2	164 ± 20

Two alleles have been found for *Lps*. These have been designated Lps^n and Lps^d (9). The alleles for *Mup-1* are $Mup\text{-}1^a$ and $Mup\text{-}1^b$. C57BL/6J mice are Lps^n, $Mup\text{-}1^b$ whereas C3H/HeJ mice are Lps^d and $Mup\text{-}1^a$. These data represent the results obtained from individual mice. These experiments have been performed with at least two mice from each of the RI strains.

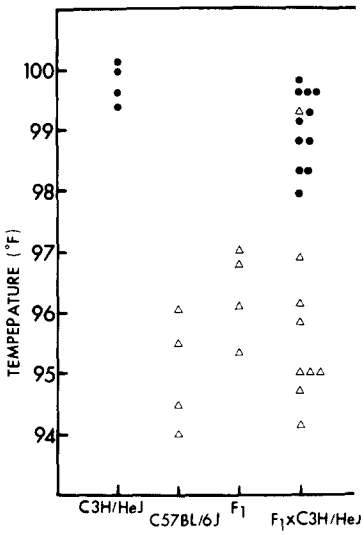

FIG. 1. Effect of LPS on hypothermia in backcross (C3H/HeJ × C57BL/6J) F_1 × C3H/HeJ mice. Individual mice kept in an environment at 22°C were injected i.p. with 50 μg *E. coli* K235 LPS and the rectal temperature recorded 2 h later. (●), $Mup\text{-}1^a/Mup\text{-}1^a$ homozygotes; (△), $Mup\text{-}1^a/Mup\text{-}1^b$ heterozygotes.

observed in C3H/HeJ mice, while the four Lps^n BXH strains (5, 11, 14, 19) showed levels which were five to eightfold higher than in C3H/HeJ mice, similar to levels observed in C57BL/6J mice treated with LPS (Table I).

A group of 20 (C3H/HeJ × C57BL/6J) F_1 × C3H/HeJ backcross mice were typed for the expression of *Mup-1*, and then injected with 10 μg *E. coli* K235

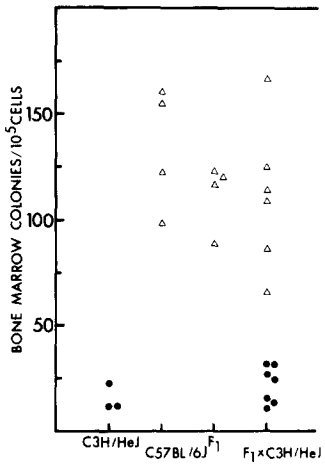

FIG. 2. Effect of LPS on serum levels of a colony stimulating factor in backcross (C3H/HeJ × C57BL/6J) F_1 × C3H/HeJ mice. Individual mice were injected i.p. with 10 μg *E. coli* K235 LPS. After 6 h serum samples were prepared and assayed in mouse bone marrow cultures for the stimulation of colony formation as described in Materials and Methods. (●), *Mup-1*^{a}/*Mup-1*^{a} homozygotes; (△), *Mup-1*^{a}/*Mup-1*^{b} heterozygotes.

LPS. After 6 h, serum samples were prepared and assayed in vitro for CSF activity. 10 of the backcross mice were *Mup-1*^{a}/*Mup-1*^{b} heterozygotes and showed high levels of CSF activity (Fig. 2). Eight mice were *Mup-1*^{a}/*Mup-1*^{a} homozygotes and showed low levels of CSF activity, similar to levels observed in C3H/HeJ mice. Two mice were presumed recombinants, being *Mup-1*^{a}/*Mup-1*^{b} heterozygotes, but exhibiting no change in serum CSF in response to LPS (Fig. 2). Again there was a correlation between the expression of *Mup-1* and LPS responsiveness.

To confirm this, we have analyzed LPS responsiveness in the same group of backcross mice in a second assay. The backcross mice were then left for 3 wk after the injection of LPS, and assayed for mitogenic responsiveness to LPS. These data are presented in Table II. Backcross mice that showed elevated serum levels of CSF after injection with LPS, supported in vitro mitogenic responses to LPS, these mice all possessing the *Mup-1*^{a}/*Mup-1*^{b} phenotype. The backcross mice that did not respond to LPS by increases in serum CSF (Fig. 2), did not support mitogenic responses to LPS (Table II). These include the eight mice with the *Mup-1*^{a}/*Mup-1*^{a} phenotype, as well as the two presumed recombinants with the *Mup-1*^{a}/*Mup-1*^{b} phenotype. Therefore LPS responsiveness in the mitogenic assay corresponds exactly with responsiveness in the CSF assay, supporting two conclusions. The expression of *Mup-1* is a useful genetic marker for the *Lps* locus, and, the expression of *Lps* is involved in the initiation of LPS responses in both the cell type that produces CSF and the cell type that supports a mitogenic response.

Effect of LPS on Levels of SAA. The data presented in Table III summarize the effect of LPS on serum levels of protein SAA in mice from the RI strains. Individual mice were injected intraperitoneally with 50 μg *E. coli* K235 LPS, and serum samples were prepared 18 h later. The BXH strains 2, 3, 4, 6, 7, 8, 9, 11, and 12 show low levels of SAA, comparable to the levels observed in

TABLE II

Correlation of Mitogenic, Hypothermal, and Serum CSF Responses to LPS to the Expression of Mup-1 in Backcross Mice

Mup-1	Mitogenic response*	
	High	Low
Mup-1$^{a/a}$	–	8
Mup-1$^{a/b}$	10	2

* Individual backcross (C3H/HeJ × C57BL/6J) F_1 × C3H/HeJ mice were typed for *Mup-1*, and assayed for serum CSF responses to LPS (data shown in Fig. 2). The mice were left for 3 wk and then assayed for their mitogenic responses in culture to 1 μg K235 LPS as described in Materials and Methods. High mitogenic responses denote spleen cultures with a stimulation index greater than sixfold above control cultures which lack LPS. Low mitogenic responses denote spleen cultures with a stimulation index less than twofold.

TABLE III

Expression of Lps and the Elevation of SAA Levels by E. coli K235 LPS in RI Strains

Strain	Lps	SAA (μg/ml)
BXH-2	d	<2
BXH-3	d	4
BXH-4	d	13
BXH-5	n	687
BXH-6	d	<2
BXH-7	d	8
BXH-8	d	2
BXH-9	d	6
BXH-11	n	47
BXH-12	d	4
BXH-14	n	828
BXH-19	n	743
C3H/HeJ	d	<2
C57BL/6J	n	401

Individual mice from each strain were injected i.p. with 50 μg *E. coli* K235 LPS. After 18 h serum samples were prepared. The SAA concentrations were determined in a radioimmune assay (Materials and Methods). The assays using all BXH strains except BXH-11 have been performed by using two mice, and the results were clear. The assay using the BXH-11 strain has been repeated in three mice, and the results have been consistently low. Although limited numbers of mice have been assayed from each of the BXH strains, the data obtained has been very reproducible.

C3H/HeJ mice. While the BXH-11 strain expressed the *Lps*^{n} allele, all other BXH strains in this group possess the *Lps*^{d} allele (Table III). The BXH strains 5, 14, and 19, all *Lps*^{n}, show significant increases in SAA, comparable to the response observed in the C57BL/6J mice.

A backcross linkage analysis was performed to compare the segregation of the *Lps* locus with the effect of LPS on SAA levels. A group of 20 backcross

(C3H/HeJ × C57BL/6J) F_1 × C3H/HeJ mice were first typed for *Mup-1*. These data are shown in Table IV. Individual mice were then injected intraperitoneally with 50 μg *E. coli* K235 LPS, serum samples prepared 18 h later, and the levels of SAA determined by a radioimmune assay (Table IV). Seven of the nine backcross mice which had the *Mup-1*^{a}/*Mup-1*^{b} phenotype showed SAA levels greater than 400 μg/ml. Two heterozygotes showed low SAA levels (BC-14 and 15). All mice that typed with the *Mup-1*^{a}/*Mup-1*^{a} phenotype showed low levels of SAA (<100 μg/ml). We have also examined the backcross mice for mitogenic responsiveness to LPS. Mice were left for a period of 3 wk after the initial injection of LPS, sacrificed, spleen cultures prepared from each mouse and tested for their mitogenic responses to LPS (Table IV). In seven of nine *Mup-1*^{a}/*Mup-1*^{b} heterozygotes, there was an exact correlation between SAA responses to LPS *in vivo* and mitogenic responses in vitro (Table IV). One backcross *Mup-1*^{a}/*Mup-1*^{b} heterozygote (BC-14) showed mitogenic responsiveness to LPS in vitro, but SAA levels in vivo were low after injection of LPS (Table IV). The phenotype of this backcross mouse is very similar to that observed for BXH-11 mice in response to LPS, namely responsiveness in the mitogenic assay, but exhibits little change in vivo in SAA levels. Another backcross animal (BC-15) typed *Mup-1*^{a}/*Mup-1*^{b} is a presumed recombinant, as it showed no LPS responsiveness in either the mitogenic or SAA assays (Table IV). In contrast, all homozygotes (*Mup-1*^{a}/*Mup-1*^{a}) failed to respond to LPS in either of the response assays.

Discussion

The injection of LPS into mice elicits a wide range of cellular and metabolic responses which are similar to the endotoxic reactions observed during infection with gram-negative bacteria. The diversity of the endotoxic reactions and the variety of cell types that appear to be involved have made it difficult to dissect the regulatory mechanisms involved. In this paper we have made use of the C3H/HeJ mouse strain to examine several aspects of the genetic control of LPS responses. We demonstrate that the responses of several different cells types to LPS are regulated by a common genetic locus *Lps*, and that the expression of a linked locus, *Mup-1*, can be used as a marker in genetic analyses of LPS responses (23, 24).

The data presented in this paper concern the effect of the expression of *Lps* on three types of LPS-induced responses: hypothermia (17), an increase in serum levels of CSF (12, 18) and in serum amyloid A protein (13). All recombinant inbred strains that express *Lps*^{d} (BXH-2-4, 6-9, 12), do not respond to an injection of LPS by a hypothermia (Table I), or by increases in serum levels of either CSF (Table I) or SAA (Table III). The *Lps*^{n} strains (BXH-5, 11, 14, and 19) all show hypothermal and CSF responses to LPS (Table I). In three of these RI strains, BXH, 5, 14, and 19, LPS induced large increases in SAA levels (Table III). However, one *Lps*^{n} strain, BXH-11, shows only a slight SAA response to LPS (Table III), which may reflect the involvement of a genetic locus in the synthesis of SAA which is distinct from *Lps*. This locus is apparently altered in BXH-11 mice.

A backcross linkage analysis has been used to examine the involvement of *Lps* in those same three LPS-induced responses. F_1 (C3H/HeJ × C57BL/6J)

TABLE IV
Effect of E. coli K235 LPS on Levels of SAA in Backcross (BC) (C57BL/6J × C3H/HeJ) F_1 × C3H/HeJ Mice

Mouse	Sex	Mup-1	SAA	Mitogenic Response
			μg/ml	*Δ cpm*
C3H/HeJ	♂	a	13	300
	♂	a	129	600
C57BL/6J	♂	b	660	5,600
	♂	b	553	6,700
F_1	♂	a/b	349	3,580
F_1	♂	a/b	281	3,200
BC-1	♂	a/b	600	3,500
BC-2	♂	a	8	950
BC-3	♂	a	5	400
BC-4	♂	a/b	467	2,400
BC-5	♂	a	9	960
BC-6	♂	a	8	−300
BC-7	♂	a/b	671	4,234
BC-8	♂	a	66	−200
BC-9	♂	a/b	376	7,814
BC-10	♂	a/b	418	12,379
BC-11	♂	a/b	591	11,535
BC-12	♀	a	15	−500
BC-13	♀	a/b	401	4,000
BC-14	♀	a/b	119	7,983
BC-15	♀	a/b	4	1,021
BC-16	♀	a	5	217
BC-17	♀	a	36	498
BC-18	♀	a	4	419
BC-19	♀	a	3	362
BC-20	♀	a	2	475

BC mice were typed for the expression of *Mup-1* and then injected i.p. with 50 μg *E. coli* K235 LPS. After 18 h serum samples were prepared and assayed for SAA levels as described previously. The mice were left 3 wk and then assayed individually for mitogenic responsiveness to LPS in spleen cultures. The mitogenic response data has been reported as the difference in radioactive thymidine incorporation between duplicate control cultures lacking LPS, and duplicate cultures treated with 1 μg/ml *E. coli* K235 LPS (Materials and Methods). An increase of more than 1,000 cpm is considered a mitogenic response.

mice were backcrossed to C3H/HeJ mice and the individual progeny examined in a number of ways. First, backcross progeny with a homozygous *Mup-1^a/Mup-1^a* phenotype, did not show either hypothermal responses to an injection of LPS (Fig. 1), or increases in serum CSF (Fig. 2), or SAA (Table IV). However, backcross progeny with heterozygous *Mup-1^a/Mup-1^b* phenotypes were LPS responders in each of the three assays (Figs. 1, 2; Table IV). Several recombinant phenotypes were observed, as would be expected upon our estimation of the recombination frequency between *Mup-1* and *Lps*.[2] Thus the expression of *Mup-1* appears to be an accurate genetic marker for the linked locus, *Lps*. To verify this, we have kept mice for 3 wk after a single injection of LPS and subsequent analysis of CSF or SAA levels (Fig. 2, Table IV), and then assayed mitogenic responses to LPS in spleen cultures. The mitogenic response

we know to be a direct assay of the expression of the *Lps* locus.[2] With the exception of several presumed recombinant backcross progeny, all *Mup-1*^{a}/*Mup-1*^{a} mice were *Lps*^{d}, whereas *Mup-1*^{a}/*Mup-1*^{b} mice were *Lps*^{n} (Tables III and IV). Therefore, there was concordance in the expression of *Mup-1* and *Lps* in each LPS response assay.

It appears reasonable to conclude that these data provide direct evidence for the expression of *Lps* in different cell types. Many of the immunologic effects of LPS appear to result from its mitogenic activity on B lymphocytes (2–9),[2] while the responses examined in this paper would appear to be due to the interaction of LPS with other cell types. While the mechanism controlling hypothermia in mice is unknown, it is a reasonable assumption that it does not involve B lymphocytes (17). CSF appears to be produced from monocytic cells (12, 18), thus the LPS-induced elevation of serum CSF levels may result from the interaction of LPS with monocytes. SAA is the precursor of the secondary amyloid protein AA whose cell of origin is unknown. It is an acute phase protein whose concentration rises several hundredfold within hours of an acute inflammatory stimulus (13). The association between a rise in SAA and a rise in neutrophil leucocyte count has been noted in human studies (25). Since almost all diseases which cause secondary amyloidosis are characterized by recurrent episodes of fever, neutrophil leukocytosis and SAA elevation, it is of interest that a single gene in the mouse controls the response of these three characteristics to a bacterial cell wall product.

We therefore suggest that the interaction of LPS with the product of the *Lps* locus in different cell types, is involved in the initiation of many of the diverse biological effects elicited by LPS. The number and variety of endotoxic reactions to LPS in mice is bewildering (1). A simplified view would be that *Lps* is required for the synthesis of a functional receptor unit for LPS, found in many cell types. Endotoxic reactions are initiated by the interaction of LPS with this receptor. We emphasize the concept of a functional receptor unit since the LPS receptor, if indeed there is a specific receptor for LPS, might not be the gene product of the *Lps* locus. The product of the *Lps* locus could be a membrane structural protein or an enzyme responsible for transmitting an intracellular signal after LPS binding. The diversity of physiologic responses resulting from this initial interaction is a reflection of the range of specialized cell types that are stimulated to some response. The use of *Mup-1* as a genetic marker will be highly useful as a means of associating an LPS response reaction with the expession of the locus *Lps* in experimental situations where it is not possible to type animals directly for the expression of *Lps* in a second LPS response assay. Knowledge of the product of the *Lps* locus in mice may lead to the development of metabolic inhibitors to block the harmful effects of an endotoxic response in a number of species.

Summary

A number of altered immunologic responses to lipopolysaccharide (LPS) in C3H/HeJ mice result from the expression in B lymphocytes of a defective genetic locus, termed *Lps*. *Lps* has been mapped to chromosome 4 between two loci, *Mup-1* and *Ps*. As it is difficult to type individual mice for LPS responsiveness in more than one type of assay, we have utilized *Mup-1* as a genetic

marker to correlate LPS responses in mice to the expression of the *Lps* locus. Three nonlymphoid responses to LPS have been examined in 12 recombinant inbred strains of mice and in a backcross linkage analysis, and are all regulated by the expression of the *Lps* locus. These responses are hypothermal changes in body temperature, and the elevation in serum levels of a colony stimulating factor and the precursor of the secondary amyloid protein AA. Therefore, the initiation of LPS responses in different cell types in mice involve the expression of a common locus. These linkage studies provide a means for analyzing the genetic control of many of the diverse reactions of the endotoxic response to LPS.

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