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Inheritance of Lipopolysaccharide-Enhanced Nonspecific Resistance to Infection and of Susceptibility to Endotoxic Shock in Lipopolysaccharide Low-Responder Mice

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In a previous study, we demonstrated that lipopolysaccharide (LPS) and other bacterial immunostimulants, in contrast to their activity in a closely related high-responder subline, failed to elicit nonspecific resistance in LPS low-responder mice against *Klebsiella pneumoniae* infection. To investigate the type of inheritance controlling the LPS-induced nonspecific resistance to infection, the present study was performed in low- and high-responder C3H sublines and in F_1 and F_2 hybrids. In addition, F_1 mice were backcrossed to each parental type. Inheritance of susceptibility to endotoxin was also tested in both sublines and their hybrids and backcross progeny. For these latter assays, mice were previously adrenalectomized because removal of this gland considerably enhances their sensitivity. Our present findings are consistent with the hypothesis that LPS enhances nonspecific resistance to infection and that susceptibility to endotoxin shock in the absence of corticoids may be determined by a single autosomal dominant gene.

Lipopolysaccharides (LPS) extracted from gram-negative organisms are potent immunogens and elicit a number of other immunological responses in mice. For example, they are nonspecific mitogens for spleen B-derived lymphocytes (1, 19) and adjuvants (10, 12), and they can enhance nonspecific resistance to infections (9, 14, 21). In the C3H/HeJ subline, which is more resistant to the lethal effect of endotoxin (11), a number of host responses to LPS have been shown to be under genetic control; e.g., these mice were more resistant to toxic shock (24, 28), adjuvant activity (22, 27), and mitogenicity of LPS (7, 26, 30). We have recently demonstrated that LPS fails to elicit nonspecific resistance in these mice against *Klebsiella pneumoniae* infection (3). Moreover, we observed that although endotoxin extracted by trichloroacetic acid and a mycobacterial preparation was mitogenic in this low-responder subline, both of these immunostimulants were still unable to protect C3H/He mice against an infectious challenge (3).

In the present study, the effect of *Corynebacterium granulosum* on *Klebsiella* and *Listeria* infections was compared in this mouse strain, which is genetically deficient in its responses to LPS, and in a very responsive related subline. Other experiments reported here were undertaken in order to investigate the type of inheritance controlling the LPS-induced nonspecific resistance to infection. Assays in adrenalectomized low- and high-responder mice were also performed to evaluate the inheritance of susceptibility to endotoxic shock, since it is well established that the susceptibility of mice to LPS is dramatically enhanced in the absence of glucocorticoids (2, 4). In addition, reciprocal breeding was conducted to produce F_1 and F_2 progeny, and F_1 mice were backcrossed to each parental type. Our results provide evidence consistent with the hypothesis that these responses may be determined by a single autosomal dominant gene.

MATERIALS AND METHODS

Mice. Five- to 6-week-old C3H/He Orl and C3HeB/Fe Orl mice were obtained from the Centre National de la Recherche Scientifique (Orléans, France). Previous experiments have shown that C3H/He Orl mice, obtained from Jackson Laboratories (Bar Harbor, Me.), have retained their unresponsiveness to the mitogenic activity of LPS (3) and that there is no significant mixed lymphocyte reaction with cells from the high-responder C3HeB/Fe subline. (C3HeB/Fe × C3H/He)F_1 and F_2 hybrids and backcrosses to each parental strain were bred at the Pasteur Institute. (C57Bl/6 × AKR)F_1 hybrids used as LPS high-responder controls were also bred in our animal facilities.

The following abbreviations are used in this paper: eB for C3HeB/Fe Orl; He for C3H/He Orl; (eB × He)F_1 for their F_1 hybrids; and F_2 for their F_2 progeny. In addition, F_1 mice were backcrossed to each

parental type and will be referred to as (F_1 × eB) and (F_1 × He). The (C57Bl/6 × AKR)F_1 hybrids will be referred to as C6AK.

Preparations. LPS was extracted from *Salmonella enteritidis* (Danysz strain) by the phenol-water procedure. Interphase material (IPM) was obtained from *Mycobacterium smegmatis* according to a method described earlier (13). *C. granulosum*, strain 5196 of the Pasteur Institute, was heat killed, and amounts were expressed as dry weight.

Infectious challenge. The *K. pneumoniae* strain was of capsular type 2, biotype d (20). The strain of *Listeria monocytogenes* was given to us by R. M. Fauve. Mice were infected intravenously with a 16-h culture. Survival was recorded after 15 days. In vivo viable counts were performed as usual by plating the blood and organ suspensions, the blood volume being estimated as previously described (18).

In vivo distribution of labeled LPS. Radioactive endotoxin was obtained after incubation with $Na_2{}^{51}CrO_4$ for 48 h at 37°C. Autoradiography on specific lines of precipitation formed in agar demonstrated the effectiveness of labeling according to a method previously described (6). The rate of blood clearance was evaluated by sacrificing mice 30 min after an intravenous injection of 10 μg of ^{51}Cr-labeled LPS. Samples of blood and organs were measured separately, the total blood volume being estimated as described previously (4, 6).

Toxicity measurements. The comparative toxicity of LPS given intravenously was established in different mouse strains and hybrids 48 h after bilateral adrenalectomy, as described elsewhere (2). After preliminary approximate evaluation, toxicity was measured by injecting five serial threefold dilutions in groups of at least eight mice. Mortality was recorded 48 h after the challenge, and the 50% lethal dose (LD_{50}) was calculated according to the method of Reed and Muench.

Statistical analysis. The adjusted chi-square method (23) was used to determine the statistical significance of differences between the observed and expected frequencies in hybrid experiments.

RESULTS

Influence of LPS and *C. granulosum* on nonspecific resistance to infection of LPS low- and high-responder mice and their F_1 hybrids. In the following experiments, C6AK, eB, He, and (eB × He)F_1 hybrid mice were used, and their resistances to an intravenous challenge with 4 × 10^4 *K. pneumoniae* were compared. Treated groups received either 1 μg of LPS or 100 μg of IPM 1 day previously or 300 μg of *C. granulosum* 7 days previously. *C. granulosum* was also administered to C6AK, He, and (eB × He)F_1 mice 7 days before challenge with 1.4 × 10^6 *L. monocytogenes*. All controls received apyrogenic saline intravenously under the same conditions as the treated groups.

Table 1 shows the following: in the case of *Klebsiella* challenge, (i) all the untreated controls and the low-responder mice treated by LPS, IPM, or *C. granulosum* died in approximately 24 h; (ii) LPS and IPM protected very effectively the high-responder C6AK and eB mice as previously observed, and also the (eB × He)F_1 hybrids; and (iii) whereas *C. granulosum* protected C6AK and eB mice, a very slight delay of mortality was only observed in the case of the (eB × He)F_1 hybrids. In contrast, *C. granulosum* protected very effectively all mice challenged with *Listeria*, including the LPS low-responder subline, and also the F_1 hybrids.

TABLE 1. *Influence of LPS, IPM, and C. granulosum on nonspecific resistance to infection in LPS high- and low-responder mice and their F_1 hybrids*

Infection[a]	Mouse strain	Pretreatment[b]	Survival at day 15
4 × 10^4 *Klebsiella pneumoniae*	C6AK		0/8
		LPS	8/8
		IPM	7/8
		Carynebacterium granulosum	7/8
	eB		0/8
		LPS	8/8
		IPM	8/8
		C. granulosum	6/8
	He		0/8
		LPS	0/8
		IPM	0/8
		C. granulosum	0/8
	(eB × He)F_1		0/16
		LPS	13/16
		IPM	14/16
		C. granulosum	0/16
1.4 × 10^6 *Listeria monocytogenes*	C6AK		2/16
		C. granulosum	15/16
			0/16
	He		
		C. granulosum	13/16
	(eB × He)F_1		0/16
		C. granulosum	12/16

[a] Mice were infected intravenously.

[b] 1 μg of LPS or 100 μg of IPM at day −1, or 300 μg of *C. granulosum* at day −7.

Inheritance of unresponsiveness to the influence of LPS on blood clearance of viable *K. pneumoniae*. Since nonspecific resistance to *Klebsiella* infection could be enhanced by LPS in F_1 hybrids like in the high-responder parents, and since there was a correlation between immunity and blood clearance (5, 17), the inheritance of unresponsiveness was studied in experiments performed in C6AK, eB, He, and (eB × He)F_1 hybrid mice. All treated groups received 1 μg of LPS 24 h previously, whereas the controls received nonpyrogenic saline. The data presented in Table 2 were obtained by plating samples of blood, liver, and spleen 5 h after inoculating 4 × 10^4 *K. pneumoniae*.

TABLE 2. *Influence of LPS on blood clearance of viable K. pneumoniae by high- and low-responder mice and their F_1 hybrids*

Mouse strain	No. of mice	No. of living bacteria		
		Total[a] ($\times 10^{-4}$)	In blood (%)	In liver (%)
C6AK				
Controls	6	159.6 ± 65 (5.3)	70.3 ± 1.2	15.7 ± 0.7
LPS treated	6	14.9 ± 5 (1.8)	42.6 ± 11.4	39.4 ± 8.3
eB				
Controls	11	176 ± 98 (5.5)	62.3 ± 14.2	12.1 ± 7.9
LPS treated	11	37.7 ± 47 (3.2)	42.9 ± 17.8	28.7 ± 9.5
He				
Controls	11	1,319.8 ± 696 (8.4)	93.2 ± 3.5	0.1 ± 0.4
LPS treated	14	1,980 ± 1,476 (8.9)	95 ± 2	0.1 ± 0.3
(eB × He)F_1				
Controls	11	410.3 ± 154 (6.7)	72 ± 7.2	10.7 ± 3.2
LPS treated	22	36.2 ± 38 (3.1)	48.4 ± 10.1	22.4 ± 7.4

[a] Organisms recovered from blood, liver, and spleen 5 h after inoculation of 4×10^4 *K. pneumoniae*, expressed as mean ± standard deviation. Value in parentheses represents number of bacterial divisions between time of inoculation and termination of experiment.

The total number of viable organisms recovered shows that, as previously reported, C6AK and eB mice were stimulated by pretreatment with LPS. Approximately 37.7×10^4 microorganisms were recovered in the LPS-treated high responders instead of 176×10^4 in the nontreated controls. As was reported earlier, the total number of organisms found in He nontreated controls was greater, indicating the higher sensitivity of the low-responder subline to this infection. Moreover, LPS stimulation had no effect on bacterial proliferation, thus confirming that endotoxin did not increase resistance of low responders (3). However, the total numbers of *K. pneumoniae* recovered from the untreated (eB × He)F_1 hybrids were comparable to those of their high-responder parents, and they were also very well stimulated by LPS. The great similarity between the pattern observed in the F_1 hybrids and in the high-responder parents was confirmed by the percentage of bacteria found in the liver. After LPS stimulation, a significant increase was observed in the F_1 progeny, and the percentages were similar to those of the high-responder mice. In the low-responder parent, almost all the organisms were recovered in the blood even after LPS treatment (Table 2).

The data obtained from individual eB and He mice were grouped according to the range of the total number of organisms recovered from LPS-stimulated mice. Although the following variations were found, 5.1×10^4 to 145.2×10^4 in eB mice, in no case was there any overlap with low-responder values (560×10^4 to $6,250 \times 10^4$ in He mice). In view of these results, the genetics of this response was analyzed by studying the effect of 1 μg of LPS under similar conditions in both parental lines, F_1 and F_2 hybrids, and backcrosses.

The F_1 progeny responded with values very similar to those of the high-responder parent, indicating dominance of the high-responder phenotype. Backcrosses of F_1 hybrid mice to the low-responder parent, irrespective of the particular sex combination, clearly segregated in 62 and 38% of low and high responses (instead of 50%), whereas backcrosses to the high-responder parent behaved uniformly like the F_1 hybrids and high responders (Table 3). Although greater numbers, particularly in the F_2 generation, are required for definitive analysis, our results can be reasonably interpreted in favor of dominance of the high-responder phenotype. This dominance in the F_1 hybrids could also be demonstrated by blood clearance of ^{51}Cr-labeled LPS.

Responsiveness of F_1 hybrids on blood clearance of ^{51}Cr-labeled LPS. It is well established that small amounts of LPS render mice refractory to a later challenge by a lethal dose. During tolerance to endotoxin, the second injection is cleared very rapidly from the circulation, as can be seen by the use of labeled endotoxin (4, 6). Therefore, rapid removal of endotoxin has been correlated with increased resistance.

In the following experiments, blood clearance of ^{51}Cr-labeled LPS was measured in C6AK, eB He, and (eB × He)F_1 hybrid mice. Percentages of injected amounts of radioactivity were measured in the blood, liver, and spleen 30 min after an intravenous injection of 10 μg of ^{51}Cr-labeled LPS. All treated groups received 1 μg of cold LPS by the same route 1 or 14 days before injection of the radioactive material. The con-

trols received an injection of apyrogenic saline under the same conditions.

In LPS-treated low-responder mice, ^{51}Cr-labeled LPS was not removed from the blood, since the levels of radioactivity were similar to those of the untreated controls (Table 4). In contrast, in all other treated groups, including the F_1 hybrids, the levels of radioactivity were smaller in the blood and greater in the liver than in their respective untreated controls.

Therefore, blood clearance experiments of viable organisms or of labeled endotoxin clearly demonstrate that the F_1 hybrids uniformly responded with values similar to those of the high-responder parent, indicating the dominance of the high-responder phenotype. Similar results were obtained when the responses were measured by evaluating the increased susceptibility to LPS of adrenalectomized mice.

Inheritance of unresponsiveness of adrenalectomized mice to LPS toxicity. It has been previously reported that HeJ mice are 20- to 80-fold more resistant to the lethal effect of endotoxin (11, 24, 28) and that this resistance is under polygenic control (24). We have recently observed that a much greater difference can be detected between this strain and its congenic subline if mice have been adrenalectomized (3).

Toxicity of LPS was evaluated in mice adrenalectomized 2 days previously, since under these conditions various strains become approximately 20,000-fold more susceptible to endotoxins (2). Thus in C6AK mice the LD_{50} of *S. enteritidis* LPS was 0.015 μg after adrenalectomy versus 400 μg before the operation.

In the following experiments, *S. enteritidis* LPS was injected in C6AK, eB, He, and (eB × He)F_1 hybrid mice. In each case, five dosage levels of LPS were administered to groups of six to eight mice, and cumulative results of two or three comparable experiments are reported (Table 5). The susceptibility to the lethal effect of LPS in adrenalectomized eB mice appeared to be of the same order as that seen in C6AK adrenalectomized controls. In contrast, in the low-responder mice the LD_{50} value was 3,500-fold greater than in the related subline. In view of these findings, toxicity measurements in (eB × He)F_1 hybrids clearly indicated that their susceptibility to the lethal activity of LPS was similar to that of the high-responder parent (Table 5).

Since there were such marked differences, the genetics of endotoxin toxicity in adrenalec-

TABLE 4. *Responsiveness of F_1 hybrids on blood clearance of ^{51}Cr-labeled LPS*

Mouse strain	Pretreatment[a]	No. of mice	Levels of radioactivity[b]	
			Blood (%)	Liver + spleen (%)
C6AK		10	47.1 ± 3.2	28.5 ± 3.2
	LPS, day −1	10	19.8 ± 3.9	59.8 ± 3.1
	LPS, day −14	10	17.2 ± 3.8	62.2 ± 5.7
eB		5	54.1 ± 0.9	19.2 ± 1.3
	LPS, day −1	5	21.8 ± 0.5	50.2 ± 3.4
He		6	60.3 ± 7.1	22.1 ± 5.2
	LPS, day −1	6	57.1 ± 5.3	21 ± 5.4
	LPS, day −14	6	67.3 ± 3.1	13.3 ± 3.8
(eB × He)F_1		6	55.8 ± 5.6	19.2 ± 5.7
	LPS, day −1	6	21 ± 3.2	64.5 ± 6.3
	LPS, day −14	6	20.8 ± 9.9	64.7 ± 9.6

[a] 1 μg of cold *S. enteritidis* LPS intravenously.

[b] Percentages of injected amount of radioactivity 30 min after intravenous injection of 10 μg of ^{51}Cr-labeled *S. enteritidis* LPS. Results are expressed as mean ± standard deviation.

TABLE 3. *Inheritance of unresponsiveness to the influence of LPS on blood clearance of viable K. pneumoniae*

Mouse strain[a]	No. of mice	Total no. of living bacteria[b] (× 10^{-4})	Responsive pattern (%)[c]			
			Low		High	
			Observed	Expected	Observed	Expected
eB	8	55.3 ± 52.1	0	0	100	100
He	10	1,628.6 ± 829.8	100	100	0	0
(eB × He)F_1	22	36.2 ± 37.8	0	0	100	100
(F_1 × eB)	10	63 ± 18.5	0	0	100	100
(F_1 × He)	21 { 13	1,307.4 ± 447	62	50	38	50
	21 { 8	67.8 ± 50.4				
F_2	9 { 3	1,323 ± 561	33	25	66	75
	9 { 6	26.2 ± 16.7				

[a] All mice received 1 μg of LPS the day before infection.

[b] Total number of organisms recovered from blood, liver, and spleen 5 h after inoculation of 4 × 10^4 *K. pneumoniae*. Results are expressed as mean ± standard deviation.

[c] Except in the case of the F_2 hybrids, which were insufficient in number for statistical analysis, the observed responses were not statistically different from the expected frequencies ($P > 0.85$).

tomized mice was analyzed. Responses to LPS were studied in F_1 and F_2 hybrids and in both F_1 backcrosses. To establish the type of response obtained in hybrid progeny, all mice were adrenalectomized and received uniformly 1 μg of LPS, which represents approximately 50 LD_{50} for both very susceptible high-responder and F_1 hybrid mice, whereas the same dosage is approximately 50-fold lower than the LD_{50} of the refractory low-responder, adrenalectomized mice.

As expected, all low responders survived whereas all high-responder parents were killed after 1 μg of LPS. In the same fashion, 21 out of 22 F_1 hybrids and all the backcrosses to the high responders were killed. As before, backcrosses of the F_1 hybrids to the low-responder parent segregated in approximately 50%, whereas F_2 hybrids segregated in approximately 25% of low responders (i.e., were refractory to endotoxin) and 75% of high responders. Although a different dosage schedule and greater numbers (particularly in the F_2 generation) may be required for definitive statistical analysis, these results suggest the dominance of the high-responder phenotype as measured by susceptibility to endotoxin after adrenalectomy (Table 6).

TABLE 6. *Inheritance of unresponsiveness of adrenalectomized mice to LPS toxicity*

Mouse strain	Mortality[a]	Response pattern (%)[b]			
		Low		High	
		Observed	Expected	Observed	Expected
eB	18/18	0	0	100	100
He	0/18	100	100	0	0
(eB × He)F_1	21/22	5	0	95	100
(F_1 × eB)	15/15	0	0	100	100
(F_1 × He)	12/28	57	50	43	50
F_2	14/20	30	25	70	75

[a] Recorded 48 h after intravenous injection of 1 μg of LPS.

[b] The observed responses were not statistically different from the expected frequencies ($P > 0.85$).

DISCUSSION

We previously reported that LPS failed to elicit nonspecific resistance in low-responder C3H/He mice against *K. pneumoniae* infection. Moreover, two other immunostimulants, such as endotoxin extracted by trichloroacetic acid and a mycobacterial preparation, were also unable to protect C3H/He mice against infection, although both preparations had a strong mitogenic activity in the low-responder subline (3). In our present study, *C. granulosum* was also unable to protect LPS low responders against *Klebsiella*. It must be noted, however, that *C. granulosum* enhanced very effectively the resistance of these mice against a *Listeria* challenge.

In view of these results, the capacity of these immunostimulants to enhance the resistance to infection of the F_1 progeny was tested. When treated by *C. granulosum*, the F_1 hybrids reacted like their low-responder parents since they were protected against *Listeria*, whereas their resistance was not significantly enhanced against *Klebsiella*. In contrast, the F_1 progeny responded like the high-responder parent when treated by LPS before *Klebsiella* challenge.

TABLE 5. *LPS toxicity in adrenalectomized high- and low-responder mice and their F_1 hybrids*

Mouse strain	LPS LD_{50}[a] (μg)
C6AK	0.015
eB	0.020
He	70
(eb × He)F_1	0.021

[a] LD_{50} of *S. enteritidis* LPS by the intravenous route.

The dominance of the high-responder phenotype in the F_1 progeny was also demonstrated in experiments involving (i) blood clearance of viable *Klebsiella* organisms, (ii) blood clearance of ^{51}Cr-labeled LPS, and (iii) susceptibility of adrenalectomized mice to endotoxin. In view of these results, the genetics of the responses of adrenalectomized mice to LPS toxicity was analyzed in F_1 and F_2 hybrids and in backcrosses with F_1 hybrids. The influence of LPS on blood clearance of viable *K. pneumoniae* was also studied in the hybrid progeny, since in both these systems there were very clear-cut differences between the high and low responders. In both assays, F_1 hybrids and their backcrosses to the high-responder parent reacted to LPS like the eB parent. In contrast, backcrosses to the low-responder parent segregated in approximately 50% of each parental phenotype, whereas F_2 hybrids segregated in approximately 25% of low responders and 75% of high responders. In the toxicity studies, there remains the possibility that different levels of resistance could have been found in the backcrosses, and particularly the F_2 generation, if minimum lethal dosages rather than 50 LD_{50} had been used. However, dominance of the high-responder phenotype was apparent in the F_1 hybrids, since different levels of endotoxin were used in their case.

Therefore, nonspecific resistance to infection, as measured by survival and blood clearance experiments, seems to indicate that the differ-

ence in response to LPS is more likely to be determined by a single autosomal dominant gene. It could be argued that if a smaller dose of LPS, instead of the 1 μg that was uniformly injected, had been used, intermediate responses could have been observed that were not detectable under the experimental conditions of our assays. It must be recalled that there is not a good dose-response relationship between LPS stimulation and infectious challenge. Thus almost identical responses were obtained when 1 μg of LPS was administered before 10^3, 10^5, or 10^7 *Klebsiella* (16). It must also be remembered that 1 μg, which was ineffective in low-responder mice, represents a very high dose since 0.01 μg of LPS protects very effectively most strains of mice against 10^5 *Klebsiella* (17). It must be noted, however, that in the case of *C. granulosum*, the responses of the F_1 hybrids were similar to those of the low-responder parent, which was protected against *Listeria* but not against a *Klebsiella* challenge. Therefore, a greater susceptibility of the F_1 hybrids and of the He parent to the latter organism could partially explain a lesser protective effect of a given immunostimulant.

The genetic control of several immune responses to LPS of C3H/HeJ mice has been variously interpreted. For Coutinho et al. (8) and Sultzer (25), F_1 progeny spleen lymphocytes incubated with LPS showed intermediate levels of deoxyribonucleic acid synthesis with no evidence of dominance. According to these investigators, the data obtained by them in hybrids and backcrosses suggest the existence of two codominant alleles controlling the mitogenic responsiveness to LPS. These results, however, stand in contrast to those reported by Watson and Riblet (30), since in their experiments F_1 hybrids were high responders, and backcross progeny from F_1 to C3H/HeJ segregated in high and low responders. These authors concluded that a single autosomal dominant gene controls LPS mitogenicity (30). Talcott et al. obtained results consistent with this postulate in a study involving LPS enhancement of antibody response to a synthetic polypeptide (27).

Our present findings concerning inheritance of susceptibility to endotoxin agree with the latter interpretation. However, more recently Watson et al. have confirmed the codominant inheritance of B-derived lymphocyte activation by LPS (J. Watson, K. Kelly, and M. Largen, *in* D. Schlessinger, ed., *Microbiology—1977*, in press). Therefore, our findings suggest the involvement of another cell type, perhaps the macrophage, in which the gene is expressed as an autosomal dominant trait. Thus the LD_{50} experiments in adrenalectomized mice clearly demonstrate that the responses of F_1 hybrids are not intermediate but are very similar to those of the high-responder subline, which is 3,500-fold more susceptible to endotoxin. Assays using F_2 and F_1 backcrosses also indicated that the susceptibility to endotoxin shock appears to be inherited as a fully dominant trait. It is noteworthy that survival of adrenalectomized mice after challenge by 1 μg, and of course higher dosages, of LPS is unique. Repeated assays have shown us that with various LPS preparations extracted from smooth gram-negative strains, the LD_{50} in adrenalectomized mice invariably is between 0.01 and 0.05 μg (instead of 300 and 600 μg in intact controls). This was found consistently for different inbred strains or even for Swiss common-stock mice. For example, by examining several experiments made over a long period of time, we observed that 10 mice out of 284 were not killed by a challenge of 0.1 μg of endotoxin, which represents the highest dosage level routinely used in those tests. Our present results contrast with what has been previously reported in non-adrenalectomized C3H/He mice. An intermediate susceptibility to endotoxic shock in F_1 and F_2 animals was recorded, suggesting a pattern typical of polygenic control (24). The presence of the adrenal glands could therefore influence in some way the expression of this phenotype.

In agreement with our earlier findings, the data reported here favor the view that C3H/He mice have a major defect of their nonspecific resistance to infection that renders them refractory to various unrelated immunostimulants. Independent studies have also shown that the C3H/HeJ strain is refractory to the adjuvant activity of *Bordetella pertussis* (15). Do low responders possess counteracting mechanisms enabling them to overcome their important immunological handicap? For instance, HeJ mice are resistant to *B. pertussis*-induced histamine sensitization (29). Moreover, a unique and extremely pronounced refractoriness to LPS toxicity in the absence of endogenous corticoids can be shown by adrenalectomy in the low-responder subline. It must be recalled that endotoxin (administered under different conditions) has been shown to generate a negative phase, decreasing the host's resistance to infection (9). Therefore, one is tempted to speculate whether the low toxicity of LPS in C3H/He mice could constitute a selective advantage compensating for the inherited immune defects previously recognized. Further studies of genetic association between the various responses to LPS and other bacterial agents should provide a useful tool to investigate mechanisms of nonspecific immunity.

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