Dissociation of Innate Susceptibility to *Salmonella* Infection and Endotoxin Responsiveness in C3HeB/FeJ Mice and Other Strains in the C3H Lineage

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Studies of various mouse strains in the C3H lineage have shown that there is no correlation between innate susceptibility to *Salmonella* infection and sensitivity to the toxic or mitogenic effects of lipopolysaccharide (LPS). C3H/HeNCrIBR mice were *Salmonella* resistant, but sensitive to the toxic and mitogenic effects of LPS, whereas C3H/HeJ mice were *Salmonella* susceptible, but LPS unresponsive. In contrast, C3HeB/FeJ mice were as *Salmonella* susceptible as the C3H/HeJ mice, yet were mitogenically responsive to LPS and sensitive to its lethal effects. Furthermore, other mouse strains (C3H/HeTex and C3H/HeDub) displayed intermediate susceptibility to *Salmonella* infection and were responders to the mitogenic and toxic effects of LPS. These results are interpreted to mean that endotoxemia cannot be a major factor in the pathogenesis of *Salmonella* infection and provide evidence for the involvement of multiple factors in the control of innate resistance to *Salmonella* infection in mice of the C3H lineage.

The discovery of the unresponsiveness of the C3H/HeJ mouse to the toxic and mitogenic effects of phenol-water-purified lipopolysaccharide (PW-LPS) (7, 23, 24, 29) has raised the question of the relationship of this unresponsiveness to the innate susceptibility of this and other mouse strains to *Salmonella* infection. The relationship is of interest for discerning whether these two phenomena are under the same type of genetic control and for clarifying the role of endotoxin in the pathogenesis of *Salmonella* infections. Several laboratories have shown that C3H/HeJ mice are hypersusceptible to *Salmonella* infection (14, 17, 30), whereas other mouse strains in the C3H lineage, such as C3H/f, C3H/DiSn, C3H/HeN, C3H/St, and C3H/BiMA, are resistant, but respond normally to the mitogenic and toxic effects of PW-LPS (14, 30). These observations have led to the suggestion that there is an inverse relationship between endotoxin responsiveness and *Salmonella* susceptibility (14, 30), a hypothesis which is consistent with the work of Hill et al., who dissociated susceptibility to *Salmonella* infection from endotoxin lethality by selective breeding (10).

Responsiveness to the toxic and mitogenic effect of LPS is believed to be determined by a pair of autosomal codominant genes (25) located

at a single locus on chromosome 4, designated as *Lps* (31). Nonresponders, such as the C3H/HeJ mouse, are homozygous for the defective allele and are designated *Lps*^d, whereas responders are normal and are designated *Lps*ⁿ (31). Genetic studies designed to map the locus controlling *Salmonella* susceptibility in C3H/HeJ mice have suggested that it may be closely linked to, or identical with, the *Lps* locus (14). It is noteworthy, however, that evidence for multiple alleles or genes controlling LPS responsiveness in mice of the C3H lineage has also been reported (6).

While studying the relationship of susceptibility to *Salmonella* infection and responsiveness to endotoxin, we found that several mouse strains in the C3H lineage are highly susceptible to *Salmonella* infection, yet respond normally to LPS, thus providing several examples in the C3H lineage in which these phenotypic traits do not show an inverse relationship.

MATERIALS AND METHODS

Mice. C3H/HeJ and C3HeB/FeJ mice were purchased from Jackson Laboratories, Bar Harbor, Maine. C3H/HeNCrIBR and CD-1 mice were purchased from Charles River Breeding Laboratories, Wilmington, Mass. C3H/HeDub mice were obtained from Flow Laboratories, Dublin, Va. C3H/HeTex

mice were purchased from Timco Breeding Laboratories, Houston, Tex., and C3H/BiMA mice were purchased from M. A. Bioproducts, Walkersville, Md. All mice, unless specified, were females weighing 19 to 21 g. Mice were acclimatized for 1 week in our mouse room before use.

Bacterial strains. *Salmonella typhimurium* strain W118-2 was used for experimental infection in all of these studies. This organism has been used extensively by our laboratory in previous studies of CD-1 and C3H/HeJ mice (1, 5). The 50% lethal dose (LD₅₀) values for intraperitoneal infection in different mouse strains were tested and are given in Table 1.

LD₅₀ determinations. A lyophile of strain W118-2 was rehydrated with 1 ml of brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.), and a loopful was streaked onto a blood agar plate, which was incubated overnight at 37°C. Several typical colonies were picked and inoculated into 10 ml of brain heart infusion broth. The culture was grown for 3 h at 37°C. The top 5 ml was inoculated into 50 ml of brain heart infusion broth and grown on a shaker at 37°C for 1.5 h. Bacterial numbers were standardized, using Petroff-Hausser counts, on bacteria suspended in 5% paraformaldehyde to stop motility. Organisms were serially diluted in sterile saline (Cutter Laboratories, Berkeley, Calif.), and the desired numbers were injected intraperitoneally in a volume of 0.5 ml. Six or ten mice were used per dilution. The actual number of organisms injected was calculated from duplicate spread plates on blood agar. LD₅₀ values were calculated based on cumulative mortality at 30 days postinfection.

Bacterial preparations. Trichloroacetic acid-extracted LPS (TCA-LPS) was prepared from *S. typhimurium* W118-2 cells as previously described (26). Subsequently, the TCA-LPS was extracted by the phenol-water method to yield endotoxin protein (EP) and an LPS fraction (PW-LPS) which contained less than 1% protein (26). For measurement of endotoxin toxicity, PW-LPS (lot no. 1604064) prepared from *S. typhimurium* was purchased from Difco Laboratories (Detroit, Mich.).

Mitogenicity assay. Spleens of three mice were teased together in cold RPMI 1640 medium with L-glutamine, pH 7.2 (M. A. Bioproducts), to which had been added a penicillin-streptomycin mixture (M. A. Bioproducts; 100 U of penicillin and 100 µg of streptomycin per ml, final concentration). Cells were transferred to conical centrifuge tubes, and large clumps were allowed to settle for 10 min. The supernatant cell suspension was collected and centrifuged at 1,000 rpm for 10 min in an International centrifuge. The cell pellet was suspended in the same RPMI medium to a final leukocyte concentration of 2×10^6 viable cells per ml. Assays were carried out in Linbro flat-bottomed, 96-well plates (Flow Laboratories, McLean, Va.). To each well was added 20 µl of mitogen suspended in medium, or medium alone for the background counts. Each mitogen was tested in triplicate. To each well was added 200 µl of the cell suspension to yield a final concentration of 4×10^5 cells per well. The plates were covered and incubated at 37°C for 42 h in a humidified atmosphere with 5% CO₂. At 42 h, 0.25 µCi of [³H]thymidine (Amersham Corp., Arlington Heights, Ill.) was added to each well in a volume of 10 µl, and the plates were reincubated for 6 h. The

contents of each well were harvested onto glass-fiber filter strips with a Bellco Microharvester (Bellco Glass, Inc., Vineland, N.J.). Filter-paper disks cut from the strips were placed in scintillation vials to which was added 3.5 ml of scintillation fluid (1 gallon [3.785 liters] of toluene plus 15.2 g of PPO [2,5-diphenyloxazole] and 190 mg of POPOP [1,4-bis-(5-phenyloxazolyl)benzene]). Vials were counted in a scintillation counter for 1 min each. Counts per minute for the triplicate cultures were averaged. The stimulation index was calculated by dividing mean counts for mitogen-stimulated cultures by mean background counts.

Bacterial count assay. Groups of 42 C3H/HeJ and 42 C3HeB/FeJ male mice and a group of 48 C3H/HeNCrIBr mice were each infected intravenously with 3×10^3 log-phase cells of strain W118-2 suspended in 0.1 ml of sterile saline. The inoculum was estimated by Petroff-Hausser counts and verified by duplicate spread plates of appropriate dilutions on blood agar. On the desired days postinfection, groups of six mice were killed by cervical dislocation, and their livers and spleens were removed and weighed separately. Each liver or spleen was individually placed in a Potter-Elvehjem homogenizer with a close-fitting Teflon pestle. Sterile water was added to each organ to yield a final volume of 10 ml. Organs were thoroughly homogenized by using a motor-driven tissue grinder to turn the pestle. The undiluted homogenate or appropriate dilutions were plated on Levine eosin-methylene blue agar (Difco) and incubated overnight at 37°C. Plates with optimal numbers of organisms were counted, and the total number of organisms per organ was calculated.

C3b receptor-mediated ingestion by macrophages. Peritoneal cells were collected from mice injected subcutaneously 24 h previously with either 1 or 20 µg of phenol-water-extracted *S. typhimurium* LPS (Sigma Chemical Co., St. Louis, Mo.) or an equal volume (0.2 ml) of sterile pyrogen-free saline. The cells were collected by intraperitoneal injection of 2 ml of cold Ca²⁺- and Mg²⁺-free phosphate-buffered saline containing 10 U of heparin per ml. Harvested cells were centrifuged at 1,000 rpm at 4°C, resuspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum, and layered onto 12-mm glass cover slips

TABLE 1. Intraperitoneal LD₅₀ values of *S. typhimurium* W118-2 in various mouse strains

Mouse strain	Breeder	LD ₅₀ (no. of cells)
C3H/HeJ	The Jackson Laboratory	<7
C3HeB/FeJ	The Jackson Laboratory	<2
C3H/HeNCrIBr	Charles River Breeding Laboratories	1.2×10^3
C3H/BiMA	M. A. Bioproducts	2.1×10^3
C3H/HeDub	Flow Laboratories	59
C3H/HeTex	Timco Breeding Laboratories	55
CD-1	Charles River Breeding Laboratories	1.0×10^4

(Bellco) which had been placed in the wells (16 mm) of tissue culture plates (Linbro Scientific, Inc., Hamden, Conn.). The final cell density was 10^5 macrophages per cover slip, as confirmed by counting cells microscopically with an ocular grid. The cells were incubated at 37°C in a humidified 5% CO₂ environment for 1 h, removed, and immersed with gentle agitation in warm RPMI 1640 medium to remove nonadherent cells, and the cover slips were placed in fresh tissue culture wells. The number of adherent cells identified as macrophages was 96% to 98% by morphological criteria, latex ingestion, and nonspecific tissue staining.

Preparation of C3b-coated erythrocytes was performed by the method of Bianco et al. (2). Washed sheep erythrocytes were reacted with rabbit anti-erythrocyte immunoglobulin M (IgM) (Cordis, Miami, Fla.) and incubated with a C5-deficient source of complement (fresh DBA/2 serum) to yield antibody and C3b-coated erythrocytes, or E(IgM)C3b. One milliliter of a 0.5% suspension of E(IgM)C3b in RPMI 1640 medium was placed over each macrophage-coated cover slip and cultured for 1 h at 37°C. The cover slips were removed, and the E(IgM)C3b which were not internalized were lysed for 5 s in a hypotonic solution. The macrophage monolayers were then Giemsa stained and examined microscopically. Ingestion was quantified by direct microscopic counts of ingested cells. Results were expressed as the ingestion index, which equals the product of the percentage of macrophages which ingested E(IgM)C3b times the average number of E(IgM)C3b ingested per 100 macrophages (2). Controls, in which macrophages were incubated with erythrocytes or with IgM anti-erythrocyte antibody, were carried out simultaneously to assure that ingestion was mediated by the C3b receptor. Little or no ingestion occurred without addition of complement.

RESULTS

Susceptibility of various mouse strains to *Salmonella* infection. Table 1 shows the LD₅₀ values for *S. typhimurium* W118-2 in six different mouse strains in the C3H lineage. All C3H/HeJ mice succumbed at a dose as low as seven cells. Tests on groups of C3HeB/FeJ mice, in which one group received 10 cells, the second received 4 cells, and the third received 2 cells, showed that all animals succumbed by day 25 of the test. The mean time to death of mice receiving two cells intraperitoneally was 13.6 days. Thus, the C3HeB/FeJ mouse, like the C3H/HeJ mouse, is hypersusceptible to *Salmonella* infection, with a theoretical lethal dose approaching a single cell. C3H/HeNCrIBR and C3H/BiMA mice were approximately 1,000-fold more resistant to the same organism, with LD₅₀ values of approximately 1,000 cells. In contrast, C3H/HeDub and C3H/HeTex mice had LD₅₀ values of about 50 cells.

Mitogenicity. These six mouse strains were each tested for the ability of their spleen cells to incorporate [³H]thymidine in vitro in response to TCA-LPS, PW-LPS, or endotoxin protein.

The data presented in Table 2 show that in contrast to the C3H/HeJ mouse, which is a poor responder to PW-LPS, the C3HeB/FeJ mouse responds normally to this and the other mitogens. Thus, no correlation is evident between the ability to give a mitogenic response to various LPS preparations and the innate susceptibility to *Salmonella* infection.

Sensitivity to the toxic effects of LPS. Table 3 shows the mortality data for groups of six mice receiving graded doses of PW-LPS. As expected from previous reports in the literature, the C3H/HeJ mouse is exceptionally resistant to the lethal effects of LPS, with 50% toxic dose values (TD₅₀) greater than 2,000 µg. The other mouse strains have TD₅₀ values between 500 and 250 µg, with the exception of the C3HeB/FeJ mouse, which is significantly more sensitive. All C3HeB/FeJ mice succumbed at the 250-µg dose. Thus, the C3HeB/FeJ mice is hypersusceptible to *Salmonella* infection, sensitive to the toxic effects of PW-LPS, but mitogenically responsive to this substance (Table 4).

In vivo growth kinetics. Figure 1 compares the growth kinetics of strain W118-2 injected intravenously into male C3H/HeJ, C3HeB/FeJ, and C3H/HeNCrIBR mice. As previously reported by Hormaeche (11), organisms inoculated into the susceptible C3H/HeJ mice showed an early "fast" growth rate, whereas the C3H/HeNCrIBR mice evidenced a "slow" growth rate in the first 5 days postinfection. C3HeB/FeJ mice also had rapid growth of the organisms in the liver and spleen, but the curve was not superimposable on that of C3H/HeJ mice. These kinetics are described quantitatively by the population doubling times shown in Fig. 1. Bacterial counts in the spleens of C3H/HeJ and C3HeB/FeJ mice at 5 days were significantly different ($P \leq 0.05$) from counts in C3H/HeNCrIBR mice as determined by the Student *t* test. Furthermore, at day 7, only 2 of 12 C3H/HeJ mice were alive, but 9 of 12 C3HeB/FeJ mice and 18 of 18 C3H/HeNCrIBR mice survived. Thus, the mortality data also support the conclusion that there is a difference in growth rate of the organism in the three mouse strains.

Ingestion via C3b receptors. To test further whether C3HeB/FeJ mice respond to PW-LPS like the refractory C3H/HeJ animals or like the responder strains, an assay was carried out which measures stimulation of ingestion of E(IgM)C3b by peritoneal macrophages. Peritoneal cells were harvested from mice 24 h after subcutaneous injection of LPS. C3HeB/FeJ mice behaved like the C3H/HeNCrIBR mice and markedly increased their ingestion of E(IgM)C3b in response to a single injection of PW-LPS at either of two doses (Fig. 2). Their response stands in contrast to C3H/HeJ mice,

TABLE 2. Comparison of the capacity of three extracts of *S. typhimurium* W118-2 to stimulate [³H]thymidine uptake in vitro of dissociated spleen cells of various mouse strains in the C3H lineage.

Mitogen	Dose (μg/well)	C3H/HeJ			C3H/HeFeJ			C3H/HeNCrIBR			C3H/BI/MA			C3H/HeDub			C3H/HeTex		
		Mean cpm ± SE ^a	S.I. ^b		Mean cps ± SE	S.I.		Mean cpm ± SE	S.I.		Mean cpm ± SE	S.I.		Mean cpm ± SE	S.I.		Mean cpm ± SE	S.I.	
None		254 ± 7	7		489 ± 89	89		499 ± 62	62		1,211 ± 266	266		455 ± 45	45		366 ± 24	24	
PW-LPS	0.1	403 ± 42	2		20,571 ± 4,839	42		55,034 ± 4,974	110		32,456 ± 1,822	27		32,361 ± 4,285	71		20,781 ± 1,290	57	
	1	738 ± 6	3		32,379 ± 2,633	66		63,054 ± 2,679	126		52,789 ± 4,172	44		33,316 ± 1,088	73		38,509 ± 443	105	
	10	1,518 ± 56	6		37,132 ± 2,838	76		69,388 ± 3,070	139		56,519 ± 3,138	47		42,546 ± 246	94		50,058 ± 938	137	
	100	3,527 ± 288	14		38,487 ± 1,837	79		47,889 ± 418	96		68,686 ± 168	57		33,402 ± 969	73		48,275 ± 1,144	132	
Endotoxin protein	0.1	8,666 ± 956	34		14,394 ± 618	29		24,593 ± 1,022	49		20,564 ± 210	17		17,637 ± 231	39		15,731 ± 945	43	
	1	22,845 ± 616	90		38,994 ± 732	80		57,190 ± 2,333	115		42,697 ± 1,368	35		38,479 ± 913	85		35,471 ± 1,221	97	
	10	29,554 ± 1,931	116		45,444 ± 3,981	93		74,156 ± 1,430	149		60,552 ± 581	50		60,095 ± 1,043	132		53,550 ± 4,469	146	
	100	23,399 ± 1,488	92		39,867 ± 3,583	82		52,349 ± 1,284	105		75,304 ± 16,929	62		36,683 ± 804	81		45,452 ± 2,184	124	
TCA-LPS	0.1	4,688 ± 130	18		16,295 ± 592	33		33,409 ± 1,596	67		19,756 ± 293	16		17,017 ± 1,253	37		18,822 ± 356	51	
	1	16,912 ± 118	67		39,767 ± 3,192	81		64,736 ± 69	130		54,847 ± 122	45		38,992 ± 1,486	86		45,777 ± 652	125	
	10	26,074 ± 952	103		58,436 ± 3,561	120		83,500 ± 1,276	167		63,404 ± 1,442	52		44,760 ± 3,837	98		58,999 ± 643	161	
	100	25,740 ± 827	100		57,386 ± 1,720	117		71,467 ± 1,247	143		53,664 ± 1,500	44		41,582 ± 2,696	91		57,703 ± 2,673	158	

^a Uptake of 0.25 μCi of [³H]thymidine by triplicate cultures of 4 × 10⁵ cultured spleen cells per well.
^b S.I., Stimulation index (mean counts per minute of mitogen well/mean counts per minute of wells without mitogen).

TABLE 3. Toxicity of PW-LPS for various mouse strains in the C3H lineage

Dose of LPS (μ g) ^a	Survival (alive/total) ^b					
	C3H/HeJ	C3HeB/FeJ	C3H/HeNCrIBR	C3H/HeDub	C3H/BiMA	C3H/HeTex
2,000	4/6	ND ^c	ND	ND	ND	ND
1,000	6/6	0/6	0/6	0/6	0/6	0/6
500	6/6	0/6	2/6	0/6	0/6	3/6
250	ND	0/6	6/6	5/6	5/6	6/6
100	ND	6/6	6/6	6/6	6/6	6/6
TD ₅₀ (μ g) ^d	>2,000	250–100	500–250	500–250	500–250	500

^a PW-LPS from *S. typhimurium* (Difco lot no. 1604064) suspended in 0.5 ml of sterile, nonpyrogenic saline (Cutter Laboratories) and injected intraperitoneally.

^b Mortality scored 72 h postinjection.

^c ND, Not done.

^d TD₅₀, Toxic dose for 50% of the animals. Two values indicate range.

which showed no increase in ingestion of E(IgM)C3b.

DISCUSSION

In these studies we used a panel of C3H mouse strains to systematically examine the correlation between their mitogenic responsiveness to LPS, their sensitivity to the lethal and macrophage-modifying effects of LPS, and their innate susceptibility to *Salmonella* infection. The results, as summarized in Table 4, fail to show any consistent relationship between these variables. Thus, the C3H/HeJ and the C3HeB/FeJ mice have over a 10-fold difference in their sensitivities to the lethal effects of LPS. Also, the C3H/HeJ strain is unresponsive to induction of C3b receptors on macrophages, whereas the C3HeB/FeJ strain is responsive. However, both are equally hypersusceptible to *Salmonella* infection, a finding which clearly establishes that reactivity to endotoxin is not a major factor in determining the outcome of an experimental *Salmonella* infection. Secondly, no clear relationship emerges between the ability of various mouse strains to give a mitogenic response to

PW-LPS and *Salmonella* susceptibility. These results contrast with those reported by O'Brien et al. (14), who also tested selected strains in the C3H lineage, but found a correlation between mitogenic responsiveness and *Salmonella* susceptibility. Results with the C3HeB/FeJ mouse provide at least one example of a mouse in the C3H lineage which is mitogenically responsive to LPS, but has an LD₅₀ of less than two cells intraperitoneally. *Salmonella* susceptibility of these two strains has also been reported by Misfeldt and Johnson (12, 13). It is noteworthy that von Jeney et al. (30), in a study comparing the mitogenicity and *Salmonella* susceptibility of a variety of inbred mouse strains, reported that C3H.SW mice were mitogenic responders, but had an LD₅₀ of 80 cells, and that C3H/DiSn mice were also mitogenic responders, with an LD₅₀ of 120 cells. Thus, it appears that there are other mouse strains in the C3H lineage, in addition to the ones we have tested, which show an intermediate susceptibility to *Salmonella* infection as well as mitogenic responsiveness to LPS.

The significance of our results with regard to

TABLE 4. Summary of susceptibility to *S. typhimurium* and endotoxin responsiveness

Mouse strain	<i>S. typhimurium</i> susceptibility ^a	Endotoxin toxicity ^b	Ingestion of E(IgM)C3b ^c	Mitogenicity ^c		
				PW-LPS	Endotoxin protein	TCA-LPS
C3H/HeJ	Sus	R	—	—	+	+
C3HeB/FeJ	Sus	S	+	+	+	+
C3H/HeNCrIBR	Res	S	+	+	+	+
C3H/BiMA	Res	S	ND	+	+	+
C3H/HeDub	Inter	S	ND	+	+	+
C3H/HeTex	Inter	S	ND	+	+	+

^a Sus, Hypersusceptible (LD₅₀ < 10 cells intraperitoneally); Res, resistant (LD₅₀ ≈ 10³ cells intraperitoneally); Inter, intermediate (LD₅₀ ≈ 55 cells intraperitoneally).

^b R, Resistant; S, sensitive.

^c —, Poor responder; +, good responder; ND, not done.

FIG. 1. Growth of *S. typhimurium* strain W118-2 in livers and spleens of three mouse strains of the C3H lineage.

the failure to find a correlation between endotoxin sensitivity and susceptibility to *Salmonella* infection was further explored by examining bacterial proliferation in the C3H/HeJ, C3HeB/FeJ, and C3H/HeNCrIBR mouse strains. The results show that bacteria proliferate to the same extent in both endotoxin-resistant C3H/HeJ and endotoxin-sensitive C3HeB/FeJ strains, reaching lethal levels when the combined bacterial load in the liver and spleen is approximately 10^8 cells. Thus, animals which are more susceptible to the toxic effects of endotoxin do not die with lower numbers of *Salmonella* organisms in their organs, a finding which argues against the hypothesis that endotoxin is a major factor in death from *Salmonella* infection. These results substantiate those of other investigators who have also failed to find a significant role for endotoxin in the pathogenesis of *Salmonella* infection in mice of disparate lineages (22, 29, 30) or in humans (7). An additional observation mitigating against the thesis that endotoxin plays a

substantive role in all gram-negative infections is the finding that C3H/HeJ mice are more resistant to *Escherichia coli* peritonitis than are endotoxin-sensitive A/HeJ mice (27). It is likely that the complexity of the factors determining virulence precludes generalizations about the role of LPS in the pathogenesis of gram-negative infection.

The implications of these results to our understanding of the genetic control of the various parameters measured is of interest. It is generally accepted that in the C3H/HeJ mouse, the defective mitogenic response to LPS and the refractoriness to its toxic effect are controlled by a single locus on chromosome 4, designated *Lps* (31). Other investigators have observed a correlation for strains in the C3H lineage between mitogenic responsiveness (*Lps*ⁿ) and *S. Salmonella* resistance, and conversely, between mitogenic unresponsiveness in C3H/HeJ mice (*Lps*^d) and *Salmonella* susceptibility (14). The findings presented in this paper, that the C3HeB/FeJ

FIG. 2. The capacity of LPS-elicited macrophages to ingest E(IgM)C3b. Macrophages obtained from the peritoneum of mice 24 h after injection of either 1 or 20 μ g of peptone-water-extracted *S. typhimurium* LPS were assessed for their capacity to ingest E(IgM)C3b. The first bars in each group represent C3H/HeJ mice; the second bars (light oblique) represent C3H/HeNCrIBR mice; and the third bars (dark oblique) represent C3HeB/FeJ mice. The broad horizontal zone represents the range of ingestion indices for peritoneal macrophages obtained from all of the mice after the injection of sterile, nonpyrogenic saline.

mouse is *Salmonella* susceptible and that this susceptibility is not associated with defective LPS responsiveness, require that other explanations be sought for the genetic control of innate resistance to this infection in this mouse strain. Two other loci have been described which influence susceptibility to murine salmonellosis. Plant and Glynn have shown that C57BL/6 mice, as well as other strains (BALB/c and DBA/2), carry a single, dominant gene (16), which they have designated *Ity* (J. Plant and A. A. Glynn, Mouse News Lett. 57:38, 1977). O'Brien et al. (14) have shown that CBA/N mice carry an X-linked gene which controls *Salmonella* susceptibility and is apparently the same gene that is involved in B-cell differentiation (15). Since female C3HeB/FeJ mice are hypersusceptible to *Salmonella* infection, the X-linked gene is not considered a factor in our results. We are currently carrying out genetic studies to try to determine whether the *Salmonella* susceptibility of C3HeB/FeJ mice is controlled by a gene closely linked to the *Lps* locus, by the *Ity* gene described above, or by some other locus. The potential complexity of the genetic factors involved is illustrated by the discovery of strains of intermediate susceptibility to *Salmonella* infection, as seen in the C3H/HeDub and C3H/HeTex mice. In our effort to understand the basis for the differences in susceptibility among

the strains tested, information was obtained concerning their genealogy. Personal communication with the various animal suppliers indicated that C3H/HeNCrIBR mice, which are bred by Charles River Breeding Laboratories, originated from C3H/He mice obtained from the National Institutes of Health in 1975. The colony was established from caesarean-delivered animals and kept germfree. Animals were eventually associated with known bacterial flora, but were kept specific pathogen free. Our results with these mice were comparable in all respects to those reported by O'Brien et al. (14) for C3H/He animals, the original stock. The C3H/HeTex colony was also started from C3H/He mice from the National Institutes of Health in 1971 by Timco Breeding Laboratories. Yet these animals show a reduced susceptibility to *Salmonella* infection. The C3H/HeDub mice were acquired by Flow Laboratories in 1968 from C3H/He stock maintained at the Oak Ridge National Laboratory. As noted, they show characteristics similar to those of the C3H/HeTex mice. Thus, spontaneous mutation, or as yet unidentified factors, has resulted in increased susceptibility to *Salmonella* infection in the C3H/HeTex and C3H/HeDub strains, even though they were both bred from C3H/He stock, and similar animals bred by Charles River have retained their innate resistance. In 1948, the C3HeB/FeJ mouse was derived from the C3H/HeJ strain by E. Fekety by ovarian transplant from the C3H/HeJ strain into a C57BL/6 foster mother to eliminate the mammary tumor virus (8). When the C3H/HeJ and C3HeB/FeJ strains diverged in terms of endotoxin responsiveness is not known precisely, but in 1965 Heppner and Weiss (9) reported that C3H/HeJ mice were considerably more resistant to the lethal effects of LPS than were other mouse strains tested. Brooke (3) also reported in 1965 that C3H/HeJ mice were more refractory than CAF₁ mice to the breaking of immunological tolerance by endotoxin. The presence or absence of the mammary tumor virus cannot be an important factor in determining innate susceptibility to *Salmonella* infection, as C3H/HeJ mice carry the agent, whereas C3HeB/FeJ and all of the C3H/He strains do not.

One must always consider the possibility that the observed differences, particularly those between the various C3H/He strains obtained from different suppliers, might be attributable to non-genetic factors such as bacterial flora or diet. Although our experiments cannot conclusively rule out these hypotheses, it is noteworthy that Schneider (19, 20), in a series of studies, reported that although diet could result in a 25% difference in the survival rate of outbred Swiss-Webster mice (19), no effect of diet was notice-

able for highly inbred strains which differed markedly in their natural resistance to *Salmonella* infection (21). Further, he observed that any dietary effect on resistance was transitory, disappearing within 2 days (20). As our mice are acclimatized to our mouse room for 1 week before use, it is probably unlikely that dietary differences are a major factor in the differential susceptibility to infection which we observed. Dubos and Schaedler (4, 18) observed that mice which were caesarean delivered and which lacked *E. coli* and *Proteus* sp. in their intestinal flora were more resistant to endotoxin than were normal mice. We found no difference in endotoxin sensitivity between the C3H/HeCr1BR mice (specific pathogen free) and the C3H/HeDub mice, although they showed marked differences in *Salmonella* susceptibility.

Breeding studies are in progress and will be necessary to unequivocally establish a genetic basis for the differences that we observed in *Salmonella* susceptibility. However, the results presented in this paper do clearly establish that factors other than the *Lps* locus determine innate resistance to *Salmonella* infection in mice of the C3H lineage.

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