Macrophages from Endotoxin-Hyporesponsive (*Lps^d*) C3H/HeJ Mice Are Permissive for Vesicular Stomatitis Virus because of Reduced Levels of Endogenous Interferon: Possible Mechanism for Natural Resistance to Virus Infection

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The C3H/HeJ mouse strain bears an autosomal gene defect, *Lps^d*, which results in a greatly diminished capacity to respond to endotoxin, the ubiquitous lipopolysaccharide derived from the cell walls of gram-negative bacteria. These mice also exhibit greater susceptibility to a variety of viral and bacterial infections than syngeneic, fully lipopolysaccharide-responsive (*Lpsⁿ*) mouse strains and possess macrophages with defects in differentiation which are reversed by treatment with exogenous interferon (IFN). To test directly the hypothesis that C3H/HeJ macrophages are deficient in endogenous IFN levels, macrophages from C3H/HeJ (*Lps^d*) and C3H/OuJ (*Lpsⁿ*) mice were compared for sensitivity to vesicular stomatitis virus. At a multiplicity of infection of 0.1, C3H/OuJ macrophages were completely refractory to infection, whereas C3H/HeJ macrophages were permissive for replication, and infection resulted in 100% cytopathic effect. These findings were confirmed with a second inbred *Lpsⁿ* and *Lps^d* strain pair. Levels of 2',5'-oligoadenylate synthetase were significantly higher in *Lpsⁿ* cells. C3H/HeJ macrophages, derived from bone marrow precursors under the influence of macrophage colony-stimulating factor, shown previously to induce IFN in macrophages, were as refractory as C3H/OuJ macrophages. Exposure of nonpermissive macrophages to anti-IFN- α/β antibody prior to infection rendered cells permissive. Our findings suggest that endotoxin provides a primary stimulus for the maintenance of normal macrophage differentiation and innate resistance via the induction of endogenous IFN by macrophages.

The C3H/HeJ mouse strain bears a single autosomal mutation on chromosome 4, *Lps^d* (40), which renders it highly refractory to the diverse biologic effects of gram-negative endotoxins in vitro (reviewed in references 24, 25, and 37). The failure of this mouse strain to respond to the toxic manifestations of lipopolysaccharide (LPS) appears to stem from a failure of their macrophages to respond to LPS to produce inflammatory products (i.e., interleukin 1 or endogenous pyrogen, tumor necrosis factor, glucocorticoid antagonizing factor, prostaglandins [16, 20, 37]), which under normal circumstances mediate many of the manifestations associated with endotoxemia. To date, neither the product of the normal allele, *Lpsⁿ*, nor the product of the *Lps^d* locus has been isolated or characterized. In addition to their profound hyporesponsiveness to LPS, C3H/HeJ mice differ significantly from their fully endotoxin-responsive counterparts in that they are highly susceptible to a wide variety of viral and bacterial pathogens (reviewed in reference 26), and in several of these studies, susceptibility to infection was genetically mapped to the *Lps^d* allele. Moreover, not only do C3H/HeJ macrophages fail to respond to LPS in vitro, but macrophages derived from this strain exhibit in vitro a significantly lowered differentiation state, as assessed by a decreased capacity to bind and phagocytose immunoglobulin G (IgG)-coated sheep erythrocytes via their Fc receptors (35). The reduced Fc receptor capacity of these macrophages could be greatly augmented by treating cultures with alpha, beta, or gamma interferons (IFN) (5, 36). This observation led us to hypothesize that C3H/HeJ macrophages produce significantly lower endogenous IFN levels than macrophages derived from fully LPS-responsive

strains. Thus, a lowered state of macrophage differentiation (secondary to a reduced level of endogenous IFN) might underlie the observed increase in susceptibility to infection. Increased susceptibility to viral infection has been induced in mice injected with high-titered antibody to IFN- α/β (2, 8-9, 10, 29), suggesting that endogenous levels of IFN may contribute to innate resistance to certain infections. However, it has been clearly pointed out by Gresser (8) that, to date, a naturally occurring genetic model has not been described.

In this report we have demonstrated that peritoneal exudate macrophages from fully LPS-responsive (*Lpsⁿ*) C3H/OuJ mice are refractory to infection with vesicular stomatitis virus (VSV), whereas LPS-hyporesponsive C3H/HeJ macrophages support the replication of VSV. Macrophage sensitivity to VSV was confirmed in a second mouse strain, C57BL/10ScN, which also bears the *Lps^d* allele (31, 39). Basal levels of 2',5'-oligoadenylate (2,5-A) synthetase were significantly higher in the nonpermissive C3H/OuJ macrophages. If the C3H/HeJ macrophages were derived from bone marrow progenitors under the influence of macrophage colony-stimulating factor (CSF-1), shown recently by Moore and his colleagues to induce endogenous production of IFN (21), the cells were as refractory to infection as the *Lpsⁿ* peritoneal exudate macrophages. Resistance to VSV infection could be reversed in either *Lpsⁿ* or CSF-1-derived *Lps^d* macrophages by exposing the resistance macrophages to anti-IFN- α/β antibody in vitro. These findings strongly support the hypothesis that in genetically normal animals natural resistance to a variety of infections is mediated by LPS via the induction of endogenous IFN by macrophages.

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MATERIALS AND METHODS

Culture of macrophages. Female C3H/HeJ (*Lps^d*), C3H/OuJ (*Lpsⁿ*), and C57BL/10J (*Lpsⁿ*) mice, 5 to 6 weeks old, were obtained from the Jackson Laboratories, Bar Harbor, Maine. C57BL/10ScN (*Lps^d*) mice were obtained from the Small Animal Section, National Institutes of Health (NIH). All mice were housed in a laminar flow hood in cages fitted with polyester filter hoods, fed standard lab chow and acid water ad libitum, and used within 2 weeks of receipt. To induce peritoneal exudates, mice were injected intraperitoneally with 3 ml of 3% fluid thioglycolate (TG; Microbiology System, BBL Cockeysville, Md.), and 5 days later macrophage-rich (>85%) exudates were collected by peritoneal lavage. Peritoneal exudates were washed and resuspended in RPMI 1640 (M.A. Bioproducts, Walkersville, Md.) supplemented with 2 mM glutamine, 30 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.2), sodium bicarbonate (3 mg/ml), penicillin (100 U/ml), streptomycin (100 µg/ml), and 2% fetal calf serum (FCS; M.A. Bioproducts). All tissue culture reagents were purchased as endotoxin free (<0.01 ng/ml) and confirmed as such in independent *Limulus* amoebocyte lysate assays. Cells were cultured in 96-well plates (Falcon Plastics, Oxnard, Calif.) at 2×10^5 per well. After an adherence incubation of approximately 4 h, nonadherent cells were removed by repeated washings in complete medium. For measurement of 2,5-A synthetase activity, peritoneal exudate macrophages were cultured at 4×10^6 cells per well in 6-well plates (Falcon).

Bone marrow-derived macrophages were derived by culture of bone marrow progenitor cells in CSF-1, partially purified by ammonium sulfate precipitation and passage over an Affigel 202 column to ensure removal of contaminating IFN- β . The purification scheme for the CSF-1 and the culture of macrophages from bone marrow progenitors in CSF-1 have been described in detail elsewhere (38). Briefly, after 7 days in culture with CSF-1 (~1,000 U/ml), mature, adherent, bone marrow-derived macrophages were removed enzymatically from the flasks with the neutral protease Dispase II. The cells were washed and recultured (in the absence of CSF-1) in the same way as the TG-elicited peritoneal exudate macrophages. All cultures were treated with complete medium (200 µl/well) or complete medium supplemented with a 1:400 final dilution of anti-murine IFN- α/β antibody (NIH reference standard G-024-501-568) or control antibody (NIH reference standard G-025-501-568), as indicated.

Virus infection of macrophage cultures. Macrophage monolayers were infected with VSV as described elsewhere (32). Briefly, at the indicated times after cell culture, culture supernatants were aspirated and replaced with 100 µl of VSV (Indiana strain) per well at a multiplicity of infection (MOI) of 0.1. At the indicated times after infection, culture supernatants were collected and frozen at -70°C until assayed for VSV yield by a modification of the method of McGowan and Wagner (18). Briefly, L929 fibroblasts were grown to confluency in 6-well culture plates (Linbro; Flow Laboratories, McLean, Va). Cells were infected for 1 h at room temperature with 1 ml of virus sample diluted in Eagle minimal essential medium (EMEM) supplemented with antibiotics and 10% FCS. After a 1-h adsorption period, the contents of the wells were aspirated, and the cells were overlaid with 1 ml of phenol red-free EMEM supplemented with sodium bicarbonate, glutamine, antibiotics, 5% FCS, and 1% Noble agar (Difco Laboratories, Detroit, Mich.) per well. Once solidified, the agar-overlaid cultures were incu-

bated at 32°C for 2 days in 5% CO_2 . Plaques were developed by overlaying the wells again with phenol red-free EMEM supplemented with 5% FCS, 1% Noble agar, and neutral red (1:150) for 4 to 6 h at 37°C . Each supernatant was assayed at multiple dilutions in duplicate in at least two separate plaque assays, and unless otherwise indicated, standard deviations associated with plaque assay data were always <10% of the mean.

2,5-A synthetase determination. Peritoneal exudate macrophages were cultured as described above for 48 h. At that time, the cells were washed with Earle balanced salt solution and then incubated on ice for 10 min. Cells were collected by scraping, and postmitochondrial cytoplasmic protein was extracted (27). The concentration of protein was measured by the method of Lowry et al. (15). A modification of the method of Stark et al. (27) was used to determine 2,5-A synthetase activity. Briefly, 0.17 mg of macrophage protein was mixed with poly(I:C)-cellulose (0.15 mg/ml) in a buffer containing 10 mM HEPES (pH 7.5), 50 mM KCl, 1.5 mM magnesium acetate, 7 mM 2-mercaptoethanol, and 28% glycerol (vol/vol). Following a 1-h incubation at room temperature, the cellulose was washed with buffer and incubated for 2 h at 37°C in an assay mixture containing 8 mM ATP (pH 7.0) and 15 mM MgCl_2 (in the same buffer). The quantity of 2,5-A in the supernatants was then determined by the radiobinding assay of Knight et al. (14). The results are expressed as picomoles of 2,5-A synthesized per hour per milligram of protein.

RESULTS

Replication of VSV in macrophages derived from endotoxin-responsive (*Lpsⁿ*) and endotoxin-hyporesponsive (*Lps^d*) mice. Our previous studies had indicated that by 48 h of incubation, peritoneal macrophages derived from endotoxin-responsive (*Lpsⁿ*) mice could bind and phagocytose IgG-coated erythrocytes significantly better than macrophages derived from endotoxin-hyporesponsive (*Lps^d*) mice (35). This defect in Fc receptor-mediated binding and phagocytosis was shown to be directly related to a paucity of Fc receptors and could be fully overcome by treatment of the defective (C3H/HeJ) macrophages with exogenous IFNs (5, 36). To test the hypothesis that the C3H/HeJ macrophage differentiation defect was related to lower endogenously produced IFN levels, peritoneal exudate macrophages from C3H/OuJ (*Lpsⁿ*) and C3H/HeJ (*Lps^d*) mice were cultured for 48 h and then compared for their ability to support replication of VSV. At an MOI of 0.1, the LPS-responsive (C3H/OuJ) macrophages were nonpermissive for VSV (Fig. 1A). In contrast, when LPS-hyporesponsive C3H/HeJ macrophages were comparably infected, virus was produced and released maximally by 16 h postinfection. Complete destruction of the cell monolayer was observed (either macroscopically in fixed and stained wells [Fig. 1B] or microscopically), in contrast to the total lack of cytopathic effect (CPE) observed in the C3H/OuJ macrophage monolayers. Comparable macrophage sensitivity to VSV infection was observed in cultures derived from C57BL/10ScN mice, which, like the C3H/HeJ strain, bear the *Lps^d* allele (31, 39). Macrophages derived from the fully endotoxin-sensitive (*Lpsⁿ*) counterpart of this strain, the C57BL/10J strain, were refractory to VSV infection (Fig. 1C).

The effect of time in culture prior to infection was also examined, since our previous studies had indicated that C3H/HeJ macrophages continue to lose Fc receptor capacity with time in culture (35). The sensitivity of C3H/HeJ mac-

FIG. 1. Replication of VSV by C3H/OuJ (*Lpsⁿ*) and C3H/HeJ (*Lps^d*) peritoneal exudate macrophages. (A) peritoneal exudate macrophages from C3H/OuJ (○) and C3H/HeJ (●) mice were cultured for 48 h, at which time the culture supernatants were aspirated and the cells were infected with VSV at an MOI of 0.1. At the indicated times, the culture supernatants were assayed for virus yield. The results represent the geometric mean $\pm$ 95% confidence limits for data pooled from five separate experiments. (B) Infected macrophage monolayers derived from C3H/OuJ (*Lpsⁿ*) and C3H/HeJ (*Lps^d*) mice 24 h postinfection, fixed and stained as described elsewhere (32). (C) Infected macrophage monolayers derived from C57BL/10J (*Lpsⁿ*) and C57BL/10ScN (*Lps^d*) mice 24 h postinfection, fixed and stained as described elsewhere (32).

FIG. 2. Effect of time in culture on replication of VSV in endotoxin-responsive and hyporesponsive macrophage cultures. Peritoneal exudate macrophages from C3H/OuJ and C3H/HeJ mice were cultured and infected with VSV at an MOI of 0.1 either on the same day (D₀) or after 48 h of incubation (D₂). At the indicated times after infection, culture supernatants were collected and assayed for virus yield. The results are from a single representative experiment in which all culture supernatants were assayed for virus yield on two separate occasions.

rophages to VSV increased with time in culture, whereas the inability of C3H/OuJ macrophages to replicate VSV was sustained over the 2-day culture period (Fig. 2). C3H/HeJ macrophages infected on the same day as they were cultured (D₀) consistently supported replication of VSV less well than the same macrophages cultured for 2 days prior to VSV infection (D₂).

Since IFN has been shown to induce an increase in cellular levels of the enzyme 2,5-A synthetase (reviewed in reference 11), levels of this enzyme were measured in macrophages derived from C3H/OuJ (*Lpsⁿ*) and C3H/HeJ (*Lps^d*) peritoneal exudate macrophages. Macrophages which were nonpermissive for VSV (C3H/OuJ) had significantly higher levels of 2,5-A synthetase (2,832 and 25,787 pmol of 2,5-A synthesized per h per mg of protein in two separate experiments) than macrophages derived from the permissive C3H/HeJ strain (26 and 418 pmol/h per mg of protein in the same two separate experiments).

Comparison of macrophages for sensitivity to VSV infection. Recent work by Moore et al. (21, 22) and Warren and Vogel (38) provided strong evidence for the induction of endogenous IFN by macrophages during their differentiation

FIG. 3. Comparison of TG-induced peritoneal exudate macrophages with CSF-1-stimulated, bone marrow-derived macrophages from C3H/HeJ (*Lps*^d) mice for sensitivity for VSV infection. C3H/HeJ TG-induced peritoneal exudate macrophages (●) and C3H/HeJ macrophages derived by in vitro culture of bone marrow progenitors in CSF-1 (○) were compared for their ability to support the growth of VSV. Macrophage cultures were infected with VSV at an MOI of 0.1 on the same day (D₀), after 24 h of incubation (D₁), or after 48 h of incubation (D₂). At the indicated times after infection, culture supernatants were collected and assayed for virus yield. The results were derived from a single representative experiment in which all culture supernatants were assayed for virus yield on two separate occasions.

from bone marrow stem cells to mature macrophages by fibroblast-derived CSF-1. Therefore, we compared the permissive C3H/HeJ peritoneal exudate macrophages with bone marrow-derived macrophage cultures from the same strain. When C3H/HeJ macrophages were derived from the bone marrow under the influence of CSF-1, they were as refractory to VSV infection as the fully endotoxin-responsive C3H/OuJ peritoneal exudate macrophages (Fig. 3; cf. Fig. 1 and 2), regardless of how long they were cultured in the absence of CSF-1 prior to infection. TG-treated C3H/HeJ macrophages treated with the enzyme Dispace II under the same conditions as the bone marrow-derived macrophages (prior to their culture in 96-well plates) were as sensitive to VSV as the control (mock-infected, Earle balanced salt solution-treated) macrophages, whether infected in D₀ or D₂ (data not shown). This experiment also reillustrates the finding (Fig. 2) that the C3H/HeJ peritoneal exudate macrophages showed increased sensitivity to VSV infection with time in culture.

Effect of anti-IFN- α/β antibody treatment on macrophage

sensitivity to VSV infection. Peritoneal exudate macrophages from C3H/HeJ and C3H/OuJ mice and bone marrow-derived macrophages from C3H/HeJ mice were cultured and treated with medium only, medium supplemented with control antibody, or medium supplemented with anti-IFN- α/β antibody for 24 h. At that time, the culture supernatants were removed, and the cultures were infected with VSV. Treatment of the nonpermissive cultures with anti-IFN- α/β antibody significantly increased their sensitivity to virus infection (Fig. 4). The concentration of antibody chosen for these experiments was based on the lowest concentration, determined in our earlier study, required to reduce Fc receptor capacity in *Lps*ⁿ macrophages to levels which approached the deficit response of C3H/HeJ macrophages (30). Treatment of C3H/HeJ TG-treated macrophages with anti-IFN- α/β antibody failed to further increase their permissiveness for VSV.

DISCUSSION

The role of endogenous IFN in natural resistance to infection has only recently been implicated as a mechanism for the maintenance of normal resistance (reviewed in reference 8). Specifically, studies carried out by Gresser and his colleagues have established their administration of anti-IFN- α/β antibody to mice increases their susceptibility to a variety of viral pathogens (including mouse hepatitis virus type 3, Sindbis virus, encephalomyocarditis virus, mouse cytomegalovirus, and herpes simplex virus type 1), even in genetically resistant mouse strains. They concluded from their findings that administration of anti-IFN- α/β antibodies

FIG. 4. Effect of anti-IFN- α/β antibody treatment on macrophage sensitivity to VSV infection. TG-induced peritoneal exudate macrophages from C3H/HeJ or C3H/OuJ mice and CSF-1-stimulated, bone marrow-derived (BM/CSF-1) C3H/HeJ macrophages were cultured in medium only or in medium supplemented with either control antibody or anti-IFN- α/β antibody for 24 h and then infected with VSV at an MOI of 0.1. Culture supernatants were harvested 22.5 h later and assayed for virus yield on two separate occasions. The results represent the mean $\pm$ 95% confidence limits of a single representative experiment.

resulted in the neutralization of endogenous IFN, which is normally a major factor responsible for natural resistance to infection. To date, the normal stimulus for the production of endogenous IFN has not been elucidated; however, some evidence suggests that a bacterial product(s) may play an important role in stimulating IFN production. Galabru et al. (7) demonstrated that in spleen and lung extracts derived from conventionally reared mice there were significantly higher levels of the IFN-induced enzymes 2,5-A synthetase and the 67,000-dalton protein kinase than in extracts from pathogen-free or germfree mice or mice which had been injected with anti-IFN- α/β antibody. Belardelli et al. (2) demonstrated that VSV and encephalomyocarditis virus multiply in only a small percentage of macrophages from the peritoneal cavities of mice (all of the strains chosen were of an *Lpsⁿ* background). However, if these mice were first injected with anti-IFN- α/β antibody, their macrophages were rendered permissive in vitro. In a recent report, Gresser et al. (11) extended these two observations by demonstrating a strong correlation between reduced 2,5-A synthetase levels and increased permissiveness for VSV in peritoneal macrophages derived from mice injection with anti-IFN- α/β antibody.

In this report, we have demonstrated that, like macrophages from anti-IFN- α/β antibody-treated mice, peritoneal exudate macrophages from the endotoxin-hyporesponsive C3H/HeJ mouse strain are permissive for VSV and exhibit low basal levels of 2,5-A synthetase, in marked contrast to macrophages derived from fully endotoxin-responsive mice. The VSV sensitivity of macrophages which bear the *Lps^d* genotype was confirmed with the C57BL/10ScN strain. Like C3H/HeJ macrophages, C57BL/10ScN macrophages, but not their fully LPS-sensitive counterparts, were permissive for VSV in vitro. The *Lps^d* genotype in C3H/HeJ and C57BL/10ScN strains arose independently (31) and is a noncomplementing genetic lesion (i.e., F_1 mice exhibit no increase in LPS sensitivity [39]). Thus, demonstration of VSV permissiveness in macrophages derived from these two strains greatly strengthens the evidence for the role of the *Lpsⁿ* allele in macrophage resistance to VSV infection.

It is important to note that the C3H/HeJ defect (i.e., the *Lps^d* allele) results in hyporesponsiveness to LPS only, not to other bacterial products or IFN inducers (reviewed in reference 25). For instance, we have found that injection of poly(I:C) (100 μ g) into C3H/HeJ mice results in the production of very high serum IFN levels (51,200 U/ml) within 2 h. Thus, our findings provide strong support for the hypothesis that LPS is a major "environmental" signal responsible for natural resistance to viral infection. Moreover, the finding in this report that direct treatment of the LPS-responsive macrophages with anti-IFN- α/β antibody significantly reversed their nonpermissiveness suggests that the macrophage is, in fact, the source of the endogenous IFN in this situation. The failure of anti-IFN- α/β to reverse completely the VSV resistance of C3H/OuJ or CSF-1-derived C3H/HeJ macrophages (Fig. 4) is most likely related to antibody concentration and degradation of antibody by macrophages (as opposed to other cell types). The anti-IFN- α/β concentration used in this study was chosen because in previous studies (30), a 1:250 to 1:500 dilution of this same antibody preparation consistently led to a reversal of Fc receptor capacity in *Lpsⁿ* macrophages which approached the deficient level exhibited by C3H/HeJ macrophages. In addition, macrophages required significantly more anti-IFN- γ antibody than L929 fibroblasts to neutralize exogenous IFN- γ -induced antiviral activity (32), suggesting that macrophages

may degrade antibody more rapidly than other cell types. The partial reversal of VSV resistance (Fig. 4) (as assessed by virus yield) correlated with approximately 50% CPE in fixed and stained plates. Preliminary experiments with a higher titered anti-IFN- α/β preparation in C3H/OuJ macrophages indicate that more complete CPE is obtainable (data not shown). Previous studies have suggested that macrophages are the primary source of LPS-induced IFN (12) and that LPS fails to induce IFN in C3H/HeJ mice in vivo (17) or in C3H/HeJ macrophage cultures (R. N. Moore, personal communication). In a previous study, attempts to measure antiviral activity directly in supernatants of *Lpsⁿ* and *Lps^d* peritoneal exudate macrophage cultures were unsuccessful due to very low levels released into the culture supernatants (30). However, Moore et al. (21) recently demonstrated low but measurable levels of antiviral activity in CSF-stimulated bone marrow cultures. The finding that C3H/HeJ macrophages derived from bone marrow progenitors under the influence of CSF-1 were phenotypically identical to C3H/OuJ (*Lpsⁿ*) peritoneal exudate macrophages with respect to VSV sensitivity suggests that the failure of C3H/HeJ peritoneal macrophages to produce endogenous IFN may be secondary to a failure to produce normal levels of CSF. Since it is known that injection of LPS also fails to induce CSF in C3H/HeJ mice (1, 19), the failure to respond to naturally occurring levels of LPS to produce CSF may underlie the deficit in endogenous IFN production.

The findings presented in this report also support the hypothesis that, in addition to increasing resistance to virus infection, endogenous IFN simultaneously provides an autostimulatory differentiative signal to the macrophage. The defective Fc receptor capacity exhibited by C3H/HeJ macrophages (as well as VSV sensitivity [32]) can be overcome to exogenous treatment of the macrophages with IFN (5, 36), whereas the normal Fc receptor capacity exhibited by C3H/HeN (*Lpsⁿ*) macrophages was shown previously to be depressed by treatment with anti-IFN- α/β antibody (30). In addition, the findings presented herein are consistent with recent observations by Moore et al. (22) and Warren and Vogel (38), which demonstrated that the Fc receptor capacity of CSF-1-derived macrophages from C3H/HeJ mice could be markedly inhibited by exposure to anti-IFN- α/β antibody either before or after removal of the CSF-1 stimulus.

The precise relationship of the endogenous IFN defect and LPS hyporesponsiveness remains elusive at this time. If one assumes that the primary *Lps^d* defect results in the failure of C3H/HeJ macrophages to respond to LPS due to a membrane defect, which is consistent with the findings of several investigators (6, 13), then the failure of the macrophage to be triggered by LPS to produce endogenous differentiative signals, such as CSF or IFN, would be compromised. Alternatively, or perhaps as a result of this primary lesion, the failure to produce endogenous cytokines may result in macrophages which are hyporesponsive to LPS due to a secondary defect, i.e., a decreased state of macrophage differentiation. This latter hypothesis is consistent with a number of observations. Specifically, potent macrophage-activating agents increase LPS sensitivity in vivo (3, 28), even in the C3H/HeJ strain (34). Treatment of C3H/HeJ peritoneal macrophages in vitro with lymphokine-rich culture supernatants increases their capacity to release interleukin 1 in response to LPS stimulation (33), and CSF-1-derived macrophages from both *Lpsⁿ* and *Lps^d* mice were found to exhibit increased sensitivity to LPS (23). Lastly, in a recent review by Beutler and Cerami (4), it was suggested

that the failure of C3H/HeJ macrophages to translate cachectin-specific mRNA could be reversed by exposure of the macrophages to IFN- γ . Thus, signals provided by cytokines to LPS-hyporesponsive macrophages have been shown in some cases to override or compensate for the failure to respond to LPS. Further experiments will be required to distinguish between these possibilities.

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