Age-Associated Differences in TNF-α and Nitric Oxide Production in Endotoxic Mice[1]

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Gram-negative bacterial infection is a common cause of septic shock in the older population in the U.S. We employed an experimental model of sepsis to study the cause of increased lethality due to LPS in older animals. Three ages of male B6JC3J/Nia mice, young (2 mo old), mature (12 mo old), and senescent (24 mo old), were treated with bacterial LPS, and the older mice were found to be 10 times more sensitive to LPS lethality. Increased sensitivity to LPS in senescent mice correlated with significantly elevated plasma TNF-α and nitric oxide levels. Abs to TNF-α afforded aged animals passive protection against a supralethal dose of LPS, establishing a central role for TNF in the increased sensitivity to LPS seen in the aged animals. Other cytokines, such as IL-1 and IFN-γ, appeared secondary to TNF and nitric oxide in the age-associated sensitivity to LPS. Plasma corticosterone levels were increased by LPS at a time when maximal levels of plasma TNF-α were observed in both age groups, although the kinetics of hormone production and the magnitude of TNF-α release varied among the age groups. Exogenously administered dexamethasone protected senescent animals given a high dose of LPS, by decreasing cytokine levels. The increased sensitivity to LPS observed in aged animals, therefore, seems to be due to excessive TNF and nitric oxide production, resulting from perturbed endogenous hormonal control of cytokine production. *The Journal of Immunology,* **1996, 156: 1525–1530.**

Aging is characterized by a decline in the ability of individuals to adapt to environmental stress. Although an effect of age on all parameters of immune function may not occur in every elderly subject, most immune functions in the elderly differ compared with those in younger subjects (1). The immune system is appealing for studies in aging because many differentiation and developmental processes at all levels have been well characterized. Also, several aspects of specific immune responses can be followed longitudinally with increasing age and correlated with known changes in physiologic function.

Septic shock, or sepsis-like syndrome with hypotension, is a clinical syndrome that has become increasingly prevalent in recent years and is reported to cause approximately 175,000 deaths annually in the United States (2). Acute and chronic sequelae of the sepsis syndrome currently represent the 13th leading cause of death in the United States (3). A prime initiator of Gram-negative bacterial septic shock is LPS, a component of the bacterial outer membrane. When administered i.v., LPS evokes a shock-like state characterized by fever, hypotension, hypoglycemia, and multiple organ failure (4). Mortality is associated with damage involving the lungs, kidneys, and gastrointestinal tract. It has become clear that LPS does not injure host tissues directly but through the action of a variety of inflammatory mediators, such as TNF-α, IL-1, IFN-γ, and nitric oxide (NO)[5] (4).

The purpose of this study was to determine the roles of cytokines and other mediators in the pathophysiology of sepsis in an aging animal model that mimics acute sepsis in elderly humans. Previous attempts to characterize the response to infection in aged animals have produced equivocal results because of the lack of a uniform approach. Some studies have shown a decreased response with age, while others do not observe any changes with increasing age (5–13).

We conducted lethality studies in inbred mice to estimate age-associated differences in LPS sensitivity and related differences in LPS sensitivity to amounts of endogenous mediator released. The LPS LD_{50} in older mice was about one-tenth of that in young or mature animals. The pattern of systemic cytokine and NO production in response to LPS was characterized, and TNF-α and NO were dramatically elevated, whereas IL-1 and IFN-γ responses were less distinct. Although a typical stress response, as indicated by elevated circulating plasma corticosterone (cort) levels, was seen in aged mice, it was apparent that the endogenous hormone levels were insufficient to diminish exaggerated TNF-α and NO release, thus explaining, in part, the increased sensitivity of aged mice to LPS.

Materials and Methods

Animals

Young (2 mo), mature (12 mo), and senescent (24 mo) B6JC3J/Nia mice were obtained from the National Institute on Aging through Charles River Mouse Farms (Wilmington, MA). Animals were housed in constant temperature- and humidity-maintained rooms, with a 12-h light/dark cycle, and were given food and water ad libitum. The average life span of male virgin B6C3F1 mice is 27.2 ± 1.1 mo (14). All experiments were performed within 1 mo of receipt of the animals and were approved by the Texas A&M University Laboratory animal care committee.

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[5] Abbreviations used in this paper: NO, nitric oxide; cort, corticosterone; Dex, dexamethasone; murTNF-α, murine recombinant TNF-α.

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Table I. Escherichia coli *O111:B4 lipopolysaccharide (LPS) median lethal dose (LD_{50}) in young (2 mo), mature (12 mo), and senescent (24 mo) B6JC3J/Nia mice*

Age	LD_{50} value[a] (mg/kg body wt)	MTD[b] (hr)
2 mo	17.84	21
12 mo	17.17	n.d.[c]
24 mo	1.84	22

[a] Median lethal dose, 72 hr after i.p. injection. Dose titrations were performed on 24 mice of each age.
[b] Median time to death.
[c] Not determined.

Agents

LPS, extracted from *Escherichia coli* 0111:B4, was purchased from LIST Biologic Laboratories, Inc. (Campbell, CA), dissolved in sterile nonpyrogenic saline, and injected i.p. Dexamethasone (Dex) was purchased from U.S. Biochemical Corp. (Cleveland, OH), dissolved in propylene glycol, and injected s.c.

Antibody preparation

Rabbit polyclonal antiserum to murine TNF-α (murTNF-α) was prepared as described previously (15). Briefly, rabbits were immunized s.c. and i.m. at various sites with murTNF-α (generously provided by Genentech, Inc., South San Francisco, CA). Following immunization with a total of 200 μg of murTNF-α, the serum Ig fraction was isolated, and TNF-α neutralizing activity was determined with the L929 cell cytotoxicity assay. One neutralizing unit of anti-TNF-α Ab is defined as the reciprocal of the dilution that resulted in 50% inhibition of cell cytotoxicity following treatment with 500 U of murTNF-α/ml. The immune rabbit IgG fraction contained 1.39×10^5 neutralizing units/ml, or 6.376×10^3 neutralizing units/mg of protein.

Blood collection

Blood was obtained from anesthetized (metafane) mice at intervals after LPS treatment by insertion of a heparinized microhematocrit tube into the ophthalmic venous plexus. Samples were collected at 1.5 h for TNF-α determination, at 3 h for IL-1 determination, and at 8 h for assay of cort, IFN-γ, and NO levels. In time-course studies of cort and TNF-α production, samples were collected 0.75, 1.5, 3, and 6 h after LPS.

Cytokine and hormone assays

L929 cells were cultured overnight at 37°C in 5% humidified CO_2 in Iscove's medium, supplemented with 5% fetal bovine serum, penicillin, streptomycin, and gentamicin, at 2×10^4 cells/well in 96-well microtiter plates. Plasma was diluted serially in medium containing actinomycin D (1 μg/ml) and was added to L929 cells. Following incubation for 18 h, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-tetrazolium bromide (MTT; 5 mg/ml in PBS) was added (25 μl/well), and incubation was continued for 2 h. After centrifugation at $600 \times g$ for 5 min, medium was removed, lysing buffer (50% dimethylformamide and 20% SDS; 100 μl/well) was added, and the plates were incubated for 4 h at 37°C. The absorbance at 570 nm was read with a Dynatech MR-700 microtiter plate reader (Dynatech Laboratories, Inc., Alexandria, VA), and cell survival was plotted against the log of the plasma dilution. One unit of TNF-α is defined as the reciprocal of the dilution that yielded 50% cytotoxicity. Immunoreactive IL-1 levels in the plasma of LPS-treated mice were measured by ELISA assay (InterTest-1αX kit, Genzyme Corp., Cambridge, MA). Plasma IFN-γ levels were determined by a IFN-γ ELISA kit (Endogen, Inc., Cambridge, MA), and plasma cort levels were measured with a RIA (^{3}H) kit obtained from ICN Biomedicals, Inc. (Costa Mesa, CA).

Measurement of plasma NO levels

NO production was assessed by measuring the amount of plasma NO_3^- and NO_2^- as described by Evans et al. (16). Briefly, plasma was diluted fourfold in sterile pyrogen-free saline, and plasma nitrate was reduced to nitrite by the addition of nitrate reductase (from *Aspergillus* species; Sigma Chemical Co., St. Louis, MO) at 0.25 U/ml at 37°C for 15 min in the presence of 5 mM NADPH. Excess NADPH was removed by incubation with 16.7 U of lactate dehydrogenase (Sigma Chemical Co.)/ml and 33 mM sodium pyruvate at 37°C for 15 min. Plasma was mixed with equal volumes of Greiss reagent in a flat-bottom 96-well microtiter plate, and the absorbance at 565 nm was determined. Nitrate amounts were calculated from a sodium nitrite standard curve.

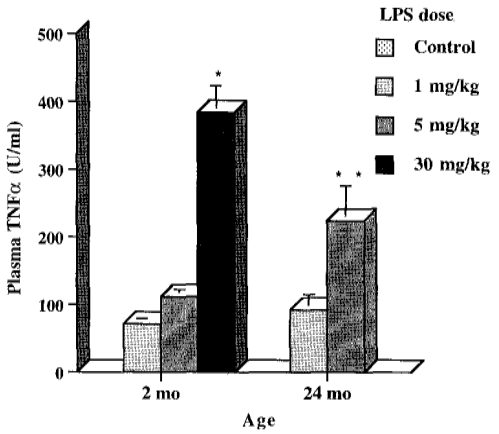

FIGURE 1. Plasma TNF-α production in response to varying doses of LPS in 2- and 24-mo-old mice. Plasma was collected 1.5 h after LPS injection. Values represent the mean ± SEM obtained from six to eight mice per group. *, Significantly different ($p \leq 0.05$) from the 1 and 5 mg/kg doses; **, significantly different ($p \leq 0.05$) from the 5 mg/kg dose in 2-mo-old mice.

Statistical analysis

Results are presented as mean ± SEM for the indicated number of determinations. Comparison between groups was made employing Student's *t* test (Microsoft Excel, Microsoft Corp., Redmond, VA). Statistical significance was assessed at the 95% confidence level ($p \leq 0.05$).

Results

Age-associated differences in sensitivity of animals to LPS

The LPS LD_{50} was determined for each age group of mice. Briefly, 24 mice of each age were divided into four subgroups of six mice each. For the young and mature mice, the LPS doses selected were 30, 20, 10, and 5 mg/kg BW, while for the senescent group, the doses were 5, 2.5, 1.25, and 0.625 mg of LPS/kg BW. The time of death as well as mortality at 72 h were recorded. LD_{50} values were determined by the method of Reed and Muench (17). The results are summarized in Table I, and it can be seen that 24-mo-old mice were approximately 10 times more sensitive to LPS than 12- and 2-mo-old mice. The LPS LD_{50} for 24-mo-old mice was 1.84 mg/kg, whereas that for 12- and 2-mo-old mice was about 17 mg/kg. The mean time to death was approximately the same for all groups (~22 h). Since the responses of 2- and 12-mo-old mice were similar, subsequent age comparisons were made between 2- and 24-mo-old animals.

TNF-α production in response to LPS in 2- and 24-mo-old mice

To characterize further the response of animals to LPS, we measured cytokine levels produced as a result of LPS challenge. To investigate whether an increase in TNF-α production correlated with the increase in sensitivity to LPS in aged animals, we treated 2- and 24-mo-old mice with different doses of LPS. The doses selected for 24-mo-old mice were 1 mg/kg (a sublethal dose) and 5 mg/kg (a lethal dose). For 2-mo-old mice, the doses were 1 mg/kg (a nonlethal dose), 5 mg/kg (a nonlethal dose), and 30 mg/kg (a lethal dose). Senescent mice were not treated with 30 mg/kg, because this dose exceeds the lethal dose for this age group by at least sixfold. As can be seen from the results in Figure 1, no age-associated differences in TNF-α levels were observed in the plasma of mice of both age groups treated with 1 mg/kg of LPS.

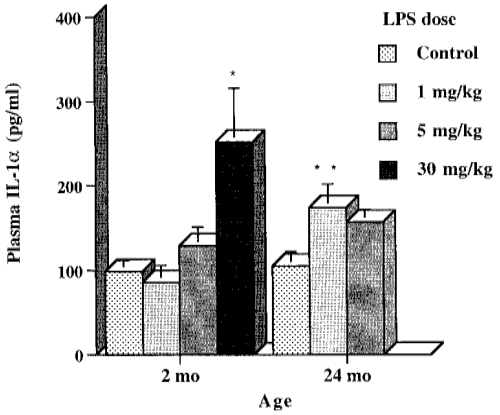

FIGURE 2. Plasma IL-1 production in response to varying doses of LPS in 2- and 24-mo-old mice. Plasma was collected 3 h after LPS injection. Values represent the mean ± SEM obtained from six to eight mice per group. The control group was injected with saline alone. *, Significantly different ($p \leq 0.05$) from the 1 and 5 mg/kg LPS doses; **, significantly different ($p \leq 0.05$) from the 1 mg/kg dose in 2-mo-old mice.

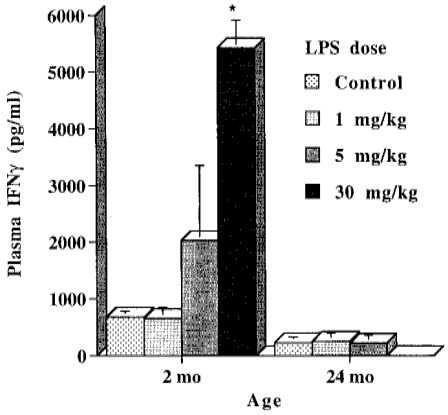

FIGURE 3. Plasma IFN-γ production in response to varying doses of LPS in 2- and 24-mo-old mice. Plasma was collected 8 h after LPS injection. Values represent the mean ± SEM obtained from six to eight mice per group. The control group was injected with saline alone. *, Significantly different ($p \leq 0.05$) from the 1 and 5 mg/kg doses.

When comparison was made between levels of TNF-α in animals given 5 mg/kg, a significantly greater increase ($p \leq 0.05$) in TNF-α production in 24-mo-old mice was observed (224 vs 111 U/ml). The control group, in which no TNF-α was detected, was injected with saline alone.

IL-1 production in response to LPS in 2- and 24-mo-old mice

As shown in Figure 2, IL-1 levels were increased significantly ($p \leq 0.05$) with increasing LPS dose in 2-mo-old mice. At a dose of 1 mg/kg, older animals demonstrated significantly increased ($p \leq 0.05$) IL-1 levels 3 h after treatment compared with 2-mo-old mice (174 ± 28 vs 86 ± 20 pg/ml). At a lethal LPS dose of 5 mg/kg for 24-mo-old animals, IL-1 production was slightly higher, but not statistically different, from that in younger mice (157 ± 22 vs 129 ± 22 pg/ml). Control animals were injected with saline alone.

IFN-γ production in response to LPS in 2- and 24-mo-old mice

To examine the effect of age on plasma IFN-γ production, we treated 2- and 24-mo-old animals with varying doses of LPS. As was the case with plasma IL-1, IFN-γ levels 8 h after LPS administration increased with increasing LPS doses in 2-mo-old mice. The amount of IFN-γ produced in 24-mo-old mice challenged with LPS, however, was significantly lower ($p \leq 0.05$) than that in 2-mo-old mice (Fig. 3) and represented only the basal (uninduced) level.

Central role of TNF-α in LPS mortality

To determine the role of TNF-α in the increased sensitivity of aged animals to LPS, we injected rabbit polyclonal anti-TNF-α Ab into mice from the two age groups 3 h before LPS (10 mg/kg) was given. This represents a supralethal dose for older mice. Control mice were given normal rabbit IgG 3 h before LPS challenge. Animals were bled at 90 min for TNF-α determination, and survival was recorded at 72 h. As shown in Table II, there was a substantial decrease in mortality in anti-TNF-α Ab-treated 24-mo-old mice (9 of 11 vs 1 of 11). Anti-TNF-α Ab neutralized circulating TNF-α levels 90 min after LPS challenge in both 2- and 24-mo-old mice (data not shown). The low mortality seen in control 2-mo-old mice was due to the LPS challenge dose (10 mg/kg) being much lower than the LD_{50} for younger animals.

Table II. *Mortality data from 2 mo and 24 mo old mice treated with normal IgG or anti-TNF antibody before LPS (10 mg/kg) challenge*

Age	Dead/Total[a]	
	Normal IgG	Anti-TNF Ab[b]
2 mo	2/11	0/11
24 mo	9/11	1/11

[a] Data are expressed as number of deaths/total number of animals, determined in two separate experiments.

[b] TNF levels in the antibody treated animals were determined by the L929 assay. Anti-TNF antibody effectively neutralized TNF levels 90 min after LPS challenge in both the 2 mo and 24 mo old mice (data not shown).

NO oxide levels in 2- and 24-mo-old mice given different doses of LPS

Excessive production of NO during endotoxemia contributes significantly to the pathophysiology of septic shock (21). A postulated role for increased NO in septic shock in young mice prompted us to examine the sensitivity of 2- and 24-month-old mice to the lethal effects of LPS challenge and NO production. As shown in Figure 4, plasma nitrate (NO_3^-) plus nitrite (NO_2^-) levels were elevated by LPS treatment in a dose-dependent manner in both 2- and 24-mo-old mice. NO levels in mice given 1 mg/kg of LPS were not different in the two age groups, although the increase in 24-mo-old mice given 5 mg/kg was significantly higher ($p \leq 0.05$) than that in 2-mo-old mice (483 vs 382 mM).

Cort production in response to LPS in 2- and 24-mo-old mice

To assess the stress response to LPS, we measured plasma cort levels in samples obtained from 2- and 24-mo-old mice 8 h after they received various doses of LPS. As shown in Figure 5, basal (control nonstressed) cort levels were not significantly different in 2- and 24-mo-old animals. Plasma cort levels in 24-mo-old mice challenged with LPS reached a maximal level at a substantially lower dose of LPS (1 mg/kg) than that required for young animals. In 2-mo-old animals, an LPS dose of 5 mg/kg was required to elicit maximal cort responses.

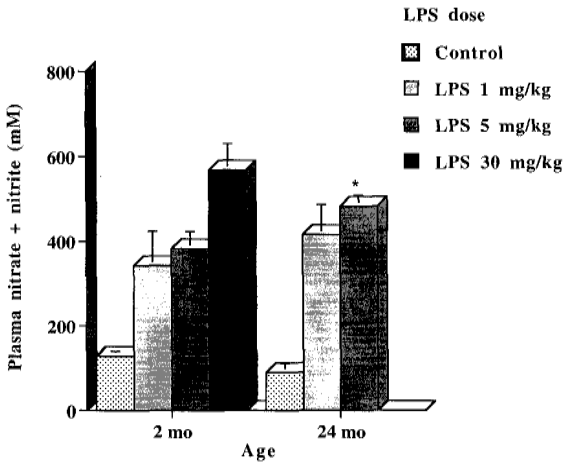

FIGURE 4. Plasma nitrate plus nitrite levels in response to varying doses of LPS in 2- and 24-mo-old mice. Values represent the mean ± SEM obtained from six to eight mice per group. The control group was injected with saline alone. *, Significantly different ($p \leq 0.05$) from the 5 mg/kg dose in 2-mo-old mice.

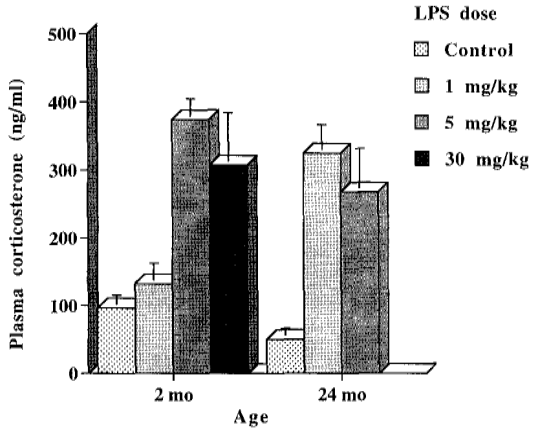

FIGURE 5. Plasma cort levels in response to varying doses of LPS in 2- and 24-mo-old mice. Plasma was collected 8 h after LPS. Values represent the mean ± SEM obtained from six to eight mice per group. The control group was injected with saline alone.

Effect of exogenous glucocorticoid on LPS lethality in 2- and 24-mo-old mice

Previous studies reported a decrease in glucocorticoid receptor expression with increasing age (18). To determine whether decreased glucocorticoid receptor function might be responsible for the increased LPS mortality seen in aged animals, we administered Dex (2.5 mg/kg), to mice 30 min before challenge with an LD_{80} dose of LPS (20 mg/kg for the 2-mo-old mice and 3.4 mg/kg for the 24-mo-old animals). As depicted in Table III, exogenous glucocorticoid was effective in preventing LPS mortality in both age groups of mice. Dex treatment reduced mortality from 64.3 to 7.1% in 24-mo-old mice and from 78.6 to 14.3% in 2-mo-old animals. We examined further the protective role of Dex on LPS lethality by monitoring TNF-α production in 2- and 24-mo-old mice 90 min after LPS treatment (5 mg/kg). As shown in Table IV, exogenous Dex was completely effective in inhibiting TNF-α production in both 2- and 24-mo-old mice, indicating the pathways for receptor-mediated glucocorticoid action to be intact in aged mice.

Table III. *Mortality data from 2 mo and 24 mo old mice treated with dexamethasone and challenged with an LD_{80} dose of LPS*

	Dead/Total[a]	
Age	LD_{80} LPS[b]	Dex[c] + LD_{80} LPS
2 mo	11/14	2/14
24 mo	9/14	1/14

[a] Data are expressed as number of deaths/total number of animals, determined in two separate experiments.
[b] LD_{80} dose for 2 mo old mice was calculated to be 20 mg/kg; LD_{80} dose for 24 mo old mice was calculated to be 3.4 mg/kg.
[c] Dex dosage was 2.5 mg/kg.

Table IV. *Dexamethasone reduction of plasma cytokine levels in 2 mo old and 24 mo old B6JC3J/Nia mice*

	TNF-α (U/ml)[a]		
Age	LPS[b]	Dex[c] + LPS	% Decrease
2 mo	88 ± 22[d]	22 ± 4	75.0
24 mo	163 ± 40	58 ± 25	64.4

[a] TNF-α measured 1.5 hr after LPS.
[b] 5 mg/kg, i.p.
[c] 2.5 mg/kg, s.c., 30 min before LPS.
[d] 7–8 mice per group.

Time course of cort and TNF-α responses to LPS in 2- and 24-mo-old mice

As indicated above, Dex decreased mortality drastically in both young and aged mice. However, aged mice showed increased endogenous cort levels compared with young mice when treated with lower doses of LPS, and cort levels equal to those in young mice at higher doses. We reasoned that perhaps the critical difference between these groups was the time at which glucocorticoid was presented. Exogenous Dex was given 30 min before LPS, whereas elevated cort was measured 8 h after LPS treatment. Therefore, we followed the kinetics of cort production and TNF-α release in both age groups at intervals after administration of 5 mg/kg of LPS.

The results presented in Figure 6 demonstrate an early (within 45 min after LPS) elevation of circulating cort levels in young and old mice. Cort levels in senescent mice appeared slightly higher than those in younger animals at 45, 90, and 180 min; however, the values were not statistically different ($p \leq 0.05$). Substantial age differences in the cort response to LPS were seen at 3 and 6 h. Six hours after LPS, cort levels in older mice were 8.3-fold greater (678 vs 82 ng/ml; Fig. 6*A*). Significantly higher levels of plasma TNF-α were observed in older mice, which peaked 90 min after LPS treatment (211 vs 133 U/ml; Fig. 6*B*). Ironically, the rise in endogenous cort in older mice before 90 min should have been sufficient to down-regulate further TNF-α production. These results confirm the inability of endogenous glucocorticoids to down-regulate excessive TNF-α production in senescent mice, although the pathways for glucocorticoid action appeared to be intact in older animals.

Discussion

The elderly have a higher incidence of morbidity and mortality due to diseases related to microbial infection (19). In our experiments we compared the responses of three age groups of mice to LPS. Results from lethality studies, which provide a measurement of the relative sensitivity of a group of animals to a specific stress, showed that senescent mice were about 10 times more sensitive to LPS than young mice. Similar LPS LD_{50} values for young and

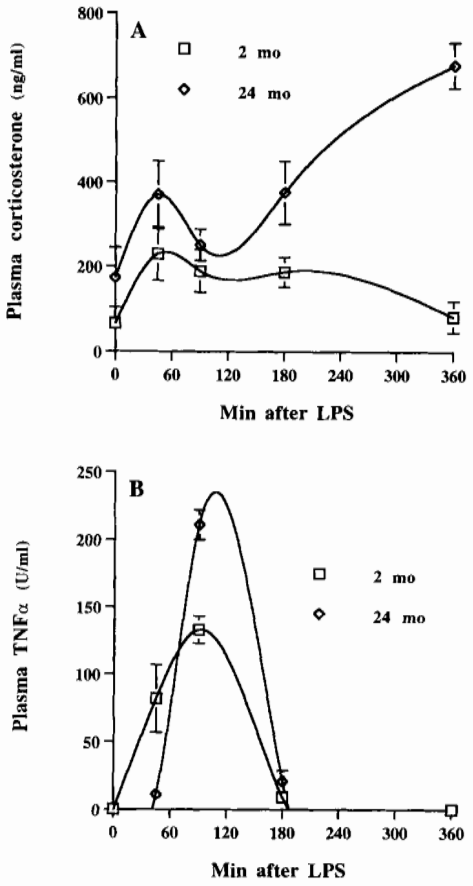

FIGURE 6. Time course of plasma cort (*A*) and TNF-α (*B*) responses to LPS (5 mg/kg) in 2- and 24-mo-old mice. Values represent the mean ± SEM obtained from four or five mice per group.

mature mice suggested a similar response in the two groups. Using AKR mice, Boggs et al. (20) also demonstrated increased sensitivity of aged mice to LPS.

Previous studies examining the effects and levels of cytokines in response to LPS in aged animals have produced equivocal results. Contradicting results are largely due to age-associated differences in sensitivity to LPS and failure to test responses at more than a single dose. We attempted to correlate circulating levels of proinflammatory cytokines and glucocorticoids following LPS treatment of young and senescent mice in a more detailed fashion by testing responses at low, median, and high challenge doses.

TNF-α has been shown to be a pivotal cytokine in the course of experimental Gram-negative infection (21). There is no consensus, however, as to the role of TNF-α in age-related responses to bacterial infection. Our results showed that TNF-α responses to sublethal doses of LPS were not different between the two age groups. However, when responses were compared at a 5 mg LPS/kg (lethal dose for senescent mice and sublethal dose for young mice), plasma TNF-α levels were significantly elevated in older mice. To substantiate the correlation between circulating TNF-α levels and LPS mortality, we administered neutralizing TNF-α Abs to mice before challenging them with a supralethal dose of LPS (10 mg/kg). The results clearly established a direct relationship between elevations in circulating TNF-α and increased sensitivity to LPS. Additional evidence recently obtained in our laboratory indicates that older mice are more sensitive to the effects of exogenous murTNF-α, as seen by more severe hypoglycemia and greater levels of circulating IL-6 in response to the cytokine (L. Mao and R. E. McCallum, manuscript in preparation). The increased TNF-α response in aged animals could be due to a number of factors. It is possible that the number of circulating TNF-α receptors, which help to down-regulate the TNF-α response, could be reduced in older animals compared with that in younger animals. Alternately, there could be a deficiency in natural inhibitors of TNF-α production, such as glucocorticoids, heparin, PGE_2, etc., in older animals. An absence or deficiency of TNF-α inhibitors could result in the uncontrolled production of the cytokine.

IL-1 is a major cytokine whose synthesis is up-regulated by LPS (22). While IL-1 showed a gradual increase with increasing doses of LPS in younger animals, IL-1 production appeared to reach a peak in older mice given a low LPS dose, with little additional IL-1 synthesized at higher doses. Reduced circulating IL-1 could be due to increased internalization of IL-1, which might act along with TNF-α to increase the sensitivity of animals to LPS.

The very low levels of IFN-γ in aged mice in response to high doses of LPS was in direct contrast to a dramatic IFN-γ response seen in younger mice. Several previous studies have examined the IFN-γ response in aged animals stimulated with a variety of activating agents and mitogens. Reports show either an increase or no difference in the response of aged animals to different Ags (8, 23). As LPS is a T cell-independent Ag, we believe that the source of IFN-γ in this model is the NK cell and that the lack of any substantial IFN-γ response in older animals might be due to a decrease in the number and the function of NK cells with increasing age (24). On the other hand, it has been shown that cytokines such as IL-6 and TNF-α increase the number of IFN-γ receptors on macrophages (25). An alternative hypothesis is that the increased sensitivity to LPS in aged animals is due to an accelerated uptake of IFN-γ, which may well synergize with TNF-α in bringing about cell death. The results reported here give no indication to the mechanism of the important finding of diminished plasma IFN-γ levels following LPS treatment in aged mice; however, ongoing studies are focused on the role of IFN-γ in regulating TNF-α production and responsiveness in older mice.

NO production in senescent mice followed a pattern similar to that of TNF-α, with significantly higher amounts of NO produced in aged animals given an LPS dose of 5 mg/kg (lethal dose). Morrison and co-workers observed hypersecretion of NO by macrophages obtained from 22-mo-old CBA/CA mice following in vitro stimulation with LPS (26). TNF-α alone or in combination with other cytokines has been shown to induce NO production, thereby exacerbating the cellular response to LPS (27–29). Thus, increased NO production in aged mice has a definite role in the increased sensitivity of these animals to LPS.

LPS is known to activate the hypothalamus-pituitary-adrenal axis, resulting in the production of glucocorticoids that are effective natural inhibitors of cytokine production (30). Our studies in aged animals did not show a decreased production of glucocorticoids; however, cort levels were maximized at a lower LPS dose. Since normal glucocorticoid production in response to LPS was found to occur in older animals, we suspected that receptor-mediated pathways for glucocorticoid action might be impaired. This possibility was posed by the results of previous studies in which we observed enhanced LPS lethality associated with elevated plasma TNF-α levels in young mice treated with the glucocorticoid antagonist, RU 486 (31). The results described here, however, demonstrate that the inhibitory action of exogenously administered Dex on cytokine production in older mice was intact, suggesting that glucocorticoid receptor function may be unchanged in aging animals. Larger LPS doses resulted in higher cort production,

which could act at the level of the pituitary to block further production of cort, thus limiting the amount of endogenous glucocorticoid produced. Additional increased LPS challenge doses were unable to induce additional cort. This finding suggests that the endogenous cort production seen in aged mice was simply insufficient to down-regulate the dramatically increased production of TNF-α following LPS.

In summary, our results show that aged animals are more sensitive to LPS challenge and that the increased sensitivity is due mainly to an overproduction of TNF-α and NO. Homeostatic regulatory mechanisms appear to undergo changes with age that bring about an excessive and uncontrolled production of the above mediators. IL-1 and IFN-γ seem to play secondary roles in the pathophysiology of sepsis in aged animals, although the normal balance between inflammatory cytokines appears to be disrupted in older mice. The hormonal pathways required for the down-regulation of TNF-α production seem to be intact in the aged animals. As unregulated TNF-α and NO production in older animals given LPS is unsuccessfully countered by endogenous glucocorticoid levels, death ensues.

We are currently examining differences in response to LPS in animals of various ages at the cellular level. Improved therapy and management of septic shock in the elderly will require a more thorough understanding of the regulatory mechanisms involved in the production of key mediators such as TNF-α and NO. Further studies may provide valuable insight into possible biologic and pharmacologic avenues effective in modulating cytokine production in the elderly septic patient.

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