Pretreatment of Young Pigs with Vitamin E Attenuates the Elevation in Plasma Interleukin-6 and Cortisol Caused by a Challenge Dose of Lipopolysaccharide[1,2]

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ABSTRACT The effect of a short-term, high-dose intramuscular injection of *d*-α-tocopherol was studied in pigs given a challenge dose of lipopolysaccharide (LPS). Twenty-four pigs surgically fitted with jugular catheters were used in a 2 $\times$ 2 factorial design. Pigs received either 0 or 600 mg *d*-α-tocopherol by intramuscular injection for 3 d before receiving an intraperitoneal injection of saline containing either 0 or 5 μg/kg body weight *Escherichia coli* LPS. Blood was collected from indwelling jugular catheters at 0, 1, 2, 4, 6, 8, 12 and 24 h after injection of LPS. Plasma α-tocopherol levels were 13-fold greater ($P < 0.01$) at time 0 in pigs pretreated with 600 mg *d*-α-tocopherol (9.9 $\pm$ 1.3 mg/L) than in those not treated with *d*-α-tocopherol (0.74 $\pm$ 0.09 mg/L). Injection of LPS increased ($P < 0.05$) plasma levels of interleukin-6 (IL-6) and cortisol at 2-h postinjection, regardless of vitamin E treatment. However, pigs that received α-tocopherol before the LPS challenge had substantially lower ($P < 0.05$) peak levels of IL-6 and cortisol than pigs not receiving α-tocopherol. These results suggest that supplementation with a surfeit level of vitamin E reduces the response of pigs to endotoxin. J. Nutr. 128: 1657–1660, 1998.

KEY WORDS: • vitamin E • lipopolysaccharide • interleukin-6 • cortisol • pigs

Cytokines released from activated macrophages are primarily responsible for the metabolic effects characterizing immunological challenge (Johnson 1997). Although many cytokines have been identified and are known to have important functions, the pro-inflammatory cytokines, interleukin-1 (IL-1),[4] interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), are thought to be the primary cytokines responsible for metabolic changes elicited by infection (Johnson 1997). Production of cytokines and the metabolic changes they induce are important in the adaptive response to infection, and it is also clear that overproduction of these molecules could have potentially deleterious effects. Thus, modification of cytokine production from activated macrophages represents a potentially useful treatment in situations in which there is excessive production of cytokines.

Exposure of macrophages to endotoxin induces the production of reactive oxygen intermediates (e.g., superoxide). In addition to their well-established toxic extracellular effects, it is now known that reactive oxygen species play a role in intracellular signaling pathways, possibly through activation of a variety of nuclear transcription factors (Eugui et al. 1994). Reactive oxygen species participate in the activation of the transcription factors NF-κB and AP-1, which are required for the expression of certain cytokine genes (Hirano et al. 1990, Shakov et al. 1990). Because activation of these transcription factors is blocked by antioxidants in vitro (Meyer et al. 1993, Schrek and Baeurerle 1994) and because antioxidants suppress the production of TNF-α, IL-1 and IL-6 from macrophages and monocytes in vitro (Eugui et al. 1994), we postulated that antioxidants may also inhibit cytokine production in vivo.

It is apparent from the information currently available that the primary role of vitamin E in nutrition is that of a biological antioxidant. As such, vitamin E is thought to play an integral role in maintaining membrane integrity in nearly all mammalian cells (Sheffy and Shultz 1979). Vitamin E also has been investigated for its effects on immunologic responses in a variety of animal species. However, until recently, these investigations have focused on antibody production and host resistance to disease challenge, with little work being conducted on how vitamin E might affect pro-inflammatory cytokine production (Finch and Turner 1996). In a recent report, Bulger et al. (1997) found that lipopolysaccharide (LPS)-induced TNF-α production was inhibited from whole blood and peritoneal macrophages obtained from rats receiving short-term, high dose enteral supplementation of vitamin E. Our in vivo study was conducted to evaluate the effects of intramuscular injection of vitamin E (*d*-α-tocopherol) on plasma IL-6 and cortisol in pigs subjected to a challenge dose of LPS. Lipopolysaccharide administration is a well-documented model of disease stress, and the effects of LPS are attributed to a cascade of events that include cytokine synthesis and release (Dinarello 1996, Webel et al. 1997).

MATERIALS AND METHODS

Pigs and housing. Weanling crossbred male pigs (cross of PIC Line 326 males with Cambourough 22 females) obtained from the

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[4] Abbreviations used: BW, body weight; IL-1, interleukin-1; IL-6, interleukin-6; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α.

University of Illinois Swine Research Center were randomly assigned to experimental treatments and pens from blocks based on ancestry and weight. Pigs were housed individually in metabolic cages (0.71 × 0.86 m) in an environmentally controlled room (24°C) in which a constant 24-h light schedule was maintained. When pigs reached an average weight of ~10 kg, jugular catheters were surgically placed, and pigs were allowed to recover for a minimum of 5 d before initiation of treatments.

Experimental protocols. Twenty-four pigs fitted with jugular catheters were used to evaluate the effects of intramuscular injections of α-tocopherol on the IL-6 and cortisol response of pigs injected with LPS. A 2 × 2 factorial arrangement of treatments was used in which *d*-α-tocopherol (0 or 600 mg) and LPS [0 or 5 mg/kg body weight (BW)] were the factors. The LPS (*Escherichia coli* serotype K-235; Sigma, St. Louis, MO) was dissolved in sterile 9 g/L (wt/v) NaCl solution so that injection of 0.1 mL/kg BW of solution would achieve the desired dosage (Webel et al. 1997). The *d*-α-tocopherol (Vital E-300; 300 g/L *d*-α-tocopherol, molecular weight = 430.7; Schering Plough Animal Health, Kenilworth, NJ) was compounded with 20% ethyl alcohol and 1% benzyl alcohol in an emulsifiable base (vegetable oil). Injections of LPS were given intraperitoneally in the lower abdominal region, and α-tocopherol was given intramuscularly in the rear flank.

The experimental period consisted of 4 d, with the first 3 d used as a pretreatment period and the final day used for sampling. For the first 3 d, pigs had free access to a corn-soybean meal/dried whey diet (Chung and Baker 1991),[5] that was analyzed to contain 22 mg/kg α-tocopherol, i.e., roughly twice the NRC (1998) requirement estimate of vitamin E for 10-kg pigs. During this 3-d pretreatment period, pigs received daily intramuscular injections of either 2 mL of sterile saline or 2 mL *d*-α-tocopherol (300 g/L). At 1800 h of d 3, food was removed, and at 0730 h the next morning, catheters were flushed and blood samples were taken from each pig. At 0800 h, pigs were weighed and LPS treatments were initiated. Blood samples were taken at 0, 1, 2, 4, 6, 8 and 12 h after LPS injection, for cortisol determination at 0, 2, 4, 8 and 12 h and for IL-6 determination at 0, 1, 2, 4, 6 and 8 h. These key sampling times after LPS administration were based on previous research in our laboratory (Webel et al. 1997). Blood samples taken before initial weight determination are described as time 0. Pigs did not have access to food during the 24-h evaluation period.

Surgical procedures. All procedures were approved by the University of Illinois Laboratory Animal Care Advisory Committee. Anesthesia was induced by intramuscular injection of a mixture of telezol (2.5 mg/kg BW), ketamine (1.25 mg/kg BW), and xylazine (1.25 mg/kg BW) and maintained with halothane (2–5%), and oxygen (300 mL/min). Pigs were given antibiotic (ceftiofur, Naxcel, Upjohn-Pharmacea, Kalamazoo, MI) injections (50 mg intramuscularly) just before surgery, and 50 mg of intravenous ceftiofur was also given postsurgically for 3 d.

Jugular catheterization procedure. The right jugular vein was surgically exteriorized and a catheter (Tygon microbore tubing; i.d., 1.02 mm; o.d.,1.78 mm; Fisher Scientific, Pittsburgh, PA) filled with heparinized saline (10^5 U/L) was advanced posteriorly 10–15 cm to the vena cava and sutured in place. The distal end of the catheter was passed subcutaneously using a straight trocar to the dorsal neck region and exteriorized between the scapula for repeat blood sample collection. Catheters were flushed twice daily with 5 mL of sterile heparinized (10^5 U/L) saline to maintain patency.

Blood collection and analysis. Serial blood samples were collected into heparinized syringes (Sarstedt, Newton, NC) via an indwelling jugular catheter and placed on ice immediately. Whole-blood samples were centrifuged and aliquots of plasma from each pig were stored at −23°C until analysis could be conducted. Total plasma cortisol was measured using a commercially available ^{125}I RIA kit (ICN Biomedicals, Costa Mesa, CA). Total plasma IL-6 was measured using the IL-6 sensitive, 7td1 B-cell hybridoma cell proliferation assay as described by Webel et al. (1997); plasma α-tocopherol was analyzed by HPLC methodology as described by Cort et al. (1983) and Mahan (1991).

[5] The diet also contained supplemental Na_2SeO_3 (0.3 mg Se/kg) and ethoxyquin (125 mg/kg).

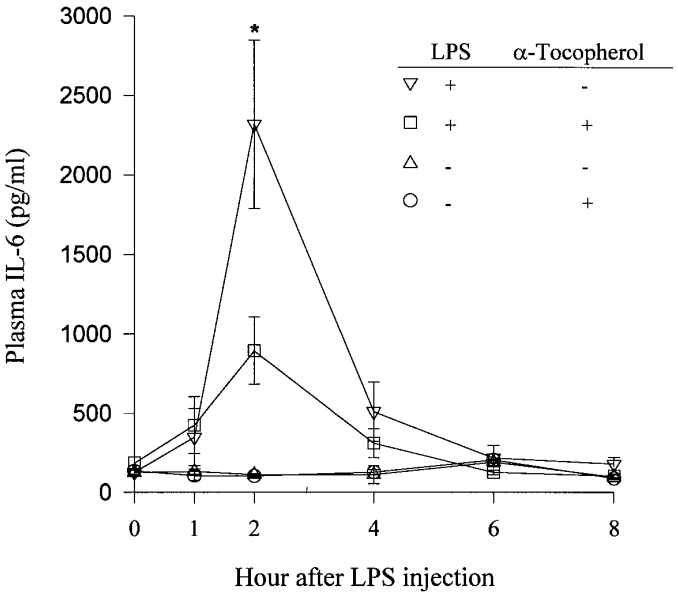

FIGURE 1 Plasma interleukin-6 (IL-6) of pigs receiving saline or *d*-α-tocopherol (600 mg) injections for 3 d before being injected with a challenge dose (5 μg/kg BW) of lipopolysaccharide (LPS). Data points represent means ± SEM, n = 6. * LPS different ($P < 0.05$) from saline and, within LPS-treated pigs, α-tocopherol pretreatment different ($P < 0.05$) from no α-tocopherol.

Statistical analysis. All data analyses were conducted using the GLM procedure of SAS (SAS/STAT Version 6, SAS Institute, Cary, NC). Data were subjected to two-factor repeated- measures ANOVA. Differences among treatments and interactions were assessed using single degree-of-freedom comparisons.

RESULTS

At time 0, plasma tocopherol levels were 13-fold greater ($P < 0.01$) in pigs pretreated with 600 mg α-tocopherol (9.92 ± 1.34 mg/L) than in those receiving saline (0.74 ± 0.09 mg/L). There was no indication at any sampling time, however, that LPS administration had any effect on the concentration of tocopherol in plasma. As shown in **Figure 1**, intraperitoneal injection of LPS increased ($P< 0.05$) plasma levels of IL-6 at 2 h postinjection, regardless of α-tocopherol treatment. However, there was a significant ($P < 0.05$) LPS × α-tocopherol interaction in that α-tocopherol pretreatment reduced IL-6 in LPS-injected pigs but not in saline-injected pigs. Indeed, in the pigs receiving LPS, IL-6 concentrations in those receiving vitamin E were only 35% of those not pretreated with vitamin E. Plasma cortisol was also elevated ($P < 0.05$) by LPS administration, regardless of α-tocopherol treatment (**Fig. 2**), with cortisol levels peaking in both groups 2 h after LPS injection. Similar to the response in IL-6, there was an LPS × α-tocopherol interaction ($P < 0.05$). Thus, pigs receiving α-tocopherol before LPS administration had lower ($P < 0.05$) peak levels of cortisol than pigs injected with LPS and not pretreated with α-tocopherol, whereas the difference did not exist in saline-injected control pigs.

DISCUSSION

We chose to measure IL-6 as a "marker" pro-inflammatory cytokine because (unlike IL-1) methodology is available for measuring this cytokine in porcine tissue (Webel et al. 1997).

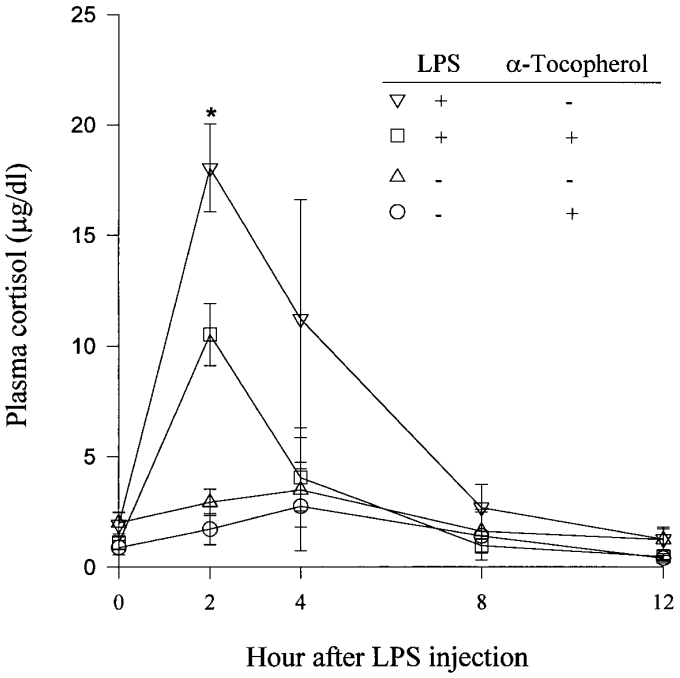

FIGURE 2 Plasma cortisol (molecular weight = 362.5) of pigs receiving saline or *d*-α-tocopherol (600 mg) injections for 3 d before being injected with a challenge dose (5 μg/kg BW) of lipopolysaccharide (LPS). Data points represent means ± SEM, n = 6. * LPS different ($P < 0.05$) from saline and, within LPS-treated pigs, α-tocopherol pretreatment different ($P < 0.05$) from no α-tocopherol.

Moreover, IL-6 is more effective than either IL-1 or TNF-α in stimulating hepatic synthesis of acute-phase proteins in response to inflammation (Johnson 1997). Also, our previous work had shown that a 5 μg/kg BW injection of LPS in pigs would cause a considerably larger elevation in IL-6 than of TNF-α. Plasma cortisol was measured because its elevation after a challenge dose of LPS occurs at the same time as the elevation in IL-6 (Webel et al. 1997). Although vitamin E is best known for its actions as an antioxidant in the protection of cellular membranes from peroxidative damage, numerous studies have suggested that vitamin E also plays an active role in the host's response to infection. Vitamin E supplementation in excess of minimal requirements has been shown to increase humoral immune responses and disease resistance in pigs (Peplowski et al. 1981), chicks (Heinzerling et al. 1974) and mice (Nockels 1979). However, we believe our study is the first to present in vivo evidence that administering high levels of vitamin E before immune stimulation attenuates the elevation of plasma IL-6.

Several in vitro studies with cultured macrophages or monocytes support our findings and suggest that vitamin E (or other antioxidants) inhibits cytokine production from activated mononuclear cells. Mendez et al. (1995) found that the LPS-induced production of TNF-α was reduced in isolated alveolar macrophages when vitamin E or the antioxidant *N*-acetyl cysteine was included in the culture media. In addition, Eugui et al. (1994) reported that a variety of antioxidants reduced the LPS-induced production of IL-1, IL-6 and TNF-α from cultured monocytes. Therefore, it seems reasonable to hypothesize that by increasing the levels of vitamin E in the plasma of animals, a reduction occurs in the in vivo production of cytokines from activated macrophages. However, few studies have sought to evaluate the effect of in vivo administration of vitamin E or other antioxidants on cytokine production. Bulger et al. (1997) found that lipopolysaccharide-induced TNF-α production was inhibited in whole blood and peritoneal macrophages obtained from rats receiving high dose, short-term enteral vitamin E supplementation. The only study that has looked at plasma levels of cytokines after administration of either antioxidants or vitamin E is the work of Eugui et al. (1994) in mice. They reported that antioxidants given shortly before LPS administration will lower plasma levels of TNF-α and IL-1. However, their studies did not evaluate the effects of vitamin E in vivo. Therefore, we believe that this study is the first to have demonstrated that high levels of vitamin E given to animals can blunt the LPS-induced increase in plasma IL-6.

We have previously reported (Warren et al. 1997, Webel et al. 1997) that LPS administration increases plasma levels of TNF-α, IL-6 and cortisol. From our previous work, it was apparent that pigs responded to LPS in a dose-dependent manner. As such, pigs receiving 5 μg/kg BW of LPS had very dramatic increases in plasma levels of TNF-α and IL-6 that were accompanied by a 10-fold increase in plasma cortisol, whereas pigs receiving only 0.5 μg/kg BW of LPS did not show these same LPS-induced elevations. Cytokines are thought to be responsible for stimulating the hypothalamic-pituitary-adrenal axis, causing secretion of corticotropin-releasing hormone (Berkenbosch et al. 1987). Therefore, it seems likely that the reduced levels of plasma cortisol observed in pigs receiving supplemental vitamin E resulted from the decreased level of inflammatory cytokines, although we can not rule out the possibility that vitamin E per se reduced the production of cortisol.

Our findings have interesting implications for both human health and animal production. For example, because IL-1 has been implicated in the regulation of atherosclerotic lesions in the arterial wall, it seems possible that reducing IL-1 production might affect the atherosclerotic process, possibly improving human health (Clinton et al. 1991, Devaraj et al. 1996, Fleet et al. 1992, Loppnow and Libby 1989). In addition, there are numerous instances in animal production in which modulation of cytokine production may be of great benefit. For example, newly weaned pigs are placed under a great deal of stress because they are removed from the lactating dam and placed in a new environment in which they are exposed to unknown pigs and numerous environmental stressors, including disease entities. Stress such as this may induce excessive production of pro-inflammatory cytokines; their reduction by vitamin E supplementation may result in improved survival and growth performance of pigs during the critical first week after weaning. In fact, an earlier report indicated that newly weaned pigs injected with vitamin E and selenium have improved survival rates compared with unsupplemented pigs (Mahan and Moxon 1980).

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