Effects of Chromium Supplementation and Lipopolysaccharide Injection on the Immune Responses of Weanling Pigs[a]

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ABSTRACT : Sixteen specific pathogen free 4-wk-old crossbred weanling pigs were allotted into a 2×2 factorial experiment to evaluate the effects of chromium (Cr) on the immune responses after lipopolysaccharide (LPS) injection. Two factors included (1) no Cr or 400 ppb Cr supplementation from chromium picolinate (CrPic) and (2) LPS injection (200 μg/kg BW, intraperitoneally) on day 21 (d 21) and 35 (d 35) as compared with saline application. Plasma samples were obtained from all piglets before (0 h) and at 2 h, 4 h, 8 h, and 24 h after LPS injection. The changes in tumor necrosis factor-α (TNF-α) and leukocyte populations after LPS injection were not significant on d 21. On d 35, the plasma TNF-α level was increased at h 2 postinjection, and supplemental Cr reduced the TNF-α level. The leukocyte populations had changed profoundly and lymphocyte subsets of $CD2^+$ and $CD8^+$ were reduced at 8 h postinjection. The blood granulocytes were increased and the percentage of $CD2^+$ was reduced in the Cr-fed group on d 35. Furthermore, Cr supplementation decreased the blastogensis of concanavalin A-stimulated peripheral blood mononuclear cells (PBMC) on d 21. These results suggest that 400 ppb Cr supplementation from CrPic in diets may modulate the immune responses in weanling pigs during LPS injection. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 10 : 1414-1421*)

Key Words : Chromium Picolinate, Lipopolysaccharide, Immune Responses, Weanling Pigs

INTRODUCTION

Trivalent chromium (Cr) may serve as an essential micronutrient for normal metabolism in human and laboratory animals (Anderson et al., 1987; Mertz, 1993). In addition to its role in the metabolic processes, substantial evidence indicates that supplemental Cr has an anti-stress effect upon calf production. In feeder calves subjected to transport-stress, supplemental Cr decreased morbidity and enhanced immune status as compared with non-Cr supplemented calves (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993). However, supplementation of Cr at 200 ppb in organic or inorganic form for weanling pigs during lipopolysaccharide (LPS) challenges did not seem to have a beneficial effect on improving the immune function (van Heugten and Spears, 1997). Conversely, Myers et al. (1995, 1997) found that Cr supplementation could modulate the cytokine production in finishing pigs and could also stimulate the peripheral blood mononuclear cells (PBMC) to produce more interleukin 2 (IL-2) and IL-6 than that in the control groups *in vitro*.

The endotoxic shock syndrome resulting from gram-negative bacterial sepsis causes high morbidity and mortality in newborn piglets (Harel et al., 1991). The LPS or endotoxin is the outer membrane component of gram-negative bacteria which is presumed to initiate a series of immunological and biochemical responses (Morrison and Ryan, 1979). Lipopolysaccharide injection has provided an opportunity to study the immune response during inflammation in piglets after dietary modulation (van Heugten et al., 1994, 1995). This study was conducted to determine the influence of LPS challenges and Cr supplementation on the immune responses in weanling pigs.

MATERIALS AND METHODS

Animals and treatments

A total of 16 specific pathogen free 4-wk-old crossbred weanling pigs (sire was Duroc and sow was Yorkshire-Landrace cross, average body weight 6.67 kg) were allotted into one of four treatments based on weight, sex, and litter origin. The experiment was performed from weaning at 28 days of age to 66 days of age (d 38). Four piglets were housed in a nursery room as one pen. The temperature in the nursery room was maintained at around 26℃ with heating supply during the first 2 weeks. Each treatment consisted of four pigs. A 2×2 factorial arrangement was used in a random design. Factors included (1) no Cr supplementation or 400 ppb Cr supplementation

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(provided by chromium picolinate, CrPic, from Prince Agri Products, Inc., USA) in the diet and (2) with LPS or saline injection. Pigs were injected intraperitoneally (i.p.) with either 1 mL of LPS solution containing LPS at 200 μg/kg BW or with saline successively on d 21 and d 35 of the experiment. LPS was obtained from *Escherichia coli* (serotype 055: B5; Sigma Chemical Co., USA).

The experimental diet (table 1) mainly consisted of corn, skimmilk, and isolated soybean protein to meet or exceed the NRC requirements for all nutrients (NRC, 1988). Feed and water were freely available. Body weight and feed intake were determined before and 3 days after each LPS injection and weekly thereafter. Rectal temperature was measured with an animal thermometer. The level of Cr in the basal diet was 1300 ppb, as determined by atomic absorption spectrophotometry (Perkin-Elmer, 5100PC, USA).

Table 1. The basal diet composition

Ingredients (%)	
Corn	79.46
Isolated soybean protein[a]	11.09
Skimmilk	5.00
Soybean oil	1.77
Dicalcium phosphate	1.29
Limestone, pulverized	0.69
Salt	0.40
Vitamin premix[b]	0.20
Trace mineral premix[c]	0.10
Calculated nutrient composition	
Metabolizable energy (kcal/kg)	3250
Crude protein (%)	19.00
Lysine (%)	1.15
Sulfur-containing amino acids (%)	0.69
Threonine (%)	0.85
Calcium (%)	0.86
Phosphorus (%)	0.65
Analyzed nutrient composition	
Chromium (ppb)	1300

[a] Pro Fam 972, ADM, Holland.
[b] Supplied per kg diet: vitamin A, 8,000 IU; vitamin D, 1,200 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_2, 8 mg; pantothenic acid, 24 mg; niacin, 80 mg; vitamin B_{12}, 40 μg and choline-chloride, 700 mg.
[c] Supplied per kg diet: Cu, 20mg; Zn, 100mg; Fe, 140 mg; Mn, 4 mg; Se, 0.1 mg and I, 0.2 mg.

Blood collection and analysis

Blood samples were collected in heparinized tubes from each pig by venipuncture in the cervical vein before (0 h) and at 2 h, 4 h, 8 h, and 24 h after LPS injection. Peripheral blood leukocytes were counted by a microcell counter (Sysmex F-800, Japan). An aliquot of blood was analyzed immediately for the leukocyte surface antigen. The other blood was centrifuged at 1,000×g for 20 min at 4℃, and the buffy coat was collected for examining PBMC blastogensis, and the plasma was frozen (-20℃) until tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) analyses were performed.

Plasma TNF-α was measured using an enzyme linked immunosorbent assay (ELISA) kit (Genzyme, USA). The intra-assay CV for TNF-α was 7.31%. Plasma CRP was measured using an ELISA method as previously described Eckersall et al. (1996). For CRP assay, rabbit anti-human C-reactive protein (Sigma Chemical Co.) and peroxidase-conjugated goat anti-rabbit IgG (Sigma Chemical Co.) were used as the first and secondary antibodies. Human CRP (Sigma Chemical Co.) was used as the standard for the calculation of unknown samples. The intra-assay CV for CRP determination was 1.64%.

The leukocyte differential cell counts and lymphocyte surface cluster of differentiation antigen (CD) expression were analyzed by flow cytometry as described by Hsu (1995). Briefly, 100 μL blood was incubated with 50 μL of monoclonal antibodies (CD2: MSA4; CD4: 74-12-4, and CD8: 76-2-11) for 30 min at 4℃ in a plastic tube. All CD antibodies were obtained from ATCC (USA) except MSA4 (from Dr. Lunny, USDA research laboratory, USA). After washing three times, the cells were incubated with 50 μL of fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin-G antibody for 30 min at 4℃ (1:80 dilution, Zymed, BK). After centrifugation, cell pellets were then treated with 2 mol/L NH_4Cl for 8 min to lyse erythrocytes and then washed three times with phosphate-buffered saline (PBS). The cells were fixed in PBS with 0.5% formaldehyde. Flow cytometry analysis was conducted on a flow cytometer equipped with Lysys software (FACStar Plus, Becton Dickinson, USA). Forward and right-angle scatter signals were displayed on a linear scale. The background fluorescense was about 1 to 3%. Differential cell counts in three-parts (monocytes, granulocytes, and lymphocytes) were performed using light scatter analysis.

The PBMC blastogenic responses to mitogen were assessed by the method of Boyum (1968) with some modifications. Concanavalin A (Con A) was used as a T cell mitogen in this study. Preliminary studies showed that 10 μg/mL Con A gave optimal blastogensis. Therefore, 10 μg/mL of Con A was chosen for the proliferation assay. The blastogensis index was calculated as count per minute (cpm) of radioactivity of mitogen stimulated/cpm of radioactivity of non-mitogen stimulated.

Statistical analysis

All data were analyzed by using the GLM

procedure of SAS (1987). Data were subjected to repeated-measures ANOVA. If a given ANOVA was significant at $p<0.05$, comparison of means was performed using Duncans multiple range test.

RESULTS

The temporal alterations in plasma TNF-α and CRP levels following the intraperitoneal injection of LPS are shown in figure 1. Injection of LPS and supplemental Cr were only shown to affect the plasma TNF-α levels at 2 h after the second LPS injection on d 35, in which the plasma TNF-α reached a peak level of 280 ± 50 pg/mL for the group without Cr supplementation. However, supplemental Cr had a lower TNF-α level of 89 ± 3 pg/mL at 2 h after LPS injection. Plasma CRP levels were increased at 8 h and at 8 h to 24 h after LPS injection on d 21 and 35, respectively. There were no differences between the Cr-fed and control group.

Figure 2 depicts the responses in numbers of monocytes, lymphocytes, and granulocytes to Cr supplementation and LPS injection on d 21 and 35. The change in scale of monocyte numbers after LPS injection was small for the first LPS injection on d 21. On d 35, a profound lowering of monocytes was noted at 2 h postinjection, which shifted to a higher monocytes concentration ($p<0.05$ vs. control) at 24 h postinjection. The changes in lymphocyte numbers after LPS injection were similar to the monocytes on d 21 and 35. Supplemental Cr did not affect the numbers of monocytes and lymphocytes. The granulocyte numbers showed an interaction between LPS injection and Cr supplementation at 24 h postinjection on d 21. On d 35, a marked granulocytopenia was observed from 2 h to 8 h postinjection, which shifted to a granulocytosis at 24 h postinjection. However, the numbers of granulocytes were significantly ($p<0.05$) higher before LPS injection in Cr-fed piglets.

The lymphocyte cluster differentiation percentages for $CD2^+$, $CD4^+$, and $CD8^+$ on d 21 and 35 are presented in figure 3. The $CD2^+$ % of lymphocytes were not significantly affected by LPS injection and Cr supplementation on d 21. Following the second

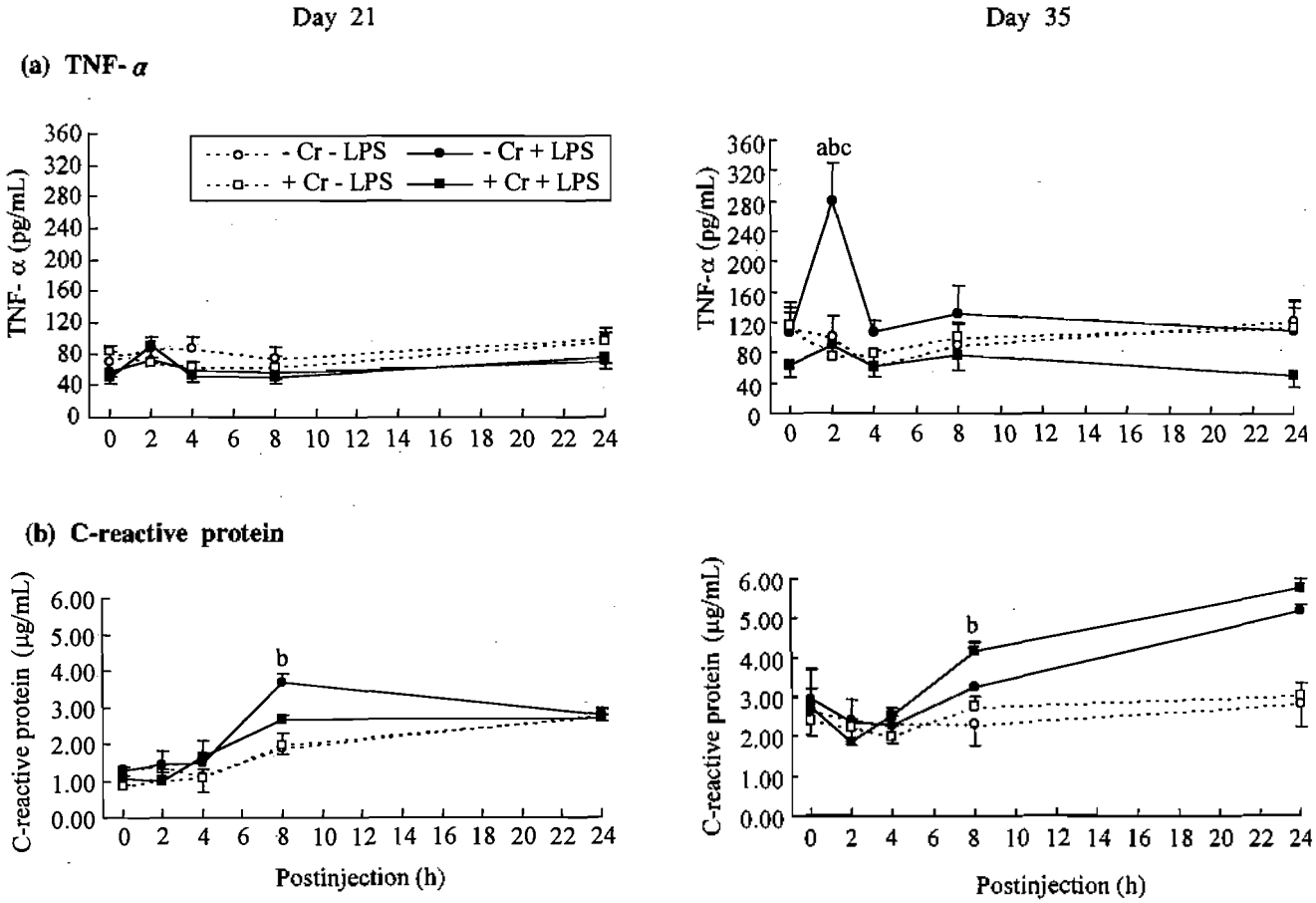

Figure 1. Effects of supplemental chromium and lipopolysaccharide (LPS) injection at day 21 and 35 on the levels of tumor necrosis factor-α (TNF-α) (a) and C-reactive protein (CRP) (b) in weanling pigs. Each point is the mean $\pm$ SE. a, b, c: Meaning the significant difference ($p<0.05$) of Cr, LPS, and the interaction, respectively.

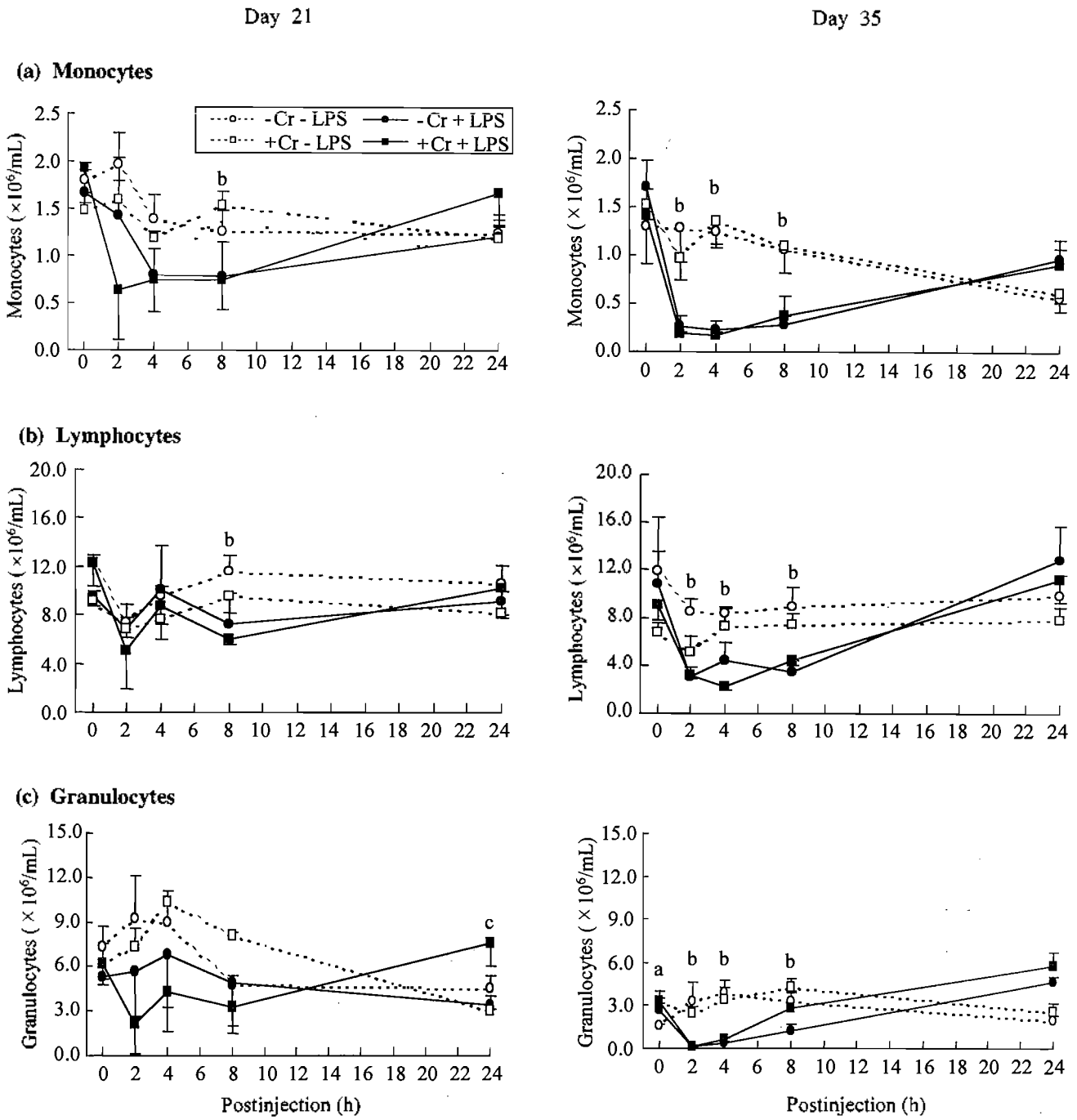

Figure 2. Effects of supplemental chromium and lipopolysaccharide (LPS) injection on the number of blood leukocytes for monocytes (a), lymphocytes (b), and granocytes (c) in weanling pigs. Each point is the mean ± SE. a, b, c: Meaning the significant difference ($p<0.05$) of Cr, LPS, and the interaction, respectively.

LPS injection on d 35, significant decreases in $CD2^+$ % were observed at 8 h and 24 h postinjection. In addition, the $CD2^+$ % was lower before LPS injection in the Cr-fed group on d 35. The $CD4^+$ lymphocyte subset had no significant changes after LPS injection on d 21 and 35, however, the percentage of $CD8^+$ lymphocytes decreased at 8 h postinjection and returned to the preinjection level at 24 h postinjection on d 35. The percentages of $CD4^+$ were decreased at 8 h and 24 h postinjection on d 21, and at 24 h postinjection on d 35 for the Cr-fed group, respectively. The ratio of $CD4^+/CD8^+$ was no different between LPS injection and Cr supplementation (data not shown).

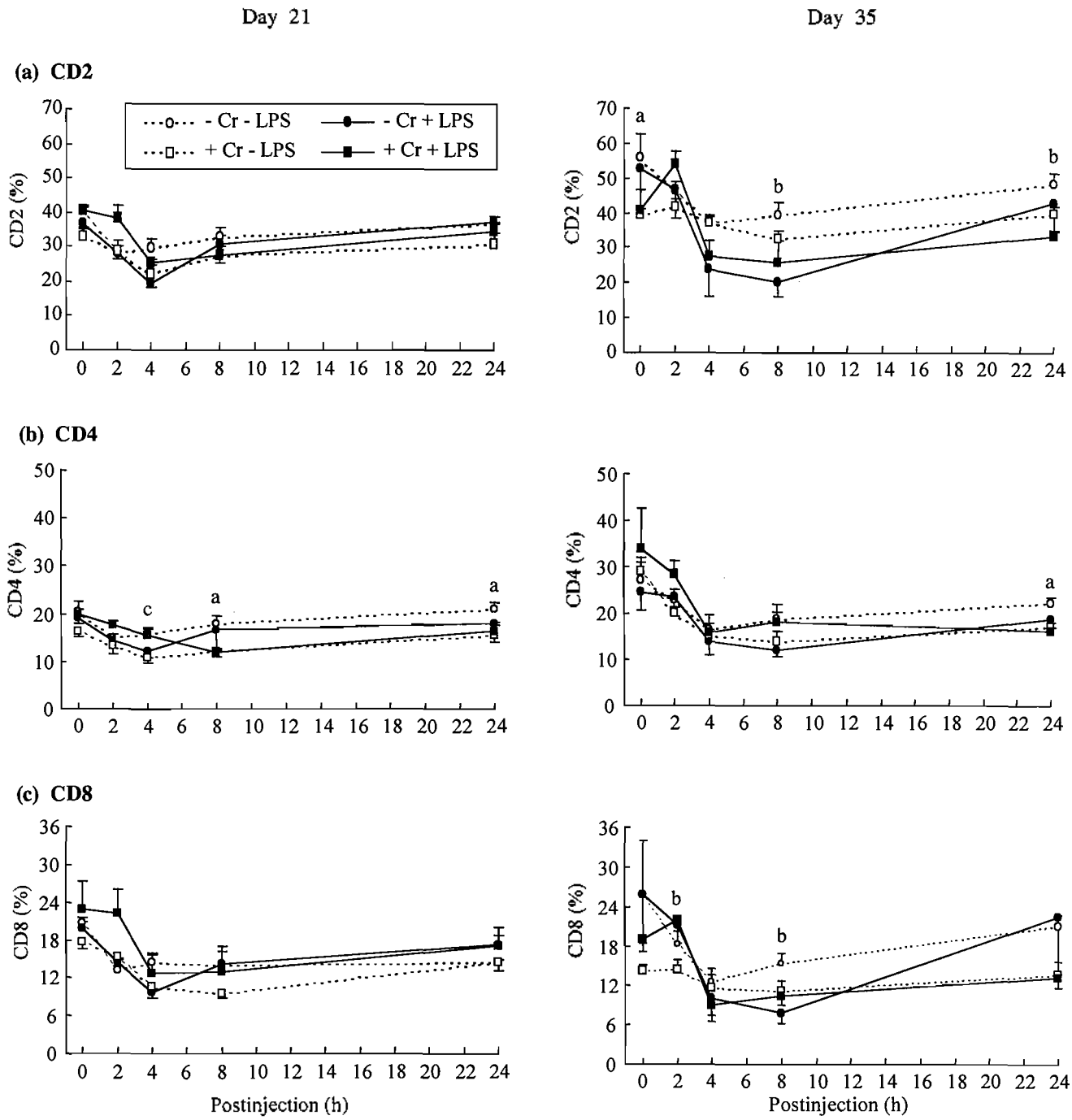

Figure 3. Effects of supplemental chromium and lipopolysaccharide (LPS) injection on the percentages of lymphocytes cluster of differentiation (CD) for CD2 (a), CD4 (b), and CD8 (c) in weanling pigs. Each point is the mean ± SE. a, b, c: Meaning the significant difference ($p<0.05$) of Cr, LPS, and the interaction, respectively.

The PBMC blastogensis after Con A-stimulation showed biphasic responses which decreased dramatically at 2 h and 4 h and was enhanced at 24 h postinjection on d 35. Before LPS injection, Cr supplementation did not alter the PBMC blastogensis after Con A-stimulation on d 21 and d 35 in weanling pigs. After LPS injection on d 21, Cr supplementation decreased the blastogensis of Con A-stimulated PBMC at 4 h postinjection. However, the means of blastogensis index of PBMC at these periods were lower than the values before injection of LPS at 0 h (figure 4).

DISCUSSION

Stress and disease can increase Cr mobilization and

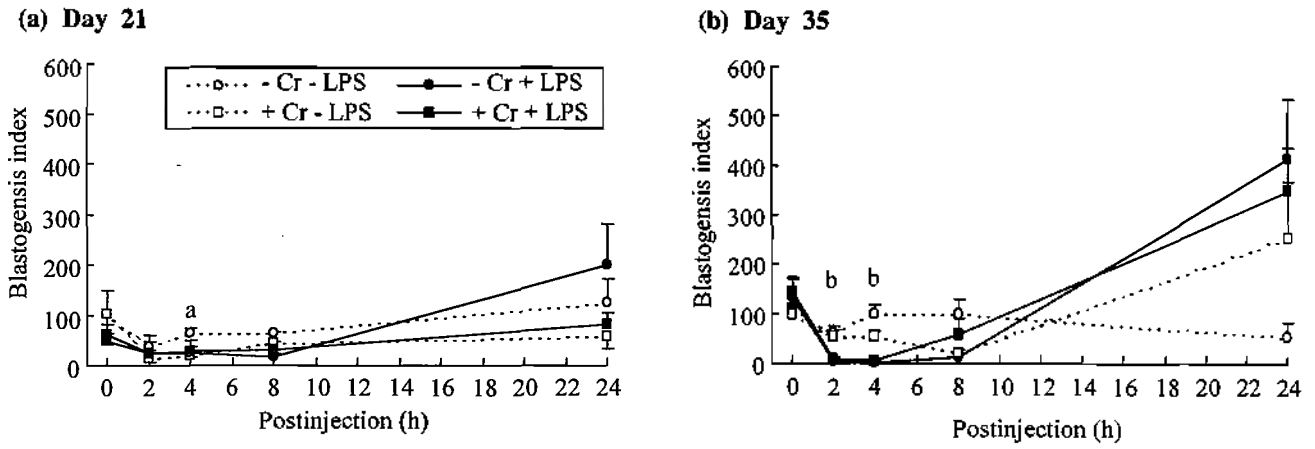

Figure 4. Effects of supplemental chromium and lipopolysaccharide (LPS) injection on the peripheral blood mononuclear cell blastogensis response to concanavalin A (Con A) on day 21 (a) and day 35 (b) in weanling pigs. Each point is the mean ± SE. a, b: Meaning the significant difference ($p<0.05$) of Cr and LPS, respectively.

excretion (Anderson et al., 1988; Borel et al., 1984; Pekarek et al., 1975) and may exacerbate a marginal Cr deficiency in humans. In this study, we used repeated LPS challenge as a stress to investigate the immune response in weanling pigs supplemented with CrPic. After LPS injection, piglets showed a typically endotoxic fever, vomiting and anastasia within 4 hours.

The temporal alterations in TNF-α level after LPS injection were similar to a previous experiment in weanling piglets. The TNF-α level was significantly increased at 2 h postinjection of LPS at 5 μg/kg BW and returned to the basal level at 8 h postinjection (Webel et al., 1997). The result of Cr supplementation reducing the plasma TNF-α levels after injection of LPS was also similar to the results of supplementation of 300 ppb Cr by CrPic after injection of LPS at 20 μg/kg BW in 90 kg pigs (Myers et al., 1997). The results in this experiment suggest that the low TNF-α levels in association with the low immune response to LPS treatment on d 35 may be due to the anti-stress effect of Cr in piglets. The phenomenon may be similar to that seen in young pigs supplemented with a surfeit level of vitamin E, which reduces the plasma TNF-α level to LPS challenge (Webel et al., 1998).

The level of CRP was alleviated at 48 hours after LPS injection in pigs (Heegaard et al., 1998). Supplemental Cr prior to shipping-stress calves in reduced the haptoglobin level at d 7 after arrival (Wright et al., 1995). Haptoglobin is an acute phase protein which is produced by the liver in response to the elevation of blood TNF-α (Murata and Miyamoto, 1993). The response was only recorded 24 hours after LPS injection in this study, which showed the effect of LPS on CRP only on the secondy injection.

The peripheral blood leukocytes alternation pattern in piglets after LPS injection was similar to the results obtained in finishing pigs (Norimatus et al., 1995) and human (Richardson et al., 1989). The evidence in human study showed that a profound granulocytopenia, lymphocytopenia, and monocytopenia is obtained before the elicited hypercortisolemia after a five-minute bolus of LPS infusion. With the rise in plasma cortisol, a reversal of the early leukocytopenia is noted after LPS injection (Richardson et al., 1989). The supplemental Cr in the present study did not affect the numbers of leukocytes. However, the numbers of granulocytes were significantly ($p<0.05$) enhanced before LPS injection when the diet was supplemented with Cr on d 35. In a study with calves, Cr as Cr-nicotinic acid complex has been reported to increase the percentage of neutrophils up to 3.50% (Kegley et al., 1996).

After LPS injection on d 35, the percentages of $CD2^+$ and $CD8^+$ were decreased at 4 h and then returned to the preinjection level at 24 h postinjection. The temporal alterations of CD subsets following the LPS injection are similar to those responses studied in human subjects (Richardson et al., 1989) and rats (Cupps et al., 1984). Calvano et al. (1992) demonstrated that the changes in CD3 (T cells), CD4, CD8, and CD20 (B cells) were due to the elevation of cortisol in human by continuous infusion of LPS. In this study, when LPS at 200 μg/kg BW was injected into piglets, the plasma cortisol level was elevated two to eight hours after injection (Lee et al., 2000). The changes in CD2 lymphocyte subset in the Cr-fed pigs after LPS injection may be correlated with vicissitude of the cortisol. A previous report (Sei et al., 1991) indicated that synthetic glucocorticoids were able to reduce $CD4^+$ lymphocytes in mice. Further studies are needed to elucidate the influence of Cr on the population and migration of lymphocyte subsets.

PBMC blastogenic response to Con A was decreased for Cr-fed piglets on d 21 after LPS injection. van Heugten and Spears (1997) observed the

increase in lymphocyte proliferation in response to pokeweed mitogen (PWM), but not in response to phytohemagglutinin (PHA) in weanling pigs when the diet was supplemented with 200 ppb organic or inorganic forms of Cr. An increase in PBMC blastogenesis with the addition of Cr to the medium (Chang et al., 1994) or the addition of serum from Cr-fed cows to the medium (Burton et al., 1995) was also found. However, Gentry et al. (1999) reported that lambs fed supplemental Cr in the diet had lower PBMC blastogensis when the cells were incubated with PWM. One possible explanation of our combined results is that Cr supplementation causes an early (before 12 h) reaction of PBMC to Con A, therefore a more rapid reaction, resulting in accelerated production and subsequent utilization of IL-2, may then reduce the PBMC blastogensis (Lee et al., 1999). The PBMC incubation time in this study was 72 h, which was longer than the 48 h in the weanling pig study (van Heughter and Spears, 1997), and similar to the study of 68 h in lambs (Gentry et al., 1999).

The decrease in Con A-stimulated PBMC blastogensis shown in this study after LPS challenge on d 21 and d 35 may be due to LPS depletion of most mature PBMC, leaving some immature cells which are hyporesponsive to mitogen, because the LPS-stimulated PBMC blastogensis was very low at 2 h and 4 h on d 35. In this study, the immune response to LPS injection changed more dramatically on d 35 than that on d 21. It seems that the endotoxic response was increased with a second challenge to the piglets. Moreover, the difference in age-related responses to endotoxin may be attributed to anatomic and physiological immaturity in the developing piglets (Li et al., 1993). Again, this work indicated that the older group appeared to present typically biphasic hemodynamic responses to endotoxin, while the younger group had a more moderate response in the early phase.

Chromium picolinate supplementation did not consistently affect the Con A-stimulated PBMC blastogensis and plasma TNF-α levels as *in vitro* cellular immunity and *in vivo* immunity indicators, respectively. However, there were some notable changes in selected blood metabolites and hormone measurements (Lee et al., 2000).

IMPLICATION

The results suggest that bacterial LPS has potent stimulating effects on immune responses in weanling piglets. Supplemental 400 ppb chromium from chromium picolinate reduced the release of TNF-α after LPS injection on d 35 and enhanced the numbers of granulocytes before LPS challenge. Thus supplemental chromium may modulate the immune responses in weanling pigs during LPS injection. The effect of Cr on PBMC blastogensis may interact with the mitogen incubation time, and must be tested in the future.

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REFERENCES

Anderson, R. A., M. M. Polansky, N. A. Bryden, S. J. Bhathena and J. J. Canary. 1987. Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. Metabolism. 36:351-355.

Anderson, R. A., N. A. Bryden, M. M. Polansky and P. A. Deuster. 1988. Exercise effects on chromium excretion of trained and untrained men consuming a constant diet. J. Appl. Physiol. 64:249-252.

Borel, J. S., T. C. Majerus, M. M. Polansky, P. B. Moser and R. A. Anderson. 1984. Chromium intake and urinary chromium excretion of trauma patients. Biol. Trace Elem. Res. 6:317-326.

Boyum, A. 1968. Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. 21(Suppl.):77.

Burton, J. L., B. J. Nonnecke, T. H. Elsasser, B. A. Mallard, W. Z. Yang and D. N. Mowat. 1995. Immunomodulatory activity of blood serum from chromium-supplemented peripartient dairy cows. Vet. Immuno. Immunopath. 49:29-38.

Calvano, S. E., A. E. Barber, A. S. Hawes, H. F. de Riesthal, S. M. Coyle and S. F. Lowry. 1992. Effect of combined cortisol-endotoxin administration on peripheral blood leukocyte counts and phenotype in normal humans. Arch. Surg. 127:181-186.

Chang, X., B. A. Mallard and D. N. Mowat. 1994. Prolifation of peripheral blood lymphocytes of feeder calves in response to chromium. Nutr. Res. 14:851-864.

Chang, X. and D. N. Mowat. 1992. Supplemental chromium for stressed and growing feeder calves. J. Anim. Sci. 70:559-565.

Cupps, T. R., L. C. Edgar, C. A. Thomas and A. S. Fauci. 1984. Multiple mechanisms of B cell immunoregulation in man after administration of *in vivo* corticosteroids. J. Immunol. 132:170-175.

Eckersall, P. D., P. K. Saini and C. McComb. 1996. The acute phase response of acid soluble glycoprotein, α1-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein in the pig. Vet. Immuno. Immunopath. 51:377-385.

Gentry, L. R., J. M. Fernandez, T. L. Ward, T. W. White, L. L. Southern, T. D. Bidner, D. L. Thompson, Jr. D. W. Horohov, A. M. Chapa and T. Sahlu. 1999. Dietary protein and chromium tripicolinate in Suffolk wether lambs: Effects on production characteristics, metabolic and hormonal responses, and immune status. J. Anim.

Sci. 77:1284-1294.

Harel, J., H. Lapointe, A. Fallara, L. A. Lortie, M. Bigras-Poulin, S. Lariviere and J. M. Fairbrother. 1991. Detection of genes for fimbrial antigens and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with diarrhea. J. Clin. Microbiol. 29:745-752.

Heegaard, P. M. H., J. Klausen, J. P. Nielsen, N. Gonzalez-Ramon, M. Pineiro, F. Lampreave and M. A. Alava. 1998. The porcine acute response to infection with *Actinobacillus pleuropneumoniae*. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid a protein are sensitive indicators of infection. Comp. Biochem. Physiol. 119B:365-373.

Hsu, J. B. 1995. Effects of essential amino acids in gestation diets on the performance and immunocompetence of first-litter sows and their piglets. Master thesis, National Chung-Hsing University, Taichang, ROC.

Kegley, E. B., J. W. Spears and T. T. Brown, Jr. 1996. Immune response and disease resistance of calves fed chromium nicotinic acid complex or chromium chloride. J. Dairy Sci. 79:1278-1283.

Lee, D. N., B. J. Chen, H. T. Yen and T. F. Shen. 1999. Effects of chromium picolinate supplementation on interleukin-2 activity of peripheral blood mononuclear cells *in vitro* and the physiological response to lipopolysaccharide injection in weanling pigs. J. Chinese Soc. Anim. Sci. 28:277-288.

Lee, D. N., C. F. Weng, H. T. Yen, T. F. Shen and B. J. Chen. 2000. Effects of chromium supplement and lipopolysaccharide injection on growth and physiological responses of weanling pigs. Asian-Aus. J. Anim. Sci. 13:528-534.

Li, J. X., J. R. Oliver, C. Y. Lu, K. D. Grantham and J. B. Philip, III. 1993. Age-related differences in response to endotoxin influsion in unanesthetized piglets. Circul. Shock. 41:40-47.

Mertz, W. 1993. Chromium in human nutrition: a review. J. Nutr. 123:626-633.

Moonsie-Shageer, S. and D. N. Mowat. 1993. Effect of level of supplemental chromium on performance, serum constituents and immune status of stressed steer calves. J. Anim. Sci. 71:232-238.

Morrison, D. C. and J. L. Ryan. 1979. Bacterial endotoxins and host immune responses. Adv. Immunol. 28:293-450.

Murata, H. and T. Miyamoto. 1993. Bovine haptoglobin as a possible immunomodulator in the sera of transported calves. Br. Vet. J. 149:277-283.

Myers, M. J., D. E. Farrell, G. M. Evock-Clover, C. V. Cope, M. Henderson and N. C. Steele. 1995. Effect of recombinant growth hormone and chromium picolinate on cytokine production and growth performance in swine. Pathobiology. 63:283-287.

Myers, M. J., D. E. Farrell, C. M. Evock-Clover, M. W. McDonald and N. C. Steele. 1997. Effect of growth hormone or chromium picolinate on swine metabolism and inflammatory cytokine production after endotoxin challenge exposure. Am. J. Vet. Res. 58:594-600.

Norimatus, M., T. Ono, A. Aoki, K. Ohishi, T. Takahashi, G. Watanabe, K. Taya, S. Sasamoto and Y. Tamura. 1995. Lipopolysaccharide-induced apoptosis in swine lymphocytes *in vivo*. Infect. Immun. 63:1122-1126.

NRC. 1988. Nutrient Requirements of Swine (9th Revised ed.), National Research Council, Washington, DC.

Pekarek, R. S., E. C. Hauser, E. J. Rayfield, R. W. Wannemacher and W. R. Beisel. 1975. Relationship between serum chromium concentrations and glucose utilization in normal and infected subjects. Diabetes. 24:350-353.

Richardson, R. P., C. D. Rhyne, Y. Fong, D. G. Hesse, K. J. Tracey, M. A. Marano, S. F. Lowry, A. C. Antonacci and S. E. Calvano. 1989. Peripheral blood leukocyte kinetics following *in vivo* lipopolysaccharide (LPS) administration to normal human subjects. Ann. Surg. 124:239-245.

SAS. 1987. SAS/STAT User's Guide (Release 6.02). SAS Inst. Inc., Cary, NC.

Sei, Y., T. Mcintyre, E. Fride, K. Yoshimoto, P. Skolnick and P. K. Arora. 1991. Inhibition of calcium mobilization is an early event in opiate-induced immunosuppression. FASEB J. 5:2194-2199.

van Heugten, E., J. W. Spears, M. T. Coffey, E. B. Kegley and M. A. Qureshi. 1994. The effect of methionine and aflatoxin on immune function in weanling pigs. J. Anim. Sci. 72:658-664.

van Heugten, E., M. T. Coffey and J. W. Spears. 1995. Effects of immune challenge, dietary energy density, and source of energy on performance and immunity in weanling pigs. J. Anim. Sci. 74:2431-2440.

van Heugten, E. and J. W. Spears. 1997. Immune response and growth of stressed weanling pigs fed diets supplemented with organic or inorganic forms of chromium. J. Anim. Sci. 75:409-416.

Webel, D. M., B. N. Finck, D. H. Baker and R. W. Johnson. 1997. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J. Anim. Sci. 75:1514-1520.

Webel, D. M., D. C. Mahan, R. W. Johnson and D. H. Baker. 1998. Pretreatment of young pigs with vitamin E attenuates the elevation in plasma interleukin-6 and cortisol caused by a challenge dose of lipopolysaccaride. J. Nutr. 128:1657-1660.

Wright, A. J., B. A. Mallard and D. N. Mowat. 1995. The influence of supplemental chromium and vaccines on the acute phase response of newly arrived feeder calves. Can. J. Vet. Res. 59:311-315.