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Embryonic exposure to lead: comparison of immune and cellular responses in unchallenged and virally stressed chickens

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Abstract Lead, a ubiquitous environmental contaminant, has been shown to modulate various functions of the immune system and decrease host resistance to infectious disease. However, limited information is available concerning the direct effects of lead on the host immune response to an infectious agent after developmental exposure. The current study utilized chickens to examine the effect of embryonic lead exposure on immune and cellular responses during viral challenge. Sublethal doses of lead were introduced into fertilized Cornell K Strain White Leghorn chicken eggs via the air sac at day 5 or day 12 of embryonic development (designated as E5 and E12, respectively). Four-week-old female chickens were inoculated with infectious bronchitis virus (IBV) strain M41. Antibody titer to IBV, delayed-type hypersensitivity (DTH) response against bovine serum albumin (BSA), the absolute number and percentage of leukocyte subpopulations, and interferon-γ (IFN-γ)-like cytokine production by splenocytes were evaluated at 5–6 weeks of age. While antibody response to IBV in juvenile chicks was unaffected by the in ovo lead exposure, IFN-γ-like cytokine production by splenocytes was significantly depressed following lead exposure at both developmental stages. In contrast with this pattern, the DTH response against BSA was unaffected following E5 exposure, but was significantly decreased after E12 exposure to lead. These changes were similar to those previously reported in chickens not exposed to IBV. While lead exposure at E5 induced significant changes in the percentage of circulating heterophils at 1 day postinfection (dpi), lead did not cause any change in relative leukocyte counts after E12 exposure. At 7 dpi, E5 lead exposure resulted in decreased absolute number and percentage of circulating lymphocytes, while total leukocyte counts, and the absolute number and percentage of circulating monocytes and heterophils were significantly reduced in E12 lead-exposed chickens. These results suggest that low-level exposure to lead has a direct effect on the developing chicken immune system, which is evident even during a postnatal infection. Furthermore, some of the changes were observed only when chicks were stressed by the viral infection. It appears that lead exposure during different stages of embryonic development is likely to result in different immunotoxic outcomes in juveniles.

Keywords Lead · Infectious bronchitis virus · Delayed-type hypersensitivity (DTH) · IFN-γ production · Leukocyte subpopulations

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Introduction

Lead is a ubiquitous environmental pollutant which has been a health concern for humans and animals (Goyer 1993). There has been growing awareness that lead, at levels below those associated with overt toxicity, can modulate various immune functions (Zelikoff et al. 1994; McCabe 1998). Previous studies have shown that lead can increase susceptibility to infectious agents (Selye et al. 1966; Hemphil et al. 1971; Cook et al. 1975; Gainer 1974, 1977; Exon et al. 1979; Lawrence 1981; Kowolenko et al. 1991; Youssef et al. 1996), as well as incidence and/or growth of tumors (Gainer 1973; Kobayashi and Okamoto 1974; Kerkvliet and Baecher-Steppan 1982). Lead appears to impair helper T lymphocyte function and regulatory processes leading to aberrant cell-mediated immunity and/or humoral immunity (McCabe and Lawrence 1991; Heo et al. 1996, 1998; Miller et al. 1998; Chen et al. 1999; Lee et al. 2001).

Developing populations (fetuses, infants and children) appear to be in the greatest risk for the toxic effects of lead exposure. Since the immune system undergoes rapid and dynamic changes during early development, even minor changes during this period can have permanent implications (Dietert et al. 2000). Pre- and postnatal lead exposure was shown to produce altered delayed-type hypersensitivity (DTH) response, T-dependent antibody response, and T lymphocyte mitogenic response in rats (Luster et al. 1978; Faith et al. 1979). Recent studies demonstrated that developing fetuses were susceptible to persistent lead-induced immunotoxicity at concentrations that did not affect the maternal immune system (Miller et al. 1998; Chen et al. 1999). In utero lead-exposed rats exhibited decreased DTH response and interferon-γ (IFN-γ) production. More recently, the possible existence of developmental windows of differential vulnerability to lead-induced immunotoxicity was supported by a study from our laboratory (Lee et al. 2001). Early in ovo exposure versus late in ovo exposure resulted in different immunotoxic outcomes in young animals in terms of DTH response and macrophage production of nitric oxide.

Limited information is available concerning the direct effects of early developmental lead exposure on the subsequent host response to disease challenge. The present study utilized chickens to evaluate the effect of differential embryonic lead exposure on immune and cellular responses to an infectious respiratory viral disease of chickens, infectious bronchitis. By using the viral challenge, the effect of early lead exposure can be compared in unstressed and disease-stressed juveniles. The chicken model can provide several advantages for developmental studies. The direct effect of lead on the fetal immune system can be examined without potential maternal effects, and lead could be introduced at precise stages of embryonic development corresponding to specific windows of immune development. Based on a prior assessment of differential lead-induced immunotoxicity in chickens (Lee et al 2001), chicken embryos were exposed to lead at day 5 or day 12 of embryonic development (E5 and E12, respectively), and then a multiple immunoassay panel was utilized to assess juvenile immune functions during a period of viral challenge.

Materials and methods

Chemicals and reagents

RPMI 1640 medium, Hank's Balanced Salt Solution (HBSS), antibiotic-antimycotic, and certified fetal bovine serum (FBS) were obtained from GIBCO (Grand Island, N.Y., USA). Diff-Quik stain set was purchased from Dade Behring Inc. (Newark, Del., USA). Sigma Chemical Co. (St. Louis, Mo., USA) supplied low endotoxin (<0.1 ng/mg detectable endotoxin) bovine serum albumin (BSA), concanavalin A (Con A), Ficoll-Hypaque (0.1077 g/ml), heparin, lead acetate, *N*-1-naphthyl-ethyl-ethylene diamine (NED), *o*-phenylenediamine dihydrochloride (OPD), phosphate-citrate buffer, sulfanilamide, disodium EDTA, sodium acetate and Tween 20. Mouse monoclonal antibody (Mab) specific to chicken IgA was prepared and characterized in the laboratory of Dr. Naqi at Cornell University (Ithaca, N.Y.) (Thompson et al. 1997). Horseradish peroxidase (HRP)-labeled goat anti-mouse conjugate was purchased from Southern Biotechnology Associates (Birmingham, Ala.). Infectious bronchitis virus (IBV)-specific IgG enzyme-linked immunosorbent assay (ELISA) kit was from Kirkegaard & Perry Laboratories (Gaithersburg, Md.).

Animals, lead exposure, and virus inoculation

Fertilized Cornell K strain White Leghorn chicken eggs were administered with a control and test solutions at days 5 or 12 of incubation, designated as E5 and E12, respectively. The sublethal dose range of lead for each developmental stage was selected based on a prior study (Lee et al. 2001). Via the air sac, groups received either lead acetate at sublethal doses for each age (5, 10 μg Pb/egg for E5; 200, 400 μg Pb/egg for E12) or sodium acetate in sterile water as the control. One hundred fertile eggs were used for each lead dose. The eggs were incubated at 37 °C with 85% relative humidity and rotated every hour. After hatching, the female chicks were housed in thermostatically controlled raised-wire batteries with a 15-h light cycle. Food (Cornell E diet) and water were provided ad libitum. A chicken embryo-adapted laboratory strain of IBV Massachusetts 41 was used for this study. At 4 weeks of age, each chicken was inoculated by eye-drop with approximately 100 chicken median embryo infective doses (EID_{50}) of the virus. There were two replicated experiments for each developmental stage examined and every experiment used a separate hatch. Protocols were approved by the Cornell University Institutional Animal Care and Use Committee and complied with NIH guidelines.

Blood lead level measurement

One day after hatching, blood was collected by cardiac puncture using powdered disodium EDTA as an anticoagulant. Lead levels in blood were determined by atomic absorption spectrometry (Parsons and Slavin 1993).

Collection and preparation of blood and respiratory lavage

Peripheral blood was collected by venipuncture using powdered disodium EDTA as an anticoagulant at 1, 7 and 14 days post-IBV infection (dpi) for total and differential leukocyte counts, and plasma collection (7 and 14 dpi only). Subsequently, chickens were sacrificed and respiratory lavages were obtained by tracheal wash (Fulton et al. 1990). Tracheas were exposed just below the glottis and the respiratory tract was flushed with 10 ml of phosphate-buffered saline (PBS). The lavage fluids were then passed through 60 μm sterile nylon mesh and centrifuged. The supernatant fluids were removed and used for the assessment of IBV-specific IgA.

IBV-specific antibody determination

IBV-specific IgA were measured in lavage fluid with the aid of an ELISA (Thompson et al. 1997). Briefly, 96-well plates were coated overnight at 4 °C with approximately 500 ng of semipurified M41 strain of IBV in carbonate coating buffer (pH 9.6). The plates were washed and treated with a blocking solution (5% dried skim milk in 0.05% Tween 20/PBS) for 1 h. Twofold dilutions of lavage fluid (starting at 1:2) were made in antigen-coated wells and incubated for 1 h at 37 °C. The plates were washed and sequentially reacted with Mabs specific to chicken IgA and HRP-labeled goat anti-mouse conjugate for 1 h with thorough washing between steps. After final washes, substrate, OPD, was added to each well and the absorbance was read at 450 nm 20 min later. Plasma anti-IBV IgG levels were determined using a commercial ELISA kit (Kirkegaard

& Perry Laboratories Inc., Gaithersburg, Md., USA) in accordance with the manufacturer's recommendations.

Delayed-type hypersensitivity (DTH)

For sensitization, 4-week-old chickens were injected subcutaneously with 0.1 ml of 20 mg/ml BSA in sterile water into the breast. On day 7, the chickens were injected again with the same amount of BSA for secondary sensitization. On day 14, 0.1 ml of heat-aggregated BSA (20 mg/ml, 80 °C for 1 h) was injected into one side of the wing web while 0.1 ml of PBS was injected into the other side of the wing web as a control. The thickness of both wing webs was measured prior to and 24 h after injection using spring-loaded calipers (Dyer Model 304, The Dyer Company, Lancaster, Pa., USA). The DTH response was calculated by comparing the difference in induration of the two wing webs using the following formula (Dietert et al. 1985):

$$\frac{\text{thickness of wing web(post} - \text{BSA injection)}}{\text{thickness of wing web (pre} - \text{BSA injection)}} - \frac{\text{thickness of wing web (post} - \text{PBS injection)}}{\text{thickness of wing web (pre} - \text{PBS injection)}} .$$

Splenocyte preparation

Spleens were aseptically removed from infected chickens in each group at 7 dpi, and single cell suspensions were prepared by forcing spleens through 100 μm sterile nylon mesh. Cells were washed and separated by centrifugation over Ficoll-Hypaque (1.077 g/ml) at 200 *g* for 30 min. Cells at the interface were collected, washed, and resuspended in 5 ml of RPMI 1640 media supplemented with 15% FBS. After incubation for 1 h at 39 °C, 5% CO_2 to remove adherent cells, splenocytes were plated at 1×10^6 cells/well in 24-well plates with 10 μg/well of the T cell mitogen (Con A) and incubated for 24 h at 39 °C, 5% CO_2. Supernatants were collected and frozen at –70 °C until analyzed for IFN-γ-like cytokine.

Interferon-γ (IFN-γ)-like cytokine bioassay

Given the lack of reagents to directly assess chicken IFN-γ, a bioassay that measures the nitric oxide induction was employed (Lowenthal et al. 1995). IFN-γ-like activity produced by splenic nonadherent cells was evaluated by measuring nitric oxide production by HD11 cells, a chicken macrophage-like cell line transformed by avian myelocytomatosis virus (Beug et al. 1979), after incubation with culture supernatants. HD11 cells in RPMI 1640 media supplemented with 5% FBS and 1% antibiotic-antimycotic were placed in 96-well plates (2×10^5 cells/well) and 100 μl of samples were added to each well. After 18 h of incubation, supernatants were removed and assayed for nitrite production by Griess reaction (Green et al. 1982).

Total and differential leukocyte counts

Total leukocyte counts of blood and respiratory lavage samples were determined with the aid of a hemacytometer. Wedge blood smears and cytospins of respiratory lavage were stained using Diff-Quik (Dade Behring Inc., Newark, Del., USA) and 200 leukocytes per sample were differentiated according to morphological criteria (Lucas and Jamroz 1961). There were two replicated experiments and 20 samples per treatment group were examined for each experiment.

Statistical analysis

Data were analyzed by a one-way analysis of variance (ANOVA) to test for overall difference among treatment groups. Fisher's least significant difference (LSD) pairwise comparison test was employed to pinpoint individual differences when the ANOVA showed difference among the groups. A P value of <0.05 was used to determine statistical significance.

Results

Blood lead levels

Lead exposure during embryonic development resulted in elevated blood lead levels in the offspring (Table 1). The sublethal lead concentrations were employed from the prior study (Lee et al. 2001). Even though a much higher administration level was used for E12 lead exposure than that of E5 lead exposure, their resulting blood lead levels were in a similar range. The lead dosages selected for this study did not cause any overt toxicity with respect to hatchability, sex ratio, or body weight.

DTH response

The DTH response against BSA in IBV-infected female offspring was compared 24 h after heat-aggregated BSA challenge by comparing the induration difference between BSA-injected and PBS-injected wing webs. The DTH function was unaffected following the E5 lead exposure when compared with controls, but was significantly depressed after E12 exposure to lead (Fig. 1). This trend and magnitude of depression at E12 was similar to that in uninfected chickens from the prior study (Lee et al. 2001).

IFN-γ-like cytokine production

IFN-γ-like cytokine production of splenic nonadherent cells was evaluated by measuring the accumulation of nitrite in the culture medium of the HD11 cell line with the splenic supernatants. As shown in Fig. 2, embryonic

Table 1 Blood lead levels in 1-day old chicks [values represent mean ± SEM ($n = 5$/group)]

Developmental stage of exposure	Treatment (μg Pb/egg)	Blood lead level[a] (μg/dl)
5-day embryo	Control	$<2.0^{b}$
	5	5.4 ± 0.7^{c}
	10	8.2 ± 0.6^{d}
12-day embryo	Control	$<5.0^{b}$
	200	11.0 ± 0.6^{c}
	400	19.4 ± 3.5^{d}

[a]All control values were below the assay detection limits. At the time the 12-day samples were analyzed, the detection limit was 5.0 μg/dl, which was reduced to 2.0 μg/dl for the 5-day sample analyses

[b,c,d]Means within a column with different superscript letters are significantly different ($P<0.05$)

exposure to lead at both developmental stages induced significantly decreased IFN-γ-like cytokine production.

IBV-specific antibody production

IgG and IgA antibody production to IBV was compared by lead treatment and exposure time point. Both E5 and E12 lead exposure did not alter anti-IBV IgG and IgA production at 7 and 14 dpi (data not shown). Mean titers of IgA (Log_2) and IgG for both lead-exposure groups were 2.9 and 960, respectively.

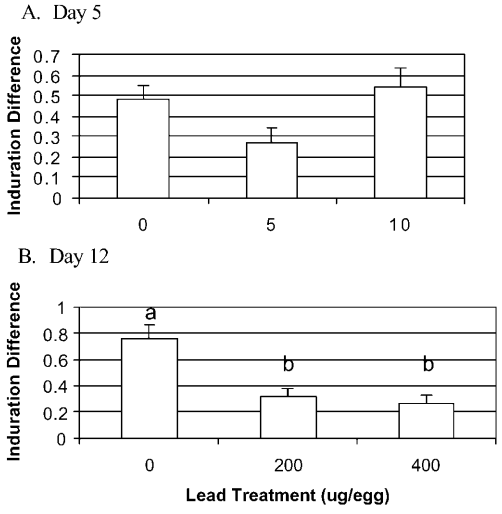

Fig. 1 Delayed type-hypersensitivity (DTH) to BSA in female chickens exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. The DTH response was measured 24 h after heat-aggregated BSA challenge, as the difference in induration between BSA-injected and control wing webs. Data shown are from two different experiments of 20 birds/group. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

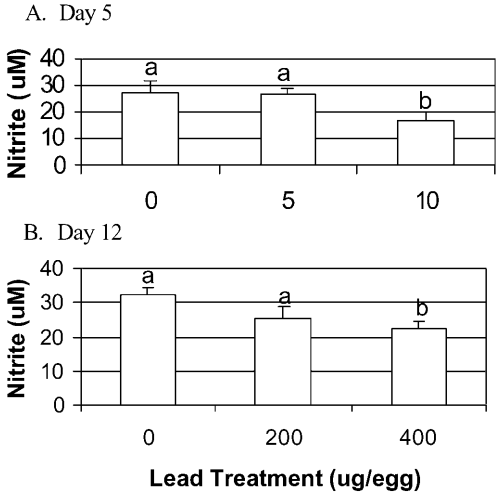

Fig. 2 IFN-γ-like cytokine production in female chickens exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. Supernatants were collected from 24-h ConA-stimulated nonadherent splenocyte cultures and then tested for their ability to induce nitric oxide secretion by HD11 cells. Data shown are from two different experiments of 20 birds/group. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

Total and differential leukocyte counts

Absolute and relative leukocyte counts were compared in the peripheral blood and the respiratory lavage (7 dpi only) at 1, 7, and 14 dpi. While lead exposure at E5 significantly decreased the percentage of circulating heterophils and increased the percentage of lymphocyte at 1 dpi, lead did not cause any change in relative leukocyte counts after E12 exposure (Fig. 3). No significant differences were noted in total leukocyte counts (TLCs) and absolute leukocyte counts at both exposure groups at 1 dpi (Fig. 4). Differential counts in the peripheral blood at 7 dpi showed major shifts in populations after embryonic lead exposure (Figs. 5 and 6). E5 lead exposure resulted in a decreased percentage of circulating lymphocytes as well as a statistically significant decrease in absolute number of lymphocytes. At 7 dpi, TLCs and absolute number of circulating monocytes and heterophils decreased in E12 lead-exposed chickens without any change in relative leukocyte counts (Figs. 5 and 6). It should be noted that in ovo lead exposure did not

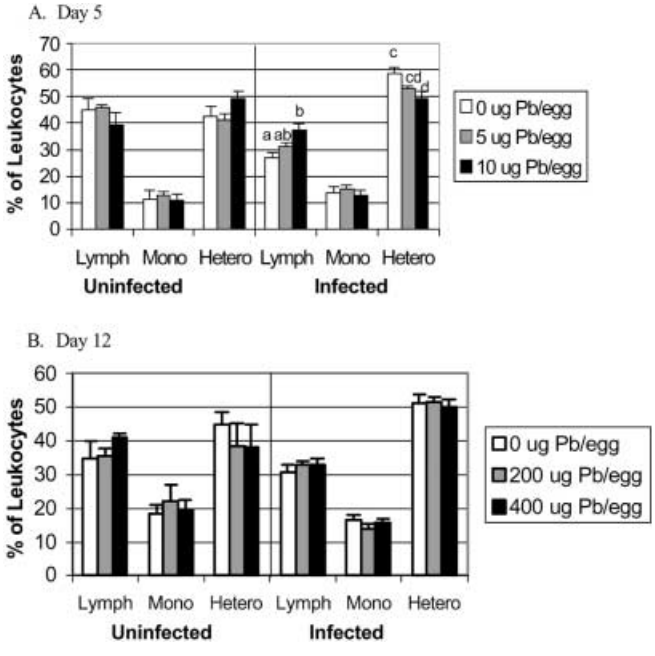

Fig. 3 Relative leukocyte counts in peripheral blood at 1 day postinfection. Female chickens were exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. There were uninfected birds for each treatment group (5–8 birds/group). Data shown for infected birds are from two different experiments of 20 samples/group. Lymph, Mono, and Hetero stand for lymphocyte, monocyte, and heterophil respectively. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

induce any juvenile change in absolute and relative leukocyte counts in the absence of infection (Figs. 3, 4, 5, and 6). While only 5–8 uninfected birds were available for each lead-exposed group, this still permitted a statistical comparison of uninfected versus infected groups. No difference in TLCs and differential counts was noted in the respiratory lavage from both exposure groups at 7 dpi (Figs. 7 and 8). Since uninfected birds have few harvestable respiratory cells, it is impossible to examine leukocyte counts from uninfected control respiratory lavage samples. Also, there was no difference in TLCs and differential counts in the peripheral blood from both exposure groups at 14 dpi (data not shown).

Discussion

Numerous studies have demonstrated that lead exposure of adults produces impaired host resistance to bacterial and viral infections. For example, Selye et al. (1966) reported that a single exposure of rats to lead markedly increased their sensitivity to otherwise well-tolerated amounts of various bacterial endotoxins, and a similar study was reported in chickens (Truscott 1970). Likewise, lead-exposed mice showed greater susceptibility to intracellular pathogens like *Salmonella typhimurium* and

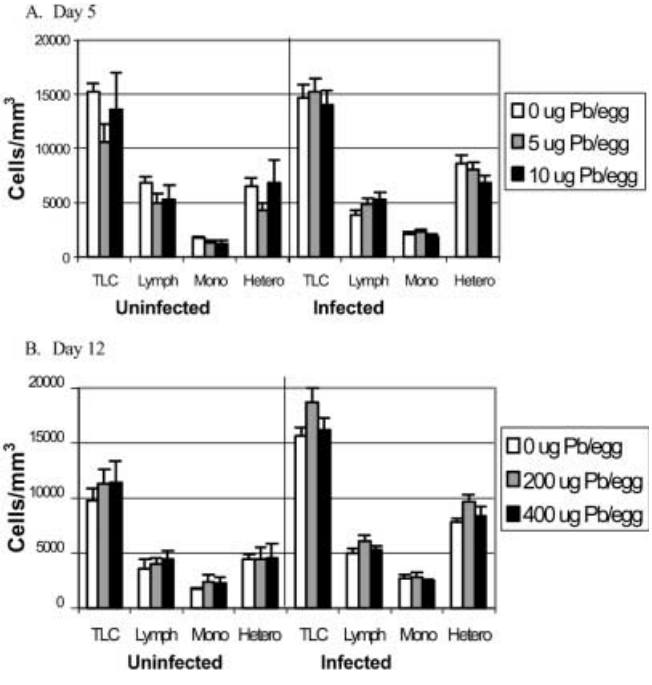

Fig. 4 Absolute leukocyte counts in peripheral blood at 1 day postinfection. Female chickens were exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. There were uninfected controls for each treatment group (5–8 birds/group). Data shown for infected birds are from two different experiments of 20 samples/group. TLC, Lymph, Mono, and Hetero stand for total leukocyte count, lymphocyte, monocyte, and heterophil respectively. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

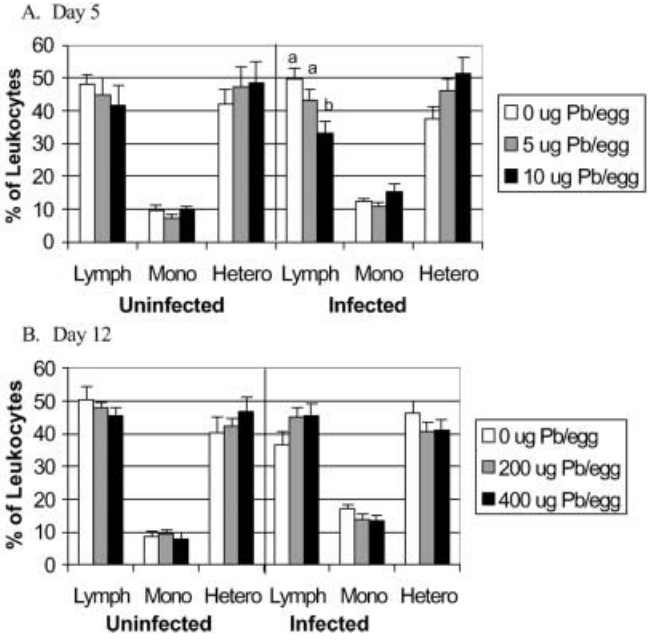

Fig. 5 Relative leukocyte counts in peripheral blood at 7 days postinfection. Female chickens were exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. There were uninfected controls for each treatment group (5–8 birds/group). Data shown for infected birds are from two different experiments of 20 samples/group. Lymph, Mono, and Hetero stand for lymphocyte, monocyte, and heterophil respectively. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

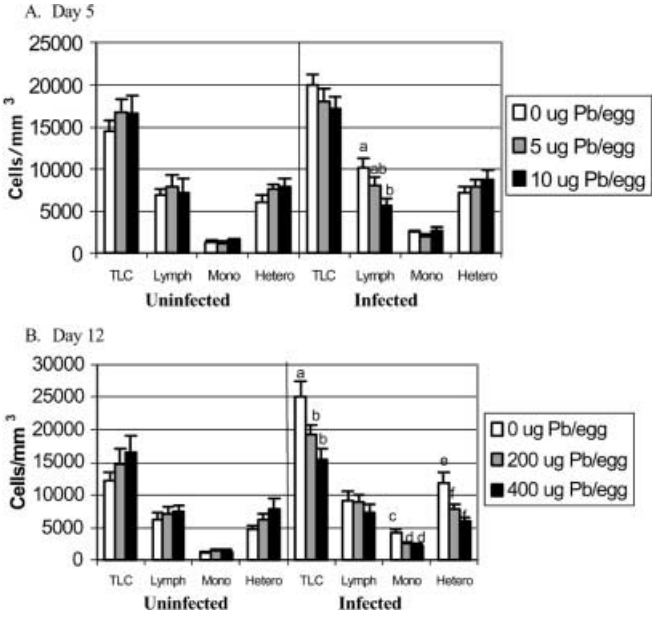

Fig. 6 Absolute leukocyte counts in peripheral blood at 7 days postinfection. Female chickens were exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. There were uninfected controls for each treatment group (5–8 birds/group). Data shown for infected birds are from two different experiments of 20 samples/group. TLC, Lymph, Mono, and Hetero stand for total leukocyte count, lymphocyte, monocyte, and heterophil respectively. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

Listeria monocytogenes (Hemphil et al. 1971; Lawrence 1981; Kowolenko et al. 1991). Antiviral and antitumor immunity was compromised by lead exposure in mice and hamsters (Gainer 1973, 1974, 1977; Kobayashi and Okamoto 1974; Kerkvliet and Baecher-Steppan 1982). Finally, an increased incidence of colds and influenza in workers with elevated blood lead levels (Ewers et al. 1982) has been reported suggesting that lead also suppresses disease resistance of humans. However, these studies were restricted to an assessment of host resistance following adult or juvenile lead exposure. Information on host immune responses and/or host defense following embryonic or neonatal lead exposure has been lacking.

The potential for persistent immunotoxic effects following early low-level chemical exposure has garnered recent attention because the developing immune system has been identified as a particularly sensitive target for chemically-induced immunomodulation (Dietert et al. 2000; Holladay and Smialowicz 2000). Pre- and/or postnatal lead exposure has been shown to induce persistent immunotoxic effects in offspring (Luster et al. 1978; Faith et al. 1979; Miller et al. 1998; Chen et al. 1999). However, information about potential differences in vulnerability to immunotoxins such as lead during the course of development has been lacking (Dietert et al. 2000). Prior results using 5, 7, 9 and 12-day chicken embryos suggested that the immunotoxic effects of lead might differ depending upon the timing of exposure (Lee et al. 2001). When chicken eggs were exposed to lead late in embryonic development (E12), female offspring exhibited significantly depressed DTH response. In contrast, the primary alteration was decreased nitric oxide production by macrophages with early exposure (E5, E7, and E9) while IFN-γ-like cytokine production would be affected at several ages. Differences in immunotoxic outcome were also reported following early gestational exposure (days 3–9) versus late gestational exposure (days 15–21) to lead in rats (Bunn et al. 2001).

The objective of the present study was to examine the potential impact of early exposure to lead on host immune and cellular responses both with and without a postnatal viral challenge. Infectious bronchitis is a highly contagious viral respiratory disease of chickens. IBV infection is known to stimulate a strong cell-mediated response with cellular infiltration which eliminates the virus from the respiratory tract (Chubb et al. 1987, 1988; Thompson and Naqi 1997); hence IBV challenge provided an excellent opportunity to examine possible persistent effects of early lead exposure on the mobilization and distribution changes associated with the host cellular responses. We hypothesized that in ovo exposure to lead at different stages of development could produce differences in subsequent immune and cellular response profiles including those associated with the viral challenge.

Lead-induced immunotoxicity observed in the present study was associated with low blood lead levels at hatching even though significantly higher levels of lead were administered at E12 compared to E5. It is also important to note that the same lead dose (200 μg/egg) given at E9 and E12 produced the same blood and bone lead levels and yet resulted in different immunotoxic outcomes (Lee et al. 2001). Therefore, differential immunotoxicity after E5 versus E12 exposure may result from different target organ susceptibilities involving immune maturation rather than from different administration levels and/or different pharmacokinetics of

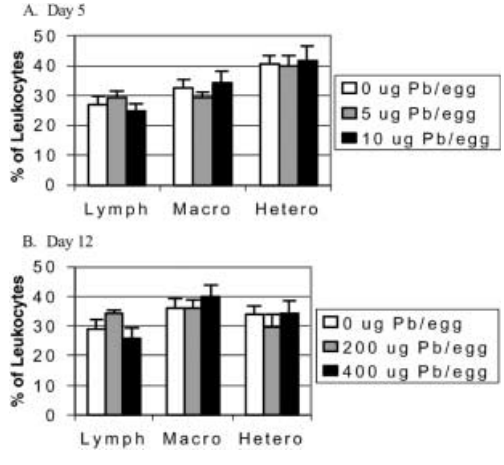

Fig. 7 Relative leukocyte counts in respiratory lavage at 7 days postinfection. Female chickens were exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. Data shown are from two different experiments of 20 samples/group. Lymph, Macro, and Hetero stand for lymphocyte, macrophage, and heterophil respectively. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

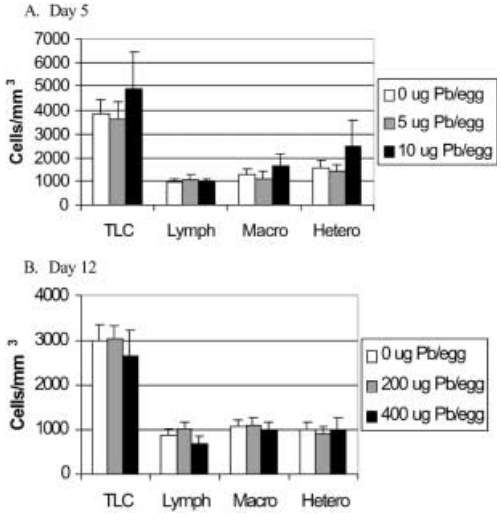

Fig. 8 Absolute leukocyte counts in respiratory lavage at 7 days postinfection. Female chickens were exposed to lead at embryonic day 5 (**A**) or day 12 (**B**) and infected with IBV at 4 weeks old. Data shown are from two different experiments of 20 samples/group. TLC, Lymph, Macro, and Hetero stand for total leukocyte count, lymphocyte, macrophage, and heterophil respectively. Values represent mean ± SEM. Means with different letters are significantly different at $P < 0.05$

lead. One possible explanation for this is that the seeding of the thymus occurs in three distinct waves during in ovo chicken development (Gobel 1996). Introduction of lead at different stages of embryonic development might result in differential risk of damage across these waves of pre-T lymphocytes.

Previous studies addressed depression of the DTH response as a hallmark of lead-induced immunotoxicity in both adults and juveniles (Faith et al. 1979; Miller et al. 1998; McCabe et al. 1999). In a prior study from our laboratory, a lead-induced decline in DTH function was not detected unless exposures were later than 9 days of embryonic development (Lee et al. 2001), and in the present study, the DTH response to BSA was depressed in the E12 (200 and 400 μg Pb/egg) lead treated groups, but not in E5 exposures. Since the magnitude of DTH depression in IBV-challenged chickens was similar to that previously observed for unchallenged chickens after E12 lead exposure, infection with IBV did not appear to either exacerbate or eliminate the extent of the decrease in cell-mediated immune response observed following embryonic lead treatment.

In the examination of host cellular responses during IBV infection, embryonic exposures to lead produced changes which differed depending upon the age of lead exposure. Lead exposure at E5 resulted in significantly decreased relative heterophil counts in the peripheral blood at 1 dpi while E12 lead exposure did not cause any change. At 7 dpi, E5 exposure to lead resulted in a decreased percentage and absolute number of circulating lymphocytes. In contrast, TLCs and absolute numbers of circulating monocytes and heterophils decreased in E12 lead-exposed chickens without any change in relative leukocyte counts at 7 dpi. Other studies have indicated that lead exposure can produce a significantly lower number of helper T lymphocytes and memory $CD8^+$ cells as seen in lead-exposed workers (Fischbein et al. 1993; Undeger et al. 1996; Sata et al. 1998). However, limited information is available concerning the effects of lead on the number of circulating polymorphonuclear leukocytes and monocytes. It is important to note that early lead exposure caused no observed peripheral blood leukocyte population changes in the absence of the IBV infection. The infection itself did produce changes, as the population profile of uninfected controls was different from that of infected birds. Therefore, infection with virus appears to result in differential lead-induced leukocyte population changes depending on the time of exposure; those changes were not observed in uninfected controls following embryonic lead treatment. Also, these changes occurred at generally lower blood lead concentrations (5.4–19.4 μg/dl) than those (19.0–74.8 μg/dl) of other studies (Fischbein et al. 1993; Undeger et al. 1996; Sata et al. 1998). Therefore, in contrast with the depressed DTH function, which has been seen in both uninfected and infected birds following E12 lead exposure, lead-induced changes in peripheral blood leukocyte profiles were observed only during the stress of host response to the virus.

The present study illustrates that exposure of the chicken embryo to lead at different stages of embryonic development can produce different immunotoxic outcomes in young animals both in uninfected animals and during disease challenge. Corresponding differences in immunotoxicity were recently observed in mammalian species based on the time of gestational exposure to lead (Bunn et al. 2001). However, it is not yet known if such stage-based differences exist in the human. The actual impact of the early lead exposure on the course of the IBV infection is not clear. Respiratory lavage antibody titer did not differ among groups nor did the numbers of elicited leukocytes. However, the observations that host immune function (DTH and IFN-γ-like cytokine production) was depressed and that inflammatory stressors revealed differential blood leukocyte profile does raise questions about the capacity of these lead-exposed animals to withstand additional disease challenges.

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