Free Radical Research, 2003 Vol. 37 (4), pp. 437–445

Taylor & Francis healthsciences

Alpha Tocopherol Protects Against Immunosuppressive and Immunotoxic Effects of Lead

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Accepted by Professor A. Azzi

(Received 11 November 2002; In revised form 19 November 2002)

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Chronic exposure to lead (Pb) is associated with multi-organ toxicity. The precise mechanism(s) involved, however, remains incompletely defined. The present study was undertaken to analyze the effect of Pb on the immune system and determine the ability of α tocopherol (AT) to reverse Pb-induced immunotoxicity. Groups of TO Mice (6 per group) were treated ip for 2 weeks with saline alone, Pb acetate alone, Pb plus AT, or with AT alone. Spleens were then analyzed for (i) cellular composition by flow cytometry, (ii) cellular response to B and T cell mitogens and (iii) production of nitric oxide (NO). Pb treatment resulted in a significant state of splenomegaly associated mainly with an influx of $CD11b^+$ myeloid cells. Surprisingly, however, these cells exhibited no upregulation in expression of activation markers and did not produce NO. The lymphocyte mitogenic responses were inhibited by $\geq 70\%$ in Pb-treated group. Concurrent treatment with Pb and AT resulted in almost a complete reversal of Pb-induced splenic cellular influx. Despite this, however, mitogenic responses in Pb + AT treated group were approximately 50% of those observed in normal (saline-treated) controls. We conclude that (1) chronic treatment with Pb acetate induces a state of splenomegaly and decreased proliferation in response to mitogenic stimuli and (2) co-treatment with AT largely reversed the cellular influx but this was associated with only a partial improvement of the mitogenic responses. These results highlight the role of AT as a potentially effective antioxidant in the immune system.

Keywords: Free radical; Lead; α Tocopherol; Immunotoxicity; Macrophages; Nitric oxide

INTRODUCTION

Persistent environmental or occupational exposure to inorganic lead (Pb) is known to adversely affect human health. The toxic effects of Pb appear to be multisystemic, influencing the nervous,[1] immune,[2] endocrine[3] and reproductive systems.[4] Most of the studies on Pb toxicity addressed the effects on the nervous system and there is good evidence demonstrating that Pb can cause serious neurotoxicity in children.[5] In comparison, less data is available on the effect of Pb on the immune system.

A limited amount of data suggest that the biochemical and molecular mechanisms of Pb toxicity involve the induction of oxidative stress in target cells, partly via the activation of reactive oxygen species (ROS), followed by DNA damage and apoptosis.[6–8] Curiously, while Pb can upregulate the production of ROS, it has been reported to have a suppressive effect on the secretion of nitric oxide, another free radical compound.[9] These findings suggest that the mechanisms governing Pb immunotoxicity may be more intricate than presently appreciated. Nevertheless, indirect support for the involvement of reactive oxygen radicals in Pb toxicity has come from studies demonstrating beneficial effects of antioxidants on Pb-induced toxicity in various tissues, including brain, liver, kidney, sperm and blood cells.[10–13]

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1071576031000076277

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A few studies have described alterations in immune system parameters in children following exposure to environmental Pb.[14,15] In animal studies, several forms of Pb-induced immunotoxicity affecting a variety of immune system cells, including B and T lymphocytes and macrophages, have been documented. Overall, the net outcome of Pb toxicity on the immune system appears to be a lowering of immune defense mechanisms and a concomitant impairment in host resistance to microbial infections.[16] This state of decreased immunity has been ascribed to a skewing of the immune system from a Th1 to a Th2-predominant phenotype.[17] In fact, one study concluded that the administration of IL-12, a Th1-promoting cytokine, can reverse the immunotoxic effects of Pb resulting in increased resistance to intracellular infections in Pb-treated animals.[18] However, the basis for the selective susceptibility of different cell types to toxic effects of inorganic Pb compounds is still unknown.

The current study has two main aims. First, we investigated the effect of systemic exposure to Pb on splenic cellular composition and functional responses in TO mice. Second, the potential influence of the antioxidant, α-tocopherol (AT), on Pb-induced immunotoxicity was evaluated. Our data indicate that Pb can induce significant alterations in the number and functional capacity of spleen cells *in vivo*. Moreover, we demonstrate that AT has the capacity to reverse, almost completely, the toxic consequences of Pb exposure on murine splenocytes.

MATERIALS AND METHODS

Mice

TO mice, purchased from Harlan Olac (Bicester, UK) and bred in the animal facility of the Faculty of Medicine and Health Sciences, UAE University, were used throughout. Animals were housed in groups of five in plastic cages with a controlled light and dark cycle of 12 h each at 24–26°C. Male mice weighing 25–32 g were used for all experiments. Mice received rodent chow and water *ad libitum*. All studies involving animals were carried out in accordance with, and after approval of, the animal research ethics committee of the Faculty of Medicine and Health Sciences, UAE University.

Reagents

Pb acetate and AT were purchased from Sigma Chemical Company (St Louis, MO). For flow cytometric analysis, conjugated mAbs specific to TCR Cβ chain, B220, CD4, CD8, Sca-1 (Ly-6A/E) and CD 11b (α_m subunit of Mac-1) were obtained from Pharmingen (San Diego, CA).

Experimental Protocol

Twenty-four age-matched TO mice were randomly assigned into four groups (6 animals per group). Group I served as control and received daily ip injection of sterile normal saline for a total period of 2 weeks. Group II (Pb group) mice received daily 1 mg/kg body weight Pb acetate (in 0.2 ml volume ip) for 2 weeks. Group III (Pb plus AT group) received Pb acetate plus a dose of 100 mg/kg body wt of AT (prepared in soya bean oil) ip daily for 2 weeks. Group IV (AT group) received daily ip injections of AT only. All animals were sacrificed by cervical dislocation one day after the end of treatment and the spleens were excised immediately and analyzed.

Flow Cytometric Analysis

Single spleen cell suspensions were prepared from treated animals following a standard protocol.[19] Following hypotonic shock to eliminate RBC, cells were washed and resuspended in staining buffer (PBS/1% FCS/0.1% NaN_3) to a concentration of 1×10^7/ml. Aliquots of 100 μl (1×10^6 cells) were dispensed into wells of a round-bottom 96-well plate and incubated with anti-CD16/CD32-specific mAb (clone 2.4G2) for 15 min on ice to block all FcγIII/II receptor sites. Cells were then double stained with a combination of directly conjugated mAbs to TCR Cβ chain (detects all $TCR\alpha\beta^+$ cells), CD4 (stains all $CD4^+$ T helper cells), CD8 (stains all $CD8^+$ T cytotoxic cells), B220 (stains all B cells), CD11b (detects myeloid lineage cells) and Sca-1 (activation marker of lymphocytes and macrophages). All antibodies were pre-titrated in preliminary experiments and used at saturating concentrations. Cell staining was done for 30 min on ice and washed cells were analyzed on a FACScan (Beckton Dickinson, Mountain View, CA). Data collected on 20,000 cells were analyzed using CELLQUEST software.

Cell Preparation and *In Vitro* Culture

Erythrocyte-depleted single cell suspensions of spleens were prepared as previously described.[20] For cell culture studies, cells were supplemented with 5% FCS, L-glutamine, sodium pyruvate, essential amino acids, non-essential amino acids, pen/strep, gentamicin and 2-ME (all reagents from GibcoBRL, Paisley, UK). Cells were cultured, without further stimulation, at a concentration of 1.0×10^7 cells/ml and incubated for 48 h at 37°C with 5% CO_2. Culture supernatants were then collected, spun free of any cells and kept at −20°C until assayed for nitrite content. The functional capacity of lymphocytes was studied by measuring the proliferative

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response to the mitogens concanavalin A (ConA; for T cells) and lipopolysaccharide (LPS; for B cells). For these studies, whole spleen cells (2×10^5 cells/well in 100 μl volume) were cultured with ConA (at final concentrations of 0.05–5.0 μg/ml) or LPS (final concentrations of 0.3–30.0 μg/ml) in a total volume of 200 μl/well in microtiter plates. After 48 h of culture, the wells were pulsed with [^{3}H]TdR (1 μCi/ml) and cells harvested 20–24 h later. Radioactivity counts were determined using a β counter.

Nitric Oxide Determination

Accumulation of NO_2^- was used to determine production of NO according to the Griess method, as detailed elsewhere.[21] Briefly, 100 μ1 of cell-free culture supernatant were mixed with an equal volume of Griess reagent and incubated at room temperature for 10–15 min. The absorbance at 562 nm was measured in an automated microplate reader. Nitrite concentration was quantitated using $NaNO_2$ as standard and expressed as μM $NO_2^-/1\times10^7$ spleen cells.

Statistical Analysis

Statistical significance was analyzed using Student's *t* test. Differences between experimental groups were considered significant when p values were <0.05.

RESULTS

Pb Treatment Induces Splenomegaly

The potential influence of Pb exposure on the immune system was first assessed by the effect on gross morphology of spleens. As shown in Fig. 1, systemic exposure to Pb acetate induces a 1.5-fold increase in spleen weights (from a mean ± SEM of 220.7 ± 7.3 mg in saline group to 332.7 ± 9.5 mg in Pb-treated group; $p = 0.0008$). The Pb-induced splenomegaly was reversed by co-treatment with AT (mean ± SEM of 235 ± 13.3; $p = 0.4615$ compared to the saline group). Interestingly, mice treated with AT alone had significantly lower spleen weights as compared to saline-injected animals (mean ± SEM of 165.3 ± 4.7; $p = 0.0027$ compared to the saline group). Taken together, the data suggest that the splenomegaly response in Pb-exposed animals may be associated with an increased state of oxidative stress and that this can be ameliorated by co-administration of AT.

Phenotypic Analysis of Spleen Cell Populations

Spleen cells obtained from the four different treatment groups were analyzed by flow cytometry

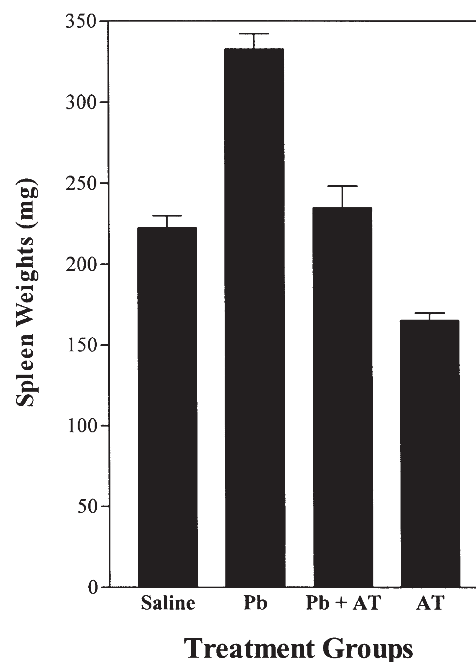

FIGURE 1 Treatment with Pb results in marked splenomegaly which is reversed by AT. TO mice were given daily intraperitoneal injections of saline alone, Pb acetate alone, Pb plus AT or AT alone for a total period of 2 weeks. At the end of treatment, spleens were removed and weighed. The data depict the mean ± SEM of three mice per group. The results are representative of three independent experiments.

for any alteration in the cellular composition of the spleen and to determine the underlying cause of the observed Pb-associated splenomegaly. As shown in Fig. 2A–D, the extent of the splenic cellular influx, delineated on the basis of FSC/SSC profile by the indicated gate, differs among the four groups of animals. In saline-treated mice (panel A), The percentage of cells exhibiting relatively elevated FSC/SSC profile is 6.6%, while the majority of splenocytes are observed outside this gate, reflecting the standard phenotype of resting spleen lymphocytes. In the Pb-treated group, the percentage of cells within the gate rises significantly to 49.7%, (panel B). This rise appears to be mostly due to the influx of inflammatory cells (mainly macrophages and neutrophils) into the spleen (see below). The degree of Pb-induced cellular influx was largely reversed by concomitant administration of AT where the percent of inflammatory cells was reduced to 18.5% of the total spleen cell population (panel C). As a control, mice treated with AT alone also exhibited a pattern that was akin to saline-treated group, with approximately 15.1% of inflammatory cells being registered within the gate (panel D).

The cellular composition of the various spleen populations was also analyzed. For the sake of clarity, the profiles of the saline, Pb and Pb + AT groups are shown only. Figure 3 shows the profile of the total, ungated, splenocyte population after

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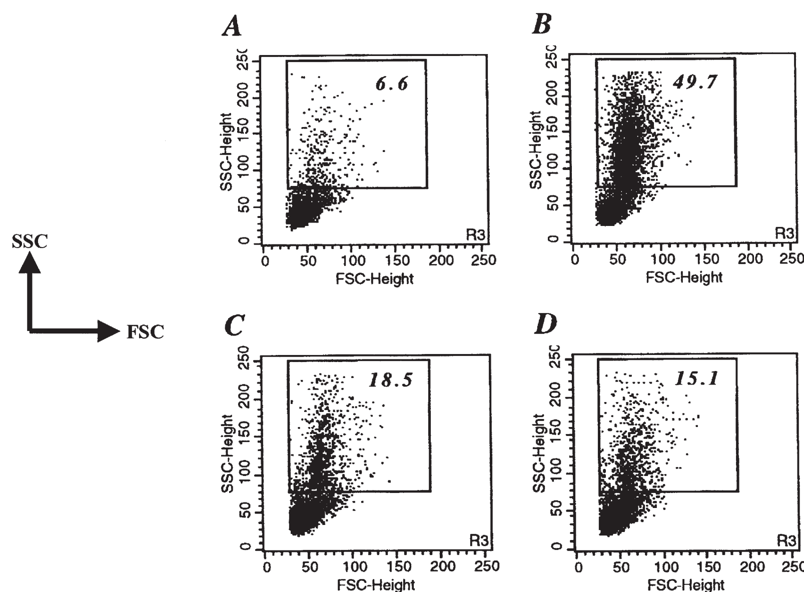

FIGURE 2 Extent of splenomegaly in Pb-treated animals. Single cell suspensions were prepared from representative spleens at the end of treatment with saline (panel A), Pb (panel B), Pb + AT (panel C) or AT alone (panel D). The results are shown as dot plots depicting FSC (indicative of cell size) vs. SSC (indicative of cellular granularity). The percentage of cells with elevated SSC (representing the cellular influx) is shown in each panel. Data from a total of 20,000 cells per group were collected and analyzed. The results are representative of three independent experiments.

staining with mAbs to TCR Cβ vs. B220 (panels A–C). In saline-treated animals, 51.3 and 35.5% of total splenocytes stained positive for B220 or TCR which represent the total B and T cell populations, respectively (panel A). Pb treatment resulted in a decrease in the percentage of both cell populations to 31.1 and 17.2%, respectively (panel B). This decrease could be the result of either lymphocyte-specific toxicity of Pb or it could be due to a dilution effect resulting from the cellular influx into the spleen. In fact, in this group, the cells with the highest representation among the spleen population, accounting for 49.0%, were negative for both B220 and TCR antigens. In the third group of animals, concurrent treatment with Pb and AT resulted in full restoration of T lymphocyte ratio to 35.7% but a partial restoration of B cells to 40.5% (panel C). Importantly, this restoration of T and B lymphocyte ratios appears to be largely due to the inhibition of recruitment of non-lymphocytic cells (non-B, non-T cells account for only 16.9% of total splenocytes in this group). Further analysis of the relationship between cell ratios and spleen cellularity revealed that despite the apparent decrease in splenic B and T cell ratios, there was in fact a slight increase in the absolute number of both types of lymphocytes in Pb-treated animals as compared to saline controls (Fig. 4A–B). This demonstrates that Pb treatment did not lead to any selective depletion of either lymphocyte subset within the spleen.

Next, we analyzed the characteristics of myeloid cells that infiltrated the spleen following Pb treatment. Figure 5 (panels A–C) illustrates the staining profile of spleen cells with mAbs to CD11b and Sca-1.

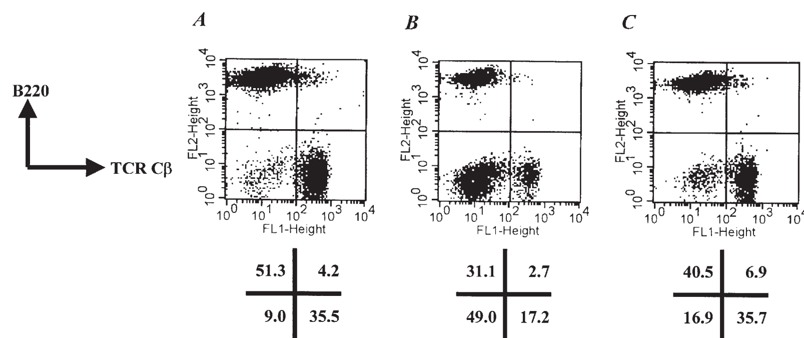

FIGURE 3 Phenotypic analysis of spleen cells. Single cell suspensions were prepared from spleens of TO mice treated for 2 weeks with either saline (panel A), Pb (panel B), or Pb + AT (panel C). The results are shown as dot plots depicting staining with mAbs specific to αβ TCR (x-axis) vs. B220 (y-axis). Data from a total of 20,000 cells per sample were collected and analyzed. The results are representative of three independent experiments.

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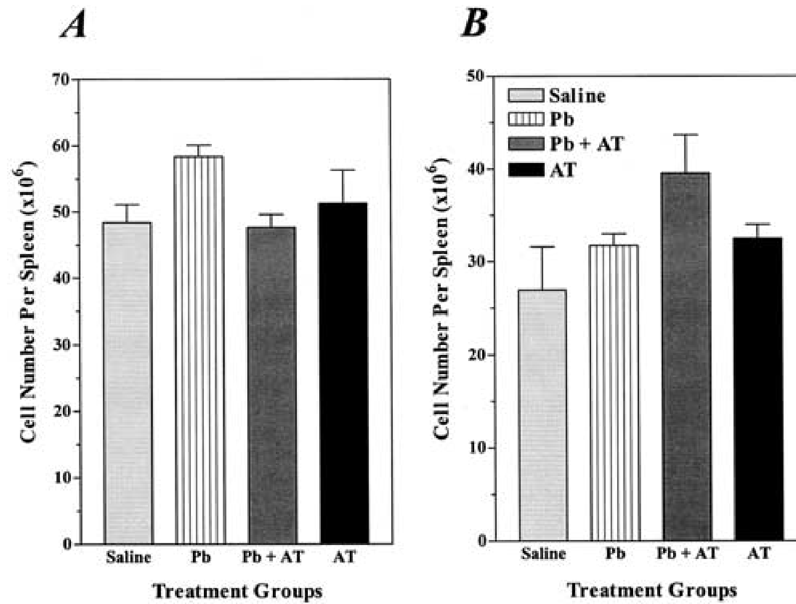

FIGURE 4 Absolute number of B (panel A) and T (panel B) lymphocytes in Pb-treated mice is not decreased as compared to saline-injected controls. Single cell suspensions were prepared from spleens of TO mice treated for 2 weeks with either saline, Pb, Pb + AT or AT alone. The absolute number of viable cells was determined after labeling with trypan blue. The results depict the means ± SEM of three mice per group and are representative of three independent experiments.

CD11b is widely expressed on myeloid lineage cells, including macrophages and neutrophils.[22] Sca-1 is an activation marker of diverse types of lymphoid and myeloid lineage cells.[23] In saline-treated mice, 5.9% of splenocytes expressed the CD11b marker, none of which was positive for Sca-1 antigen (panel A). These CD11b$^+$/Sca-1$^-$ cells most likely represent resting cells of myeloid origin. In addition, 40.3% of total spleen cells were CD11b$^-$/Sca-1$^+$, indicating cells of lymphoid origin with endogenous Sca-1 expression. By sharp contrast, the ratio of CD11b$^+$/Sca-1$^-$ cells was increased to 42.3% in Pb-injected mice, while the representation of the remaining two cell populations, CD11b$^-$/Sca-l$^+$ and CD11b$^-$/Sca-1$^-$ cells, were proportionately lowered (panel B). Co-treatment with Pb and AT largely prevented myeloid cell influx into the spleen (ratio of CD11b$^+$ cells was reduced to 15.0%; panel C) thus maintaining the proportion of the other spleen cell populations at normal levels, as observed in the saline group. A summary of the overall flow cytometry analysis in the different groups of animals is presented in Table I.

Analysis of the Functional Capacity of Spleen Cells

Given the difference in the splenocyte populations in the various treatment groups, the potential impact on their functional capacity to respond to mitogenic stimulation was next investigated. Spleen cells were incubated with varying concentrations of ConA or LPS and their proliferative responses were determined by the extent of tritiated thymidine incorporation in a 72-h assay. As shown in Fig. 6A and B,

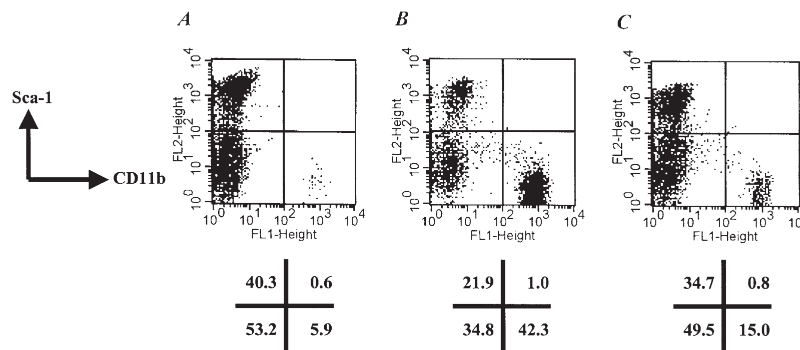

FIGURE 5 Myeloid cells make up the majority of the Pb-induced splenic cellular influx. Single cell suspensions were prepared from spleens of TO mice treated for 2 weeks with either saline (panel A), Pb (panel B), or Pb + AT (panel C). The results are shown as dot plots depicting staining with mAbs specific to CD11b (x-axis) vs. Sca-1 (y-axis). Data from a total of 20,000 cells per sample were collected and analyzed. The results are representative of three independent experiments.

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TABLE I Flow cytometric analysis of spleen cells in treated animals

Treatment group*	Percentage of spleen cells positive for the indicated antigen† (Cell type)				
	TCR cβ (Total T cells)	CD4 (Th cells)	CD8 (Tc cells)	B220 (Total B cells)	CD11b (Myeloid cells)
Saline	30.3 ± 5.2	22.3 ± 2.8	7.2 ± 3.1	54.4 ± 3.0	9.5 ± 3.6
Pb	16.6 ± 0.6	10.6 ± 0.8	5.2 ± 0.1	30.4 ± 0.9	44.2 ± 1.9
Pb+AT	32.3 ± 3.3	21.8 ± 5.9	9.1 ± 2.3	38.9 ± 1.6	26.3 ± 11.3
AT	32.5 ± 1.4	25.6 ± 1.3	8.3 ± 0.4	51.3 ± 4.9	10.9 ± 1.9

* Mice were treated for 2 weeks with saline, Pb alone or Pb + AT, as detailed in the "Methods" section. Individual spleens were excised (6 per treatment group) and 3 were randomly selected for flow cytometric analysis. The results shown are mean ± SEM of 3 mice per group. The experiment was repeated twice with similar results.
† Spleen cells (1×10^6 per sample) were stained with mAbs specific to the indicated antigenic markers, as described in the "Methods" section. The TCR cβ is expressed. on all αβ T lymphocytes, while the B220 antigen is found on all B lymphocytes. The CD4 antigen is expressed on all helper T (Th) cells while CD8 is a marker for all cytotoxic T (Tc) cells. CD1lb is widely expressed on all myeloid lineage cells, including macrophages and polymorphonuclear cells (PMNs).

at optimal mitogenic doses, significant inhibition of mitogen-induced proliferative responses of Pb-treated splenocytes was evident. Compared to the saline group, there was 81 and 68% suppression of proliferative responses to LPS and ConA, respectively. This inhibition was partially reversed in mice co-treated with Pb and AT, where the responses to LPS and Con A were 51 and 50% of control group, respectively. The Pb-induced suppression of mitogenic responses is unlikely to be solely due to a dilution of potential responder cells. This is evident from the observation that despite a decrease of ~45% in the ratios of B and T cells in Pb-treated mice (compared to saline controls), the mitogenic responses were inhibited by 81 and 68%, respectively.

Production of Nitric Oxide

Spleen cell suspensions, prepared from the various treatment groups, were cultured at 1×10^7 cells/ml for 48 h without any further stimulation, and cell-free culture supernatants were assayed for NO content by the Griess's assay. The results are shown in Table II. As can be seen, the NO production was not detectable in any of the four treatment groups. As a positive control, cell-conditioned medium collected from spleen cells of mice infected with an attenuated strain of *Salmonella typhimurium*[19,20] showed approximately 59.0 μM nitrite per 10^7 cells, which is indicative of a high level of NO production.

DISCUSSION

Pb is a common environmental pollutant with documented deleterious effects on human health. Despite the introduction of Pb-free gasoline more than a decade ago, environmental exposure to Pb continues through rapid industrialization and urbanization in many regions of the world.[24] In the USA alone, tens of thousands of children aged 2–4 years of age are exposed to Pb every year, giving rise to blood Pb levels >15 μg/dl, the threshold for clinical

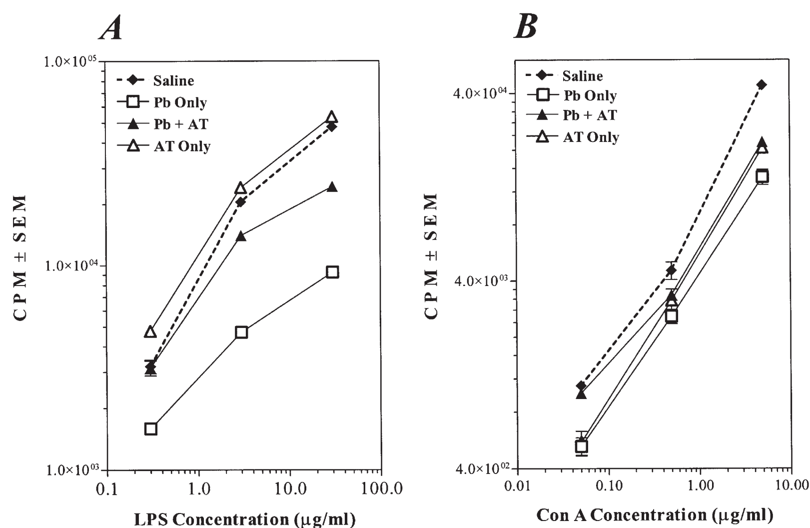

FIGURE 6 Proliferative responses of spleen cells to mitogenic stimulation. Spleen cells from the indicated treatment groups were seeded at 1×10^5/well and stimulated with LPS (A) or Con A (B) at the indicated concentrations. Cells were pulsed with 1 μCi/well of ^{3}H-TdR after a 48 h culture and harvested 24 h later. Triplicate wells were set up for each group. Data denote the mean CPM ± SEM of triplicate cultures per group and are representative of three independent experiments.

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concern.[25] Long-term exposure to even low levels of Pb in children is associated with neurotoxicity and impaired cognitive potential.[26,27] Importantly, once the children are exposed, the development of symptoms is largely unaffected by chelation treatment to reduce blood Pb levels.[28] These findings suggest that prevention is the best course of action to avert Pb-induced toxicity.

In addition to its neurotoxic effects, Pb can also negatively influence the immune system. A small number of studies reported on the immunotoxic effects of Pb in animals as well as in human subjects following exposure.[14,15,17,29] The present report describes the results of a detailed morphological and functional analysis of the immunological consequences of systemic administration of Pb acetate.

In the present study, systemic administration of Pb led to an alteration in spleen cellularity and suppression of immune cell functions. One of the intriguing findings in our study relates to the nature of the splenic cellular influx that is induced by Pb treatment. Despite the fact that myeloid cells are predominantly recruited and that they are the major cell type in the enlarged spleen, they appear to be surprisingly quiescent. This conclusion is supported by two observations. First, all the recruited myeloid cells fail to express Sca-1 (Ly-6A/E), an activation marker of lymphoid as well as myeloid cells.[19,30] Second, they also fail to produce NO, another indicator of activated macrophages.[31] It has been well established that optimal activation of macrophages requires host-derived cytokines in combination with microbial-derived ligands acting on a set of conserved macrophage receptors such as Toll-like receptors and scavenger receptors.[32] The data indicate that Pb-induced toxicity is associated with recruitment of inflammatory cells, but signals for the full activation of these cells may be absent. This appears to be fundamentally different than splenomegaly induced by a microbial infection, such as by *S. typhimurium* for example, where recruited myeloid cells are fully activated (see Table II; Refs. [19,20]). Thus, the immunological sequelae of Pb exposure illustrate how the above two phenomena, namely cellular recruitment and functional activation, are clearly distinct.

An alternative, but by no means mutually exclusive, explanation for the lack of activation of cells recruited into the spleen could be that the presence of Pb is inhibitory. This would be in line with findings from other laboratories demonstrating inhibitory effects of Pb on macrophages and Thl lymphocytes.[9,17,33] Support for this hypothesis comes from a careful analysis of the data on functional responses to mitogens, which is presented in Fig. 6. It is clear from this data that mitogenic proliferative responses in Pb-treated mice are suppressed. In mice exposed to Pb in the presence of AT, the ratio of the T cell population, as well as that of the major $CD4^+$ subpopulation, in the spleen are restored to their normal levels. Despite this, however, the maximal mitogenic ConA response remained at approximately 50% of normal (saline-treated) group, suggesting that Pb may have a direct toxic effect on T cell function, independent of its induction of the cellular influx into the spleen. The situation with the B cell response appears to be different. The percentage of splenic B cells in Pb-treated animals was about 56% of normal and this was restored to 72% of normal in the Pb + AT group. Interestingly, the mitogenic proliferative responses to LPS correlated well with the overall ratio of B cells in the whole spleen. This suggests that B lymphocytes may not be as susceptible to the suppressive action of Pb as T cells. This observation is in agreement with data showing that Pb exerts more of its inhibitory influence on T lymphocytes but not B cells and may in fact be stimulatory for the latter cells.[14,34]

TABLE II Production of NO by spleen cells

Treatment group*	$CD11b^+$ cells (%)†	Nitrite (μM)‡
I. Saline	5.5	<1.0
II. Pb	46.6	<1.0
III. Pb+AT	16.2	<1.0
IV. AT	12.9	<1.0
V. 7-day *Salmonella*-infected spleen	22.0	59.0

*For groups I–IV, mice were treated for 2 weeks with saline, Pb alone, Pb + AT or AT alone, as detailed in the "Methods" section. For group V, spleen cells were prepared 7 days after ip administration of 1×10^6 organisms of attenuated *Salmonella typhimurium*, strain BRD509, as described previously.[19] The experiment was repeated twice with similar results.
†The ratio of $CD11b^+$ (myeloid lineage) cells, shown here as a percentage of the total spleen cell population in each treatment group, was determined by flow cytometric analysis using a specific mAb, as described in the "Methods" section.
‡Production of NO was assessed by the Griess assay which measures the accumulation of NO_2 in culture medium following 48 h of cell culture at 37°C, as described in the "Methods" section. It is expressed as μM nitrite per 1×10^7 spleen cells.

Generation of ROS appears to be central for the immunotoxic effects of Pb.[8,35] The net outcome on target cells can vary from an inhibition of function to genotoxic effects to apoptosis. The antioxidant AT is a low molecular weight, lipid-soluble, compound that interacts directly with ROS radicals and inhibits lipid peroxidation.[36] Recently, AT was reported to protect against Pb-induced lipid peroxidation, and consequently improve cell survival, in brain, kidney and liver tissue.[13] Other antioxidants have also been shown to reverse many of Pb-initiated toxicities. Gurer *et al.*, demonstrated that treatment with taurine reversed many of the consequences of Pb-induced oxidative stress, including glutathione and malondialdehyde (end-product of lipid peroxidation) levels, resulting in increased cell survival but without affecting Pb levels in the blood or in the tissues.[37] Thus, taurine is effective in protecting

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cells against Pb-induced oxidative insult, but has no chelating action and does not affect blood levels. Another recent study reported on the effectiveness of melatonin in reversing toxic effects of Pb on the immune system.[38] Melatonin was shown to restore spleen and liver weights, antibody responses to various antigens and mitogenic responses of B and T lymphocytes following Pb exposure. In the present study, AT is also shown to be effective in reversing Pb immunotoxicity. However, subtle, cell-specific, differences in Pb and AT action were also noted. For example, while Pb induced a state of splenomegaly by activating the recruitment of myeloid (mainly) and lymphoid (some) cells, AT normalized splenic cellularity for all cell types examined. As already mentioned, our findings also indicate that the B and T cell splenic compartments are differentially influenced by Pb and AT. The differential susceptibility may be associated with differences in the signaling pathways affected in the various cell types of the immune system.[39] The direct influence of ROS on the different immune cell types is a subject for further investigation.

In summary, there are three main conclusions to be drawn from the present findings: (1) systemic exposure to Pb results in profound alteration in spleen cellularity due mainly to a large influx of myeloid cells into the spleen; (2) Pb exposure also appears to have a direct toxic or inhibitory effect on the function of splenic lymphocytes, in particular T cells; and (3) Pb-associated immunotoxicity is largely reversed by co-treatment with the antioxidant AT.

Acknowledgements

We wish to thank Sarabjit Singh, Ghada Bashir and Ebtisam M. Saghayer for excellent technical assistance. This work was funded by grants from the Research Committee of the Faculty of Medicine and Health Sciences, UAE University, United Arab Emirates to B.K. al-Ramadi, M.Y. Hassan and M.A. Fahim. Additional support was provided by a grant from the Terry Fox Fund for Cancer Research to B.K. al-Ramadi.

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