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INORGANIC COMPOUNDS

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Modulation of tumor necrosis factor α expression in mouse brain after exposure to aluminum in drinking water

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Abstract Aluminum, a known neurotoxic substance and a ground-water pollutant, is a possible contributing factor in various nervous disorders including Alzheimer's disease. It has been hypothesized that cytokines are involved in aluminum neurotoxicity. We investigated the alterations in mRNA expression of tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and interferon γ (IFNγ), cytokines related to neuronal damage, in cerebrum and peripheral immune cells of mice after exposure to aluminum through drinking water. Groups of male BALB/c mice were administered aluminum ammonium sulfate in drinking water ad libitum at 0, 5, 25, and 125 ppm aluminum for 1 month. An additional group received 250 ppm ammonium as ammonium sulfate. After treatment, the cerebrum, splenic macrophages and lymphocytes were collected. The expression of TNFα mRNA in cerebrum was significantly increased among aluminum-treated groups compared with the control, in a dose-dependent manner. Other cytokines did not show any aluminum-related effects. In peripheral cells, there were no significant differences of cytokine mRNA expressions among treatment groups. Increased expression of TNFα mRNA by aluminum in cerebrum may reflect activation of microglia, a major source of TNFα in this brain region. Because the aluminum-induced alteration in cytokine message occurred at aluminum concentrations similar to those noted in contaminated water, these results may be relevant in considering the risk of aluminum neurotoxicity in drinking water.

Key words Aluminum · Drinking water contaminant · Neurotoxicity · TNFα · Reverse-transcription (RT)-PCR

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Introduction

Aluminum is a known neurotoxic substance. Aluminum administration to sensitive experimental animals such as cats and rabbits produced neurological and neuropathological changes in the central nervous system (CNS; De Boni et al. 1976; Selko et al. 1979; Wen and Wisniewski 1985). However, the mechanism of aluminum neurotoxicity is unknown. In epidemiological studies, low level exposure to aluminum has been postulated as a possible contributing factor in several neurodegenerative disorders such as Alzheimer's disease (Crapper-McLachlan and De Boni 1980; Crapper et al. 1973, 1976, 1980; McLachlan et al. 1996; Neri and Hewitt 1991; Perl et al. 1980), dialysis dementia (Arieff et al. 1979; Elliott et al. 1978), amyotrophic lateral sclerosis (ALS; Garruto 1991; Perl et al. 1982), and Parkinson's disease (Hirsch et al. 1991). Low levels of aluminum are reported to be a contributing factor for cognitive impairment (Jacquin et al. 1994; Rifat et al. 1990). Because the role of aluminum in the pathogenesis of these diseases remains unclear, studies on the neurotoxic mechanism of aluminum are needed.

Aluminum had been considered to exist predominantly in forms not biologically available to humans and animals (Beliles 1991; Walton et al. 1995). However, the amount of available aluminum in biological ecosystem has dramatically increased in recent years because of solubility of this metal in acid rain water (Boegman and Bates 1984). Aluminum contamination of ground water has been reported at levels as high as 380 μg/l (Davis and Turlington 1987). Treatment of drinking water using aluminum compounds, such as aluminum sulfate and aluminum ammonium sulfate (Cohen and Hanna 1971), can substantially increase the dissolved aluminum concentration, although the exact values of such an increase are not available in the literature. The uptake of aluminum into rat brain from aluminum-treated drinking water has been demonstrated (Walton et al. 1995). In addition, the systemic absorption of aluminum in the

water-soluble form is higher than that of water-insoluble forms. The experimental exposure of laboratory animals to water-soluble forms of aluminum through drinking water may be a better model for the study of the effects of environmental aluminum contamination.

Several mechanisms for aluminum neurotoxicity have been proposed. The effect of aluminum-induced alterations on cytokines may be involved in the mechanism of aluminum neurotoxicity for the following reasons.

(1) Aluminum alters mRNA expression and protein synthesis in brain (Exss and Summer 1973; Magour and Maser 1981; Miller and Levine 1974).
(2) Cytokines such as interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and interferon γ (IFNγ) are involved in neurotoxic mechanisms (Boje and Arora 1992; Chao et al. 1992; Skaper et al. 1995).
(3) A previous study demonstrated the depression of IL-2, IFNγ, and TNFα induction in splenic lymphocytes in Swiss Webster mice by administration of 1 mg aluminum lactate/g diet for 6 months (Golub et al. 1993).

In this study involving treatment of animals via aluminum-supplemented feed, cytokine determination using enzyme-linked immunosorbent assay (ELISA) was limited to peripheral cells of the immune system (Golub et al. 1993). In the current study, aluminum was administered through drinking water to elucidate the relationship between altered cytokines and neurotoxicity. A sensitive method to detect changes in cytokine expression, reverse transcriptase-polymerase chain reaction (RT-PCR), was employed for TNFα, IL-1β, and IFNγ in the cerebrum. The mRNA expression for these cytokines in peripheral cells, that is, TNFα, and IL-1β in macrophages and IFNγ in lymphocytes, was also examined.

Materials and methods

Animals and treatment

Adult, 7- to 8-week-old, male BALB/c mice from Harlan Sprague Dawley, (Indianapolis, Ind.), were acclimated for 1 week at 21 °C, 50% humidity, and a 12:12 h light/dark cycle. The mean initial body weight was 23.0 ± 1.7 g. Mice were housed in groups (five per group), and maintained on commercial rodent chow (Harlan Teklad 22/5 rodent diet, Harlan Teklad, Madison, Wis.). The animal protocols followed the guidelines of Public Health Service Policy on human care and use of laboratory animals.

Mice were administered aluminum ammonium sulfate (alum, J.T. Baker, Phillipsburg, N.J.) in deionized drinking water ad libitum at levels of 0, 5, 25, and 125 ppm as aluminum for 1 month. The solution for 125 ppm aluminum was prepared by dissolving 2.1 g $AlNH_4(SO_2)_2 \cdot 12H_2O$ into 1 l distilled water, other solutions were made after appropriate dilutions. An additional group was administered 0.611 g/l of ammonium sulfate to provide an ammonium ion concentration of 250 ppm. The results of this additional group were compared with those of the control to determine whether ammonium or sulfate ions affected the results. The weights of mice were recorded weekly and water consumption measured daily. Animals were observed daily for general behavior such as appearance, activity, grooming, and locomotion.

Sampling and extraction of RNA

On the last day of treatment, brains were isolated and the cerebrum was dissected from brain. In this study, a representative sample of whole cerebrum rather than of a specific area was employed. Cerebrum samples were placed in TRI-LS reagent (Molecular Research Center, Cincinnati, Ohio) to extract RNA according to the manufacturer's protocol. Splenic macrophages and lymphocytes were collected according to the method described previously (Dugyala and Sharma 1996). Each spleen was collected and maintained in 10 ml of cold RPMI 1640 medium with 100 Units/ml of penicillin G and 100 μg/ml of streptomycin (Gibco, BRL, Grand Island, N.Y.).

Cell suspensions were prepared by using a Stomacher lab blender (model 80; Seward Medical, London, UK) with Quality Seward Stomacher Bags (Tekmar Co., Cincinnati, Ohio). Red blood cells were removed by adding 5 ml of ACK lysing buffer [0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM ethylenediaminetetraacetic acid, disodium salt (Na_2EDTA), pH 7.4] and centrifugation. After discarding the supernatant, cells were resuspended in 10 ml of RPMI 1640 medium with 5% fetal bovine serum (Atlanta Biologicals, Atlanta, Ga.). Cells were counted in a hemocytometer and aliquoted in 24-well culture plates at 5×10^6 cells/well. After 1 h incubation at 37 °C, medium containing nonadherent cells (lymphocytes) was removed. Macrophages (adherent cells) were activated with 5 μg/ml lipopolysaccharide (LPS) in 2 ml of medium. Lymphocytes (non-adherent cells) were activated by 0.5 μg/ml phytohemagglutinin (PHA) in 2.75 ml of medium. For macrophages, the medium was removed after 6 h of incubation, cells were scraped, and RNA extracted as for cerebrum. For lymphocytes, 12 h later cell pellets were collected by centrifugation (400 rpm × 5 min), and RNA extracted. The exposure periods for optimum mRNA expression were based on our preliminary work (Dugyala and Sharma 1996).

Measurement of RNA

Total RNA of each sample was extracted as mentioned above and dissolved into 0.1% diethyl pyrocarbonate (DEPC) water. The RNA concentration was quantified by spectrophotometric absorbance at 260 nm and RNA solutions stored at −85 °C until RT-PCR. A semiquantitative RT-PCR was performed by following the manufacturer's protocol (Life Technologies, Grand Island, N.Y.). The first-strand complementary DNA (cDNA) was synthesized from mRNA of cerebral tissue, splenic macrophages, and lymphocytes; a semiquantitative PCR was performed for each cytokine. For the first-strand cDNA synthesis, 1 μl of oligo $(dT)_{12-18}$ (500 μg/ml) (Life Technologies) was mixed with 1 μg of total RNA in a sterilized microcentrifuge tube and the final volume of mixture adjusted to 12 μl. The mixture was heated at 70 °C for 10 min and quickly chilled on ice; 4 μl of 5× X first-strand buffer, 2 μl of 1 M dithiothreitol (DTT; Life Technologies) and 1 μl of 10 mM dNTP mix (adjusted from 100 μM of dATP, dCTP, dGTP, and dTTP (Promega, Madison, Wis.) were added. This mixture was preincubated at 42 °C for 2 min and mixed with 1 μl (200 Units) of Superscript-II RNase H- reverse transcriptase (Life Technologies). The mixture was incubated for 50 min at 42 °C, and the reaction was inactivated by heating at 70 °C for 10 min. To remove residual RNA, 1 μl of RNase H was added to the mixture, followed by incubation at 37 °C for 20 min.

For the polymerase chain reaction (PCR), 1 μl of cDNA from first strand reaction was used. In addition, 5 μl of 10× PCR buffer (100 mM TRIS-HCl, pH 8.3, 500 mM KCl, 1% Triton X-100), 1 μl each of dNTP mix, sense primer (10 μM), antisense primer (10 μM), and *Thermus aquaticus* DNA polymerase (*Taq* polymerase) were mixed in a tube on ice. Cytokines determined were TNFα, IL-1β, and IFNγ. As an internal standard, β-actin from cDNA was also amplified. The primer sequences of cytokines and β-actin are shown in Table 1 (Benavides et al. 1995; Reiner et al. 1993). The optimum magnesium concentration for each substance was determined in preliminary trials; 3 mM for TNFα, IL-1β, and IFNγ, and 2.5 mM for β-actin. For β-actin amplification, 2 μl of dimethyl sulfoxide was also added to each tube. The mixtures were adjusted

Table 1 Primer sequences of cytokines and the internal standard employed for cDNA amplification (*PCR* Polymerase chain reaction, *TNFα* Tumor necrosis factor α, *IL-1β* interleukin-1β, *IFNγ* interferon γ, *cDNA* complementary DNA)

cDNA	Primer sequence sense/antisense	Size of PCR (bp) product	Reference
TNFα	sense 5′-GTTCTATGGCCCAGACCCTCACA-3′ antisense 5′-TCCCAGGTATATGGGTTCATACC-3′	383	Reiner et al. (1993)
AlternateTNFα	sense 5′-CTCTTCAAGGGACAAGGCTG-3′ antisense 5′-CGGACTCCGCAAAGTCTAAG-3′	253	Primer3[a]
IL-1β	sense 5′-GCAACTGTTCCTGAACTCA-3′ antisense 5′-CTCGGAGCCTGTAGTGCAG-3′	382	Benavides et al. (1995)
IFNγ	sense 5′-AACGCTACACACTGCATCT-3′ antisense 5′-AGCTCATTGAATGCTTGG-3′	398	Benavides et al. (1995)
β-actin	sense 5′-ATGGATGACGATATCGCT-3′ antisense 5′-ATGAGGTAGTCTGTCAGGT-3′	569	Benavides et al. (1995)

[a] Primer3 program for primer selection, Whitehead Institute, MIT Center for Genome Research, Cambridge, Mass

Table 2 Body weights (g)[a] and organ weights (g/100 g b. w.)[a] of mice treated with aluminum or ammonium sulfate for 1 month (*b.w.* Body weight)

Group	Final body weight (g)	Liver (g/100 g b.w.)	Kidney (g/100 g b.w.)	Spleen (g/100 g b.w.)
Control	26.02 ± 0.855	5.730 ± 0.178	1.722 ± 0.024	0.355 ± 0.011
Aluminum, 5 ppm	26.10 ± 0.607	5.567 ± 0.054	1.765 ± 0.018	0.341 ± 0.008
Aluminum, 25 ppm	24.74 ± 0.895	5.634 ± 0.076	1.671 ± 0.034	0.343 ± 0.011
Aluminum, 125 ppm	25.32 ± 0.975	5.723 ± 0.156	1.770 ± 0.031	0.354 ± 0.015
Ammonium sulfate	26.32 ± 0.599	5.532 ± 0.148	1.662 ± 0.063	0.365 ± 0.036

[a] Mean ± SE ($n = 5$)

Table 3 Weight gain, water intake, and water-borne aluminum dose in different treatment groups[a]

Group	Weight gain (g)/mouse during 1 month	Water consumption (ml/mouse per day)	Dose of Al via water (mg/kg per day)
Control	2.62 ± 0.43	4.574 ± 0.182	0
Aluminum, 5 ppm	2.74 ± 0.67	4.981 ± 0.090*	0.954 ± 0.017
Aluminum, 25 ppm	2.10 ± 0.44	4.271 ± 0.258	4.316 ± 0.260
Aluminum, 125 ppm	2.46 ± 0.25	4.310 ± 0.088	21.278 ± 0.434
Ammonium sulfate	3.18 ± 0.21	4.484 ± 0.081	0

[a] Mean ± SE ($n = 5$)
* Significantly different from other aluminum-treated groups ($P < 0.01$) and the group treated with ammonium sulfate ($P < 0.05$), but not from the control group by Fisher's post-hoc least significant difference (PLSD) test

to 51 µl as final volume of reaction, overlaid by mineral oil (Sigma, St. Louis, Mo.), and the PCR reaction started with a hot start in Tempcycler (Coy Lab, Ann Arbor, Mich.) at 95 °C for 5 min. The cycling consisted of denaturing at 94 °C for 30 s, annealing for 30 s (TNFα, 56 °C; IL-1β, 52 °C; IFNγ and β-actin, 48 °C, respectively), and extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. The number of cycles was also determined in preliminary trials for each cytokine, reactions were linear up to 40 cycles. Results for TNFα were confirmed using alternate primers indicated in Table 1.

Amplified products were mixed with 10× DNA dye (5 mg/ml bromophenol blue, 50% glycerol, 100 mM TRIS, 20 mM NaCl, and 1 mM EDTA) at the ratio 10:1 (v/v) and separated by electrophoresis at 150 V on 2% agarose (J.T. Baker) gels containing 0.476 mM ethidium bromide (Sigma). The gels were photographed in a backlighted UV transilluminator (Ultra Lum, Carson, Calif.). The photographs were scanned using the ScanMan model 256 with Foto Touch Color software version 1.3 (Logi Tech, Fremont, Calif.). The scanned bands were quantified using UN-SCAN-IT software (Silk Scientific, Orem, Utah). Mean quantified value of the band for each cytokine was adjusted by respective β-actin band.

Statistical evaluation

The log-transformed data of control and treatment groups were compared by one-way analysis of variance (ANOVA) followed by Fisher's post-hoc least significant difference (PLSD) test using the Statview software (Abacus Concept, Berkeley, Calif.). For statistical analyses different treatment groups were compared to the group given deionized water.

Results

There were no treatment-related differences in the final body weight and in liver, kidney, and spleen weights normalized to the body weight (Table 2). No signs of gross behavioral alterations were observed in any animal during the treatment period. There were also no significant differences among control and treatment groups in weight gain (Table 3). Food consumption for the various treatment groups was 4.52 g/mouse per day for the control, and 4.63, 4.52, and 4.49 g/mouse per day for 5, 25, and 125 ppm aluminum groups, respectively, and 4.63 g/mouse per day for the ammonium sulfate-treated group. Aluminum concentration measured by inductively coupled plasma spectrometry in the basal diet was 122 ppm. Based on this concentration, the aluminum

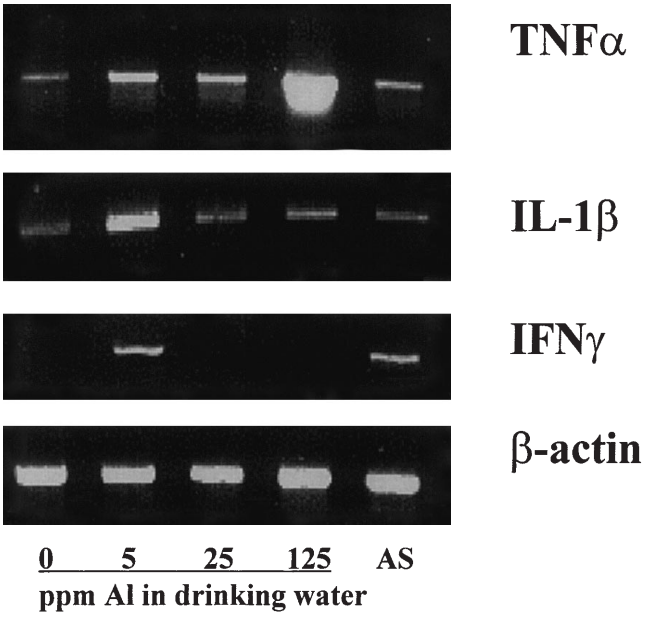

Fig. 1 Polymerase chain reaction (PCR) amplified products of tumor necrosis factor α (TNFα), interleukin 1β (IL-1β), interferon γ (IFNγ), and β-actin in cerebrum of controls, aluminum-treated mice, and ammonium-treated mice. *Numbers* above "ppm Al in drinking water" represent aluminum concentration (ppm) in drinking water. *AS* indicates the group treated with ammonium sulfate

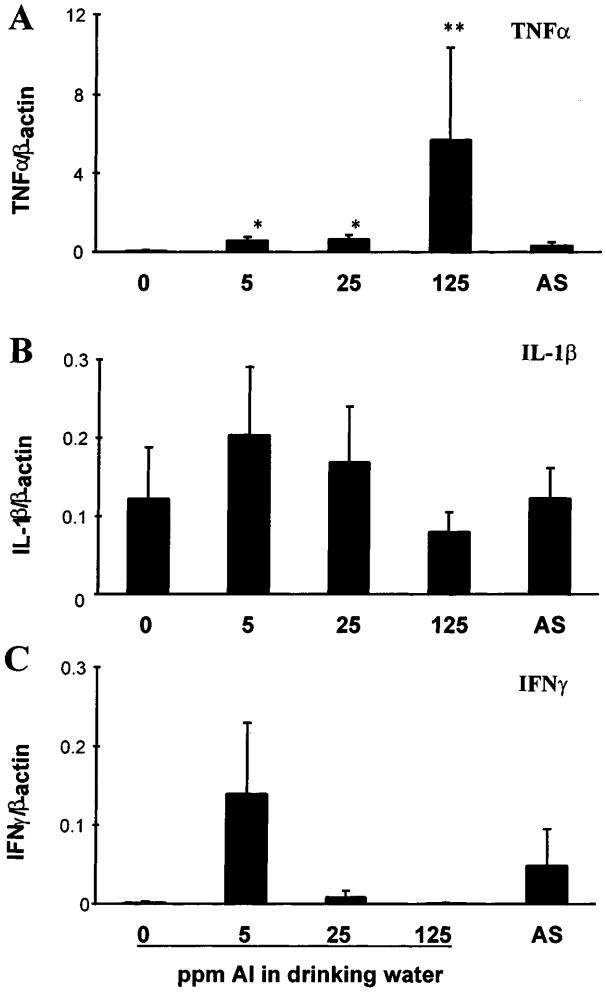

Fig. 2 Relative mRNA expression of **A** TNFα, **B** IL-1β, and **C** IFNγ in the cerebrum samples treated with aluminum or ammonium sulfate by semiquantative reverse-transcription polymerase chain reaction (RT-PCR). $*P < 0.05$, $**P < 0.01$. See legend to Fig. 1 for explanation of abscissa labels. Each *bar* represents mean for each cytokine normalized to respective β-actin. *Error bars* represent standard error ($n = 5$)

taken from food was calculated to be 21.19, 21.64, 22.29, 21.63, and 21.46 mg/kg body weight per day for the control, 5, 25, 125 ppm aluminum groups, and ammonium sulfate group, respectively. However, the amount of aluminum absorbed from food has been reported to be very low, in the range of 0.01–0.04% (Greger and Poers 1992), as long as aluminum compounds are not water soluble. Water consumption of treatment groups is shown in Table 3. Water consumption (ml/mouse per day) by the 5 ppm aluminum group was significantly higher than other groups except the control. However, because of relatively higher mean body weight of this group, the dose of aluminum via water (mg/kg body weight per day) was proportional among the treatment groups (Table 3).

Figure 1 presents typical examples of the bands of amplified product for TNFα, IL-1β, IFNγ, and β-actin in cerebrum of different treatment groups. The relative expression of TNFα, IL-1β, and IFNγ in the cerebrum is indicated in Fig. 2A–C. There were no significant differences for any cytokine between the control and ammonium sulfate treated groups. Aluminum-treated groups demonstrated a significant increase of relative expression of TNFα in the cerebrum compared with controls. A marked increase of TNFα expression was observed in the group treated with the highest aluminum concentration. Because TNFα from the cerebral tissue was the only cytokine showing significant differences, the results were confirmed using an alternate set of primers as indicated in Table 1. The results were essentially similar to those obtained with the first set of primers (data not shown). For IL-1β, the effect of aluminum treatment did not show any differences in cerebrum. The relative expression of IFNγ in cerebrum was low, therefore, the effect of aluminum was not apparent. Because of clear bands in two samples of the 5 ppm aluminum group, the mean value of the relative expression for IFNγ in cerebrum of 5 ppm aluminum group was high but not statistically different from the control.

Figure 3 presents typical examples of the gel bands obtained after RT-PCR amplification for TNFα, IL-1β, and β-actin in LPS-activated splenic macrophages (Fig. 3A), and IFNγ and β-actin in PHA-activated splenic lymphocytes (Fig. 3B) of mice. Figure 4A–C represents the relative expression of TNFα and IL-1β in LPS-activated macrophages, and of INFγ in the PHA-activated splenic lymphocytes. There were no significant

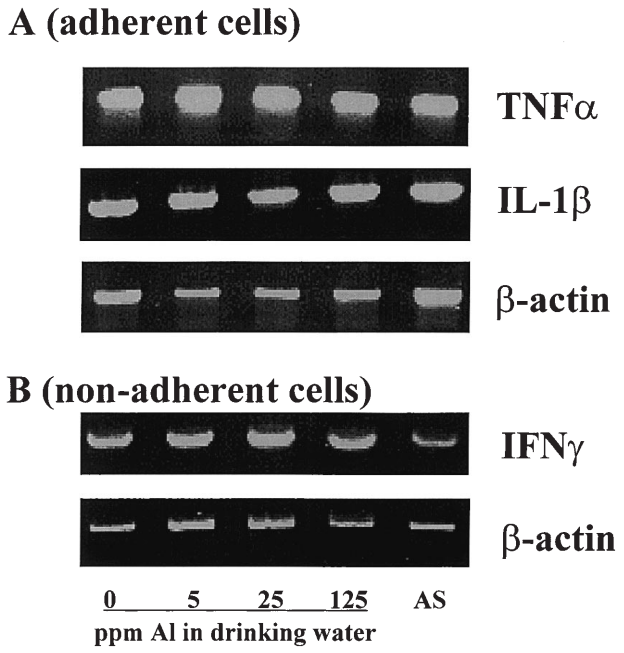

Fig. 3 Amplified products of **A** TNFα, IL-1β, and β-actin in lipopolysaccharide (LPS)-activated adherent cells (splenic macrophages), and **B** IFNγ and β-actin of phytohemagglutinin (PHA)-activated non-adherent cells (splenic lymphocytes) of controls, aluminum-treated mice, and ammonium-treated mice. See legend to Fig. 1 for explanation of labels on abscissa

differences between control and treatment groups, although there was a tendency towards a dose-dependent decrease in the expression of IFNγ in splenic lymphocytes.

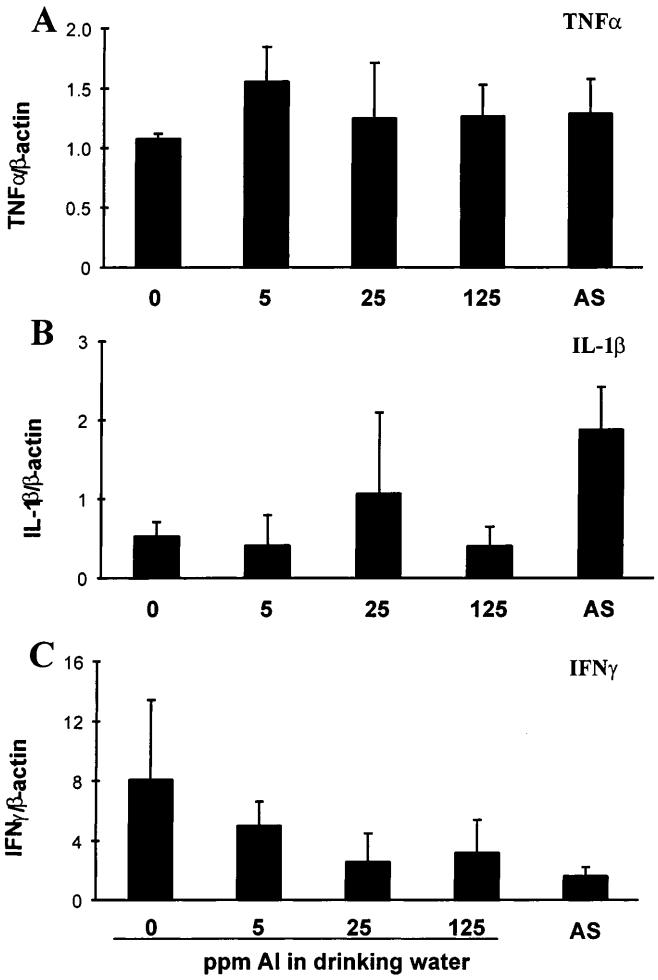

Fig. 4 Relative mRNA expression of **A** TNFα and **B** IL-1β in LPS-activated adherent cells (splenic macrophages), and **C** IFNγ in PHA-activated non-adherent cells (splenic lymphocytes) of controls, aluminum-treated mice, and ammonium-treated mice by semi-quantative RT-PCR. See the legend to Fig. 2 for further explanation

Discussion

The mechanism of aluminum neurotoxicity is of interest because aluminum has been postulated as a possible contributing factor in several neurodegenerative diseases such as Alzheimer's disease (Crapper-McLachlan and De Boni 1980; Crapper et al. 1973, 1976, 1980; Mclachlan et al. 1996; Neri and Hewitt 1991; Perl et al. 1980). Aluminum administration to aluminum-sensitive experimental animals produced neurological changes or experimental aluminum neuropathy (De Boni et al. 1976; Selkoe et al. 1979; Wen and Wisniewski 1985). Treatment of pregnant mice with aluminum sulfate, either intraperitoneally (200 mg/kg daily) or via drinking water (750 mg/l) during days 10–13 of gestation caused alterations of choline acetyltransferase activity in different brain regions of the offspring (Clayton et al. 1992). For the expression of neurotoxicity, the bioavailable form of aluminum is important. The water-soluble form of aluminum is more readily absorbed than the insoluble forms; therefore, aluminum exposure via drinking water could be an important route of exposure. Neurochemical alterations involving brain biopterin synthesis were reported after treatment of rats with aluminum in drinking water but not when this metal was incorporated in a pelleted diet (Amstrong et al. 1992). We have recently reported decreased dopamine turnover in the hypothalamus but not in the striatum of mice treated with aluminum via drinking water in an identical protocol as that used in the current study (Tsunoda and Sharma 1999). The level of aluminum exposure in the current and in the previously reported study were only one level of magnitude higher than contamination levels reported in ground water by Davis and Turlington (1987).

In the current study, the aluminum content of the brain samples were not determined. Limited entry of this metal through the blood-brain barrier has been suggested in previous reports. Aluminum is distributed to mouse brain after an intravenous infusion (Radunovic et al. 1997) or after a percutaneous application (Anane et al. 1995). Julka et al. (1996) reported that after repeated intraperitoneal administration of aluminum lactate, aluminum was distributed in all areas of rat brain, with maximal accumulation in the hippocampus. After

an intraventricular injection of aluminum gluconate, aluminum was eightfold higher in frontal and parietal cortex compared to the hippocampus of rats (Szerdahelyi and Kasa 1988). On the other hand, exposure of rats to aluminum citrate either as oral gavage or in the drinking water for 15 weeks did not increase brain aluminum levels over the control values (Garbossa et al. 1998). In another study to determine the effect of age on aluminum entry into brain, young (21 day old) rats accumulated some aluminum (twofold increase over the controls) in brain after 6.5 months but not after 3 months of treatment with aluminum nitrate; no difference in the brain aluminum content was noted when the treatment was started in adult (8 month) or old (16 month) rats (Gomez et al. 1997).

It is possible that the effect of aluminum in brain could be produced by peripheral events without sufficient accumulation of this element in brain. Biochemical changes in brain have been noted through afferent branches of the vagal nerve (Hansen et al. 1998; Simons et al. 1998). A biphasic effect of aluminum on cholinergic enzymes was reported after oral treatment with aluminum chloride at 14 versus 60 days (Kumar 1998); the author speculated on a slow accumulation of aluminum in brain although no such measurements were conducted.

The modulation of mRNA and protein synthesis may be one of the mechanisms of aluminum neurotoxicity (Boegman and Bates 1984). Aluminum accumulates within the nucleus of neurons (Crapper-McLachlan and De Boni 1980), and the chromatin-bound aluminum causes neurofibrillary degeneration in vitro (De Boni et al. 1980). Brain protein synthesis in vivo was inhibited following intraperitoneal administration of aluminum chloride (Magour and Maser 1981). However, neurons from aluminum phosphate-treated rabbit brain in vitro showed increase in the incorporation of leucine into protein (Exss and Summer 1973). In neuroblastoma cell lines, incorporation of leucine into protein and total cell protein biosynthesis were increased following addition of aluminum phosphate (Miller and Levine 1974). Thus, the alteration of mRNA expression and protein synthesis by aluminum may be highly variable.

The proinflammatory cytokines (such as IL-1β, TNFα, and IFNγ) are closely involved in the mechanism of neuronal death. Boje and Arora (1992) demonstrated that the neurotoxic effects of IFNγ, TNFα, IL-1α, and IL-6, could be mediated by immunostimulation of glial cells to produce nitric oxide or other reactive nitrogen oxides. Chao and coworkers (1992) demonstrated that microglia activated with IFNγ and LPS decreased neuronal cell survival via nitric oxide production. In the study of Skaper et al. (1995), a combination of IL-1β and TNFα resulted in a marked decrease of neuronal cell survival. Meningeal fibroblasts stimulated by TNFα provoked a significant decrease in survival of cerebellar granule cells by nitric oxide synthesis. In the current study, we hypothesized that alteration in mRNA of proinflammatory cytokines occurs after exposure to aluminum in drinking water and is related to the neurotoxic mechanism.

TNFα in CNS is secreted by astrocytes and microglia (Sawada et al. 1989; Woodroofe 1995). Microglia are considered to be the major producer cells of TNFα in brain, because they are more sensitive than astrocytes to LPS-induced TNFα production (Sawada et al. 1989). Microglia are monocyte-macrophage lineage cells (Frei et al. 1988). The mechanism for this effect of aluminum may be CNS specific, should the initial effect be attributed to microglia. Although saturable transport of TNFα through the blood-brain barrier has been reported (Gutierrez et al. 1993), in the current study the gene expression of TNFα in brain was investigated. Peripheral macrophages were not influenced by aluminum and may not be a good indicator of the potential for aluminum-induced cytokine production in cerebrum.

Van Rensburg et al. (1997) hypothesized that aluminum neurotoxicity involves sequestering aluminum and iron followed by formation of immature plaques in brain. Microglia are then activated in an attempt to destroy the plaques. If microglia are activated, the activated microglia could produce TNFα leading to further neuronal degeneration. It has been reported that IL-1 in CNS is also produced by activated microglia (Giulian et al. 1986; Woodroofe 1995). If microglia are activated by aluminum, the reason the increased expression occurred only for TNFα remains unclear. Astrocytes have also been reported as another source of TNFα and IL-1 production in brain (Fontana and Grob 1984). Skaper et al. (1996) reported that by releasing TNFα, mast cells are also involved in the mechanism of neuronal death via nitric oxide production. Possible interactions between aluminum and zinc can also be considered as a mechanism of aluminum-induced neurotoxicity (Sugawara et al. 1987).

In the current study, TNFα expression in cerebrum was significantly increased by aluminum treatment. This effect was somewhat dose dependent, statistically significant at 5 to 125 ppm and markedly increased at 125 ppm of aluminum. In all samples from the aluminum-treated animals, the amount of mRNA for TNFα was higher than those in the control group. Although the increase in TNFα message was confirmed in the same cerebral tissues using a different set of primers, the results obtained here should be considered preliminary and need to be replicated in future experiments using specific cortical regions. Our attempts to measure the amount of TNFα protein using a commercial ELISA kit were unsuccessful; a high background in the post-nuclear supernatant of the cerebral samples prevented an accurate assessment (data not shown). It appeared, however, that the aluminum-induced increase in mRNA expression was limited to TNFα in brain. Other cytokines in cerebral tissue were not significantly altered. In the peripheral cells, no cytokine including TNFα showed any change in the mRNA expression.

The expression of mRNA for IFNγ in brain was quite low. The mRNA expression of IFNγ showed a higher

mean value in cerebral samples of the mice treated with the lowest dose of aluminum; however, it did not reach the level of statistical significance due to a large variability in this treatment group. In brain, infiltrated lymphocytes secrete cytokines, including IFNγ (Woodroofe 1995).

Our present results on peripheral expression of cytokines in the immune cells revealed no differences. Immunological alterations including cytokine changes have been found in aluminum-treated animals (Golub et al. 1993). These authors measured concentrations of IL-2, IFNγ, and TNFα in the supernatant of spleen cells activated by concanavalin A by ELISA. In their study (Golub et al. 1993), Swiss Webster mice were exposed to dietary aluminum (1 mg aluminum/g diet, as aluminum lactate) from conception to 6 months of age. Because aluminum lactate is water soluble, it would be bioavailable. The percentage of CD4+ cells in spleen, quantified by using a flow cytometer, was 31% lower in aluminum-treated mice compared with controls. The authors suggested a T-helper cell deficit and reported a lower total T lymphocyte number in aluminum-treated mice. Differences in the previous study and current results can be accounted for by the dose of aluminum and duration of treatment. Flaten et al. (1996) have pointed out that infants could be particularly susceptible to aluminum accumulation and toxicity.

The relationship between aluminum and Alzheimer's disease has been controversial. However, several studies suggest a possible relationship between cytokines and Alzheimer's disease. In an epidemiological study by Fillit et al. (1991), TNFα was elevated in sera from Alzheimer's disease patients compared with controls. Rossi and Bianchini (1996) demonstrated that the β-amyloid peptides deposited in the brain of Alzheimer's disease patients induce the production of nitric oxide in astrocytes in the presence of IFNγ, TNFα, and IL-1β. Microglia play an important role for Alzheimer's disease (McGeer et al. 1993). The amyloid fibrils of Alzheimer senile plaques were interdigitated with the plasma membrane of microglia (Perlmutter et al. 1990). Dickson et al. (1993) indicated that activated microglia, which have immunoreactivity with antibodies to IL-1 and TNFα, participate in a local inflammatory cascade that promotes tissue damage and amyloid formation. Therefore, aluminum neurotoxicity and Alzheimer's disease may have a common mechanism by involving TNFα and cell activation that produces similar conditions. In future studies we intend to include other brain regions, employ longer treatment duration, and attempt to identify the specific cellular sources of cytokine increases that may be influenced by aluminum in brain tissue.

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