BioMetals **16:** 359–368, 2003.

Early onset of virus infection and up-regulation of cytokines in mice treated with cadmium and manganese

Pankaj Seth[1], Mirza M. Husain[2], Pratibha Gupta[2], Bruce A. Schoneboom[3], Franziska B. Grieder[1,4], Haresh Mani[1,5] & Radha K. Maheshwari[1,*]

[1]*Center for Combat Casualty Care and Life Sustainment Research, Department of Pathology, Uniformed Services University of the Health Sciences (USUHS), Bethesda, MD-20814, USA;* [2]*Industrial Toxicology Research Center, Lucknow, India;* [3]*Neuroscience Program, USUHS, Bethesda, MD-20814, USA;* [4]*Comparative Medicine, National Center for Research Resources, NIH, Bethesda, 20892, USA;* [5]*Armed Forces Medical College, Pune, India;* [*]*Author for correspondence (Phone: 301-295-3394; Fax: 301-295-1640; E-mail: rmaheshwari@usuhs.mil)*

Received 5 February 2002; accepted 3 June 2002; published on line September 2002

Key words: Encephalomyocarditis virus, infection, Semliki Forest virus, Venezuelan Equine Encephalitis virus, virus

Abstract

A substantial database indicates that a large number of environmental pollutants, chemicals and therapeutic agents to which organisms are exposed cause immunotoxicity. The suppression of immune functions may cause increased susceptibility of the host to a variety of microbial pathogens potentially resulting in a life-threatening state. Evaluation of the immunotoxic potential of chemical xenobiotics is of great concern and, therefore, we have investigated the impact of exposure of inorganic metals, specifically cadmium (Cd) and manganese (Mn) on Encephalomyocarditis virus (EMCV), Semliki Forest virus (SFV), and Venezuelan Equine Encephalitis virus (VEEV) infection. Pretreatment with a single, oral dose of Cd or Mn increased the susceptibility of mice to a sub-lethal infection of these viruses as observed by increased severity of symptoms and mortality compared to untreated controls. An early onset of virus infection was found in brains of Cd and Mn treated animals. Histopathological observations of the brain indicate evidence of inflammation and greater tissue pathology in Cd-or Mn-exposed mice compared to control animals. Meningitis and vascular congestion was seen in virus infected mice in all the metal treated groups, and further, the perivascular inflammation appeared earlier in treated mice compared to control. Encephalitis was maximum in Cd pretreated mice. Widespread environmental contamination of metals and the potential for their exposure and subsequent infection of humans or animals is indicative that further studies of these and all other metals are important to understand the effect of environmental pollution on human health.

Introduction

There is a wide spread low dose repeated exposure of the population to a variety of chemical pollutants of environmental or occupational origin. Experimental animal data indicate that a large number of environmental contaminants and chemicals agents, in addition to manifesting toxic effects, have potential to impair the functional integrity of the immune system (Burns *et al.* 1996; Burchiel 1999). It is apparent that the suppression of the immune system is characteristically associated with the increased susceptibility to different kinds of infectious diseases. Numerous metals are responsible for immunologically mediated diseases in humans (Pelletier & Druet 1995). Cadmium (Cd) and Manganese (Mn) have noticeable clinical importance and are known to elicit a number of immunomodulatory effects leading to enhanced susceptibility to microbial agents. The mechanisms involved in these processes are not completely elucidated (Bernier 1995).

The combination of significant toxicity at low doses, long biological half-life and the low rate of excretion from body, categorizes Cd as a unique metal,

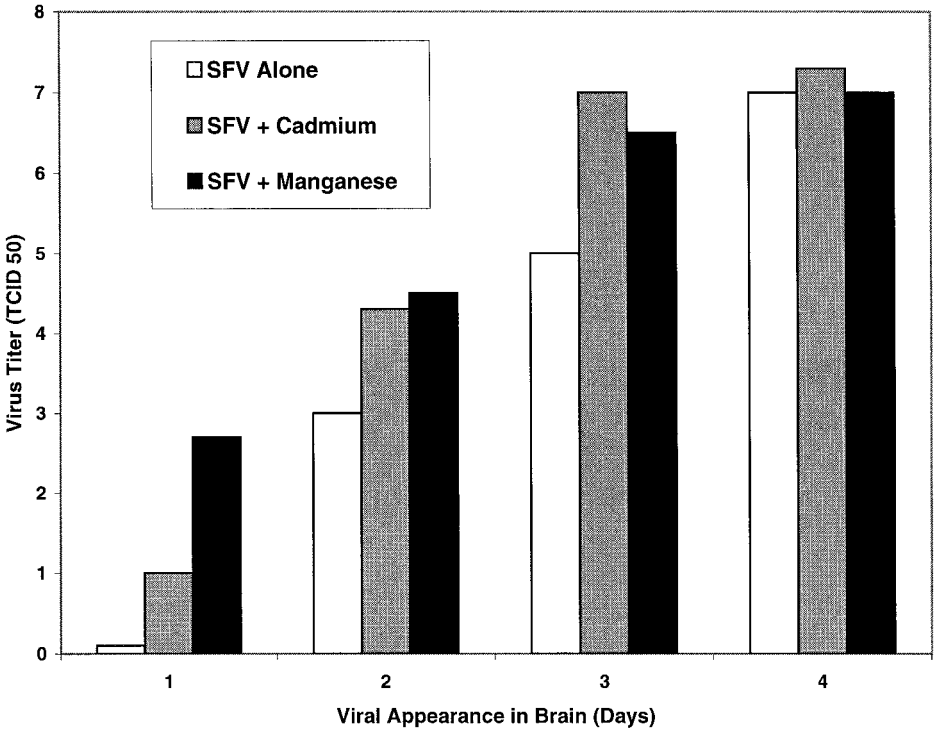

Fig. 1. Effect of Cd and Mn on the viral kinetics in mice brain infected with SFV. The hollow bars represent the virus titers of brain samples collected from mice without metal but SFV infected (SFV controls) while the filled bars represent the viral titers of brain from metal pretreated and SFV infected mice. Viral titers were measured as $TCID_{50}$/ml of 10% brain homogenate.

as exposure at low doses become toxic over the period of time, due to accumulation in the body (Belilies 1994). Levels of Cd in urban environments are rising due to its increased use in industrial settings as electrodes in nickel-Cd batteries, pigments in plastics, ceramics and glasses, stabilizers for polyvinyl chloride, as well as steel coatings and alloys (ATSDR: Toxicological Profiles for Cd -update 1999, Alonso *et al.* 2001). Human exposure to Cd occurs in form of food and water intake. Furthermore, cigarettes and tobacco leaves contain large amounts of Cd and cigarettes smoke may contribute up to 3 μg per day to the smoker's daily Cd intake (ATSDR, Toxicological Profile for Cadmium). Major toxic effects of Cd include lung damage, emphysema and cancer, kidney and liver damage, osteomalacia, testicular tumors, teratogenic malformations, anemia, hypertension, and deficiencies of iron, copper and zinc (Beliles 1994), and alterations in synaptic neurotransmission (Minami *et al.* 2001).

Studies on the evaluation of host resistance during co-exposure of Cd to herpes simplex virus (HSV) (Thomas *et al.* 1985), EMCV (Exon *et al.* 1986), cytomegalovirus (Daniels *et al.* 1987) and Influenza virus (Chaumard *et al.* 1991) have been reported earlier. More recently, Fawl and colleagues reported reactivation of HSV in latently infected mice after Cd administration and Glynn (1998) has shown an increase in intestinal absorption of Cd during Coxsackie virus infection in mice.

Manganese (Mn), while ubiquitous in the environment, is the twelfth most abundant element in the earth's crust. Metallic Mn is mainly used in steel production, whereas its compounds are commonly used in dry cell batteries, fertilizers, varnishes, ceramics, fungicides, as disinfectant, and as preservative of flowers and fruits. Mn is a natural component of most foods and is an essential element. Food is the main route of exposure to humans. The central nervous system is the primary target of Mn toxicity, however, elevated incidents of respiratory problems, bronchitis, and pneumonia have also been reported (Beliles 1998, ATSDR: Toxicological Profile for Mn 2000). Alterations of humoral and cellular immunity following Mn treatment to mice have been studied earlier (Srisuchart

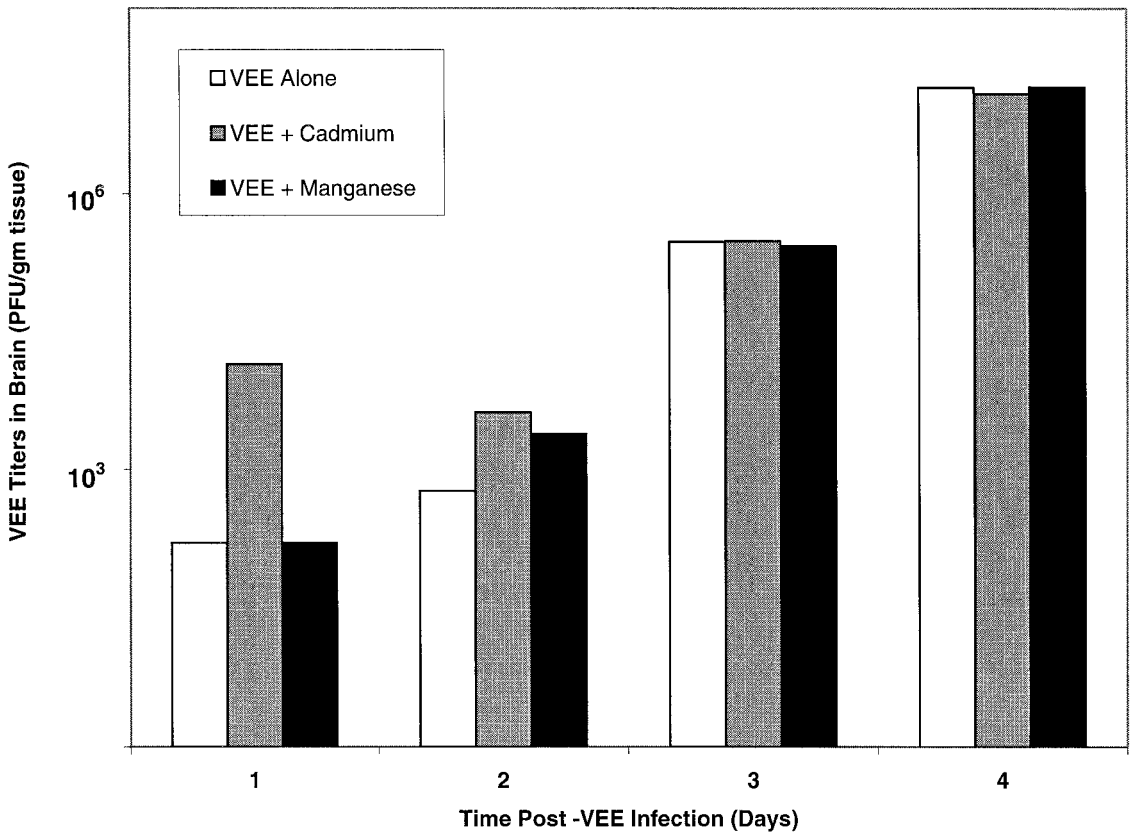

Fig. 2. Represents the pattern of appearance of the VEE virus at different time intervals in brain tissue of mice pretreated with either cadmium (0.13 mg kg) or manganese (419 mg kg). Vehicle treated mice were used as controls (untreated). Level of detection was at 160 plaque forming units (PFU)/gram tissue. Virus titers are represented as the (PFU)/gram tissue.

1987), but its interaction with viral multiplication has not yet been attempted.

Evaluation of the immunotoxic potential of chemical xenobiotics is of great concern and we have therefore initiated studies on the impact of inorganic metals during viral infections. Mice were orally gavaged with Cd or Mn followed by a sub-lethal challenge of either SFV or EMCV, or lethal VEE infection. Animals were monitored for morbidity, mortality, mean survival time (MST), severity of symptoms/infection, histopathology and expression of inflammatory cytokine mRNA levels. Data reported in this paper demonstrate that mice given Cd or Mn prior to viral infection showed earlier appearance of symptoms and higher mortality, reduced survival time, significantly enhanced viral titers in brains, greater neuronal tissue damage and up regulated expression of mRNA levels of IL-12p40, IL-1Ra, IL-1alpha and beta.

Materials and methods

Chemicals

$CdCl_2$, $MnCl_2$ and all other chemicals used were of highest purity grade procured from Sigma Chemicals Co.

Animals, cells, and viruses

CD-1 mice of either sex, weighing 10–12 g and six-week-old female C57BL/6 mice were obtained from Charles River Laboratories and National Cancer Institute, respectively. All the animals were maintained in compliance with the United States Public Health Service policy on humane care and use of laboratory animals in an AAALAC-accredited facility and approved by the institutional ethical committee. SFV was obtained from the American Type Culture Collection, Rockville, MD, and EMCV was originally obtained from C. Buckler (NIAID, NIH). Both SFV and

EMCV were passaged by intracerebral (I.C.) injection in 10–12 g old CD-1 mice. Mice showing symptoms were sacrificed and 10% brain (w/v) homogenate was made in phosphate buffered saline (PBS). Viral titerations were carried out by cytopathic effect assay and were represented as $TCID_{50}$/ml. Cell lines were grown in Eagle's minimum essential medium with 10% fetal bovine serum. Molecularly cloned virulent VEE strain, V3000 (Davis *et al.* 1989; Grieder *et al.* 1996) was used in these experiments and was 100% fatal in mice regardless of the routes of infection. Virus stocks were generated by standard procedure and titer were determined using plaque assay with BHK cells, VEE virus stocks were stored at −80 °C and experiments were carried out in a biosafety level 3 laboratory.

Treatment

Acute, oral, median lethal dose (LD_{50}) in mice (10–12 g body weight) was determined based on the scientific values reported in the literature and was 2.6 mg kg for $CdCl_2$ and 2.09 g kg for MnCl2 respectively. Respective metal salts were dissolved in sterile water and gavaged orally. Control animals were gavaged with equal amount of sterile water. Mice were administered with a single oral dose of either 0.13 mg kg (1/20 LD_{50}), 0.26 mg kg (1/10 LD_{50}) $CdCl_2$ or 419 mg kg (1/5 LD_{50}) $MnCl_2$. All animals were subsequently inoculated either with SFV, EMCV, or VEE. Animals were observed daily morning, noon, and evening for the appearance of virus-induced symptoms, morbidity and mortality. The animals were assigned to groups, which consisted of 6 mice in the group for survival studies and 3 mice for viral titration studies. All experiments were repeated at least three times. Animals were sacrificed on the third day for EMCV and on fourth day for SFV. Finally, brains from same group were collected and pooled and divided in three parts: the first was used for virus titration; the second for RPA; and the remaining was placed in 10% formalin. For viral kinetics studies, animals were sacrificed every 24 h post infection till day 5. We also observed effects of repeated dose of metal on viral multiplication, where mice were gavaged for two more consecutive days after the first day.

Viral titration and mean survival time

Viral titers (TCID50) in brains were determined by cytopathic effect assay in a 10% brain (W/V) homogenate. SFV titers were assayed in BHK cell lines, while EMCV was assayed in LB cells. BHK and

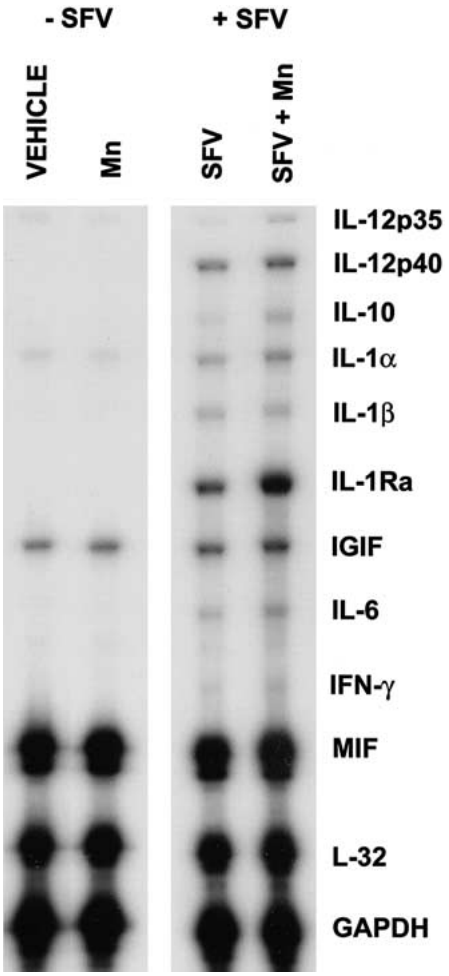

Fig. 3a. Effect of Mn pretreatment (419 mg kg) on cytokine mRNA levels in brain of mice infected with SFV at day 3 post-infection. Figure is representative of a typical Ribonuclease protection assay (RPA) autoradiograph and represents results from three independent experiments.

LB cells were grown in Eagle's minimum essential medium with 10% fetal bovine serum. Viral kinetics was performed from day 2 post infection and was followed till day 5. Mean survival time (MST) was calculated as reported earlier (Maheshwari *et al.* 1991). For VEE titrations, tissues were homogenized in PBS containing 1% donor calf serum resulting in a 20% (W/W) suspension, which was aliquoted, and frozen at −80 °C prior to plaque assay titration's on BHK cells, in BL-3 lab.

Histopathology

To evaluate tissue damage, formalin fixed and paraffin-embedded sections of brains of mice infected with

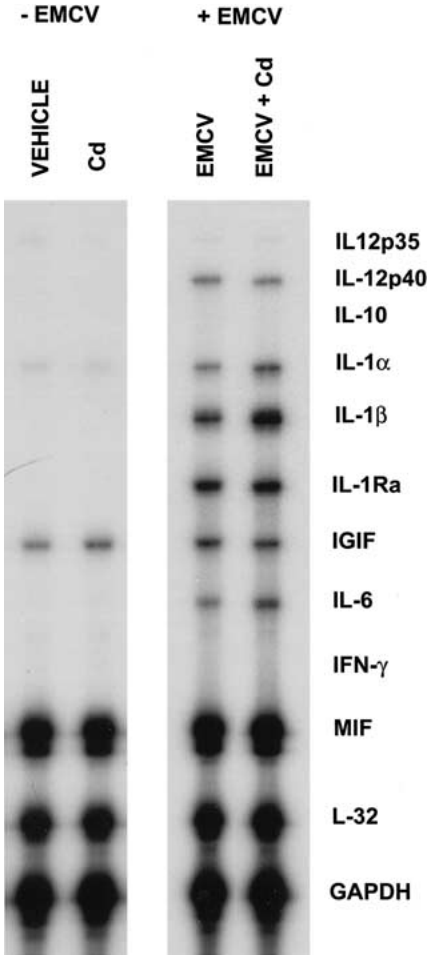

Fig. 3b. Effect of Cd pretreatment (0.13 mg kg) on cytokine mRNA levels in brain of mice infected with EMCV at day 4 post-infection. Typical Ribonuclease protection assay (RPA) autoradiograph representing results from three independent experiments.

SFV or EMCV, and treated with either metals were examined post-infection. Tissue sections (5 μ) were stained with hematoxylin and eosin (H&E), and examined microscopically. Leukocyte migration in the blood vessels, perivascular inflammatory infiltrate, and neuronal destruction in the form of neuronophagia, perikaryolysis and karyorrhexis were looked for as features of encephalomyelitis.

Ribonuclease protection assay of cytokines

We analyzed the expression of cytokine mRNA levels in brains, spleens and livers of mice treated with Cd or Mn, and infected with SFV or EMCV, using the RiboQuant Multiprobe Ribonuclease Protection Assay (RPA) kit (Pharmingen). RPA was also performed on samples from untreated infected mice. Animals from each group were sacrificed and brains were collected, snap-frozen, and stored at -80 °C. RNA was isolated from the frozen tissue samples using TRIzol (Life Technologies Inc.), quantitated, and equalized on formaldehyde agarose gels. For RPA, the protocols used were according to the manufacturer's instructions. Briefly, 20 mg of each RNA sample was hybridized at 56 °C for 12–14 h with a ^{32}P-UTP-labeled probe. The probe was prepared by transcribing the mouse cytokine mck-2b template set using T7 RNA polymerase. After hybridization, samples were subjected to RNase digestion for 45 min at 30 °C. Ribonuclease-protected bands were then resolved on denaturing urea-PAGE gels, followed by autoradiography. The template used allowed us to study the differential regulation of IL-1 α, IL-1β IL-10, IL-12(p35), IL-12(p40), IL-1Ra, interferon (IFN), IGIF, and macrophage inducing factor (MIF). L-32 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNAs served as housekeeping gene controls in the assay, to assure equal loading of RNAs. Quantitations for the band intensities were done using the NIH Image 1.62 software. The mRNA expression was expressed in terms of arbitrary units.

Results

Effects of Cd and Mn on mortality and mean survival time (MST) mice

Mice administered with a single oral dose of 0.13 mg kg (1/20 LD_{50}) $CdCl_2$ or 419 mg kg (1/5 LD_{50}) $MnCl_2$ and infected with virus showed the early appearance of disease symptoms such as roughening of fur and paralysis of hind limbs followed by increased mortality when compared to controls (Table 1). We also studied the effect of co-exposure of repeated doses of Cd and Mn in mice, where mice were treated for three consecutive days. Data showed that both Cd at 0.26 mg kg and Mn at 419 mg kg enhance the mortality with a consistently shorter MST compared to control (Table 2).

Effect of Cd and Mn on virus titers

Kinetic studies with SFV showed that virus appeared on day 1 post-infection (p.i.) in both the Cd- and Mn-treated groups, while the virus titer was below detectable levels in the brain of untreated mice. A

Table 1. Effect of Cd or Mn on sub lethal infection of SFV or EMCV on mice.

Virus	Treatment[a]	# Died/Total #	Mortality (%)
EMCV	Virus alone	0/6	0
	$CdCl_2$ (0.13 mg kg)	1/5	20
	$CdCl_2$ (0.26 mg kg)	2/6	33
	$MnCl_2$ (419 mg kg)	2/6	33
SFV	Virus Alone	2/10	20
	$CdCl_2$ (0.26 mg kg)	10/10	100
	$MnCl_2$ (419 mg kg)	7/10	70

[a]Mice were given $CdCl_2$ or $MnCl_2$ (dissolved in sterile water) by oral gavage in a single dose. Sub lethal dose of EMCV or SFV was injected i.p. and s.c. respectively in 0.25 ml PBS per mouse. Virus alone animals were gavaged with sterile water. Animals were observed for 14 days to record the mortality.

Table 2. Effect of repeated administration of Cd and Mn in infection in mice.

Treatment[a]	Days of observation (Cumulative mortality)														No. Died/Total No.	Mortality (%)	MST (Days)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14			
Virus Alone						2	3				4				4/6	66	9.7
$CdCl_2$ (0.26 mg kg)					3	6									6/6	100	5.5
$MnCl_2$ (419 mg kg)					3	4	6								6/6	100	5.9

[a]Mice were given $CdCl_2$ or $MnCl_2$ (dissolved in sterile water) by oral gavage. Lethal dose of SFV was injected 5 hours after $CdCl_2$ or $MnCl_2$ administration. Mice were continued to be gavaged with the same dose of Cd or Mn for the next two consecutive days post infection. The lethal dose of SFV and repeated doses of toxic chemicals may account for the increased mortality. Virus alone group represents control mice where test agents were replaced with equal volume of water. Mean survival time (MST) was calculated as described.

time-dependent progression of virus titer was observed (Figure 1). Brain tissue from Cd or Mn groups exhibited significantly higher virus titers on earlier days as compared to virus control untreated groups (Figure 1), however, no significant difference in viral titers was found at later time points.

Treatment of mice with Cd, 5 h prior to infection with 10^3 plaque forming units (PFU) of virulent VEE resulted in earlier appearance of VEE in the brains of infected mice. Both Cd (0.13 mg kg) as well as Mn (419 mg kg) treatment increased the viral titers in brains of infected mice at early time points (Figure 2). Specifically, following rear footpad inoculation of VEE in low dose Cd-treated mice, VEE titers of approximately 10^4 PFU/gram fresh tissue were detected at 24 h p.i. In contrast, from vehicle-treated mice, virus could not be isolated from brain tissues at that time point at a detection level of 160 PFU/gram tissue. At 48 h p.i, VEE titers in the brains of Cd- and Mn-treated mice was more than 8-fold elevated when compared to those in untreated controls. At 72 and 96 h p.i, VEE brain titers in both Cd- and Mn-treated and untreated mice were determined to be at similar levels.

Up-regulation of cytokine mRNAs

Cytokine profile studies with RPA reveal that SFV and EMCV infection led to an up-regulation in mRNA levels of various cytokines including IL-12p40, IGIF, IL-1Ra, IL-10 and IL-1α & β as compared to the vehicle-treated brain RNA levels. We also observed that following treatment with Cd or Mn, there was a further significant increase in RNA transcripts for IL-1Ra (0.4675 ± 0.017 Units from 0.2481 ± 0.0183 Units), IL-10 0.1382 ± 0.043 Units from 0.0831 ± 0.003 Units) and IL-12 (p40) (0.1926 ± 0.013 Units from 0.1391 ± 0.006 Units) in brains of SFV-infected mice. RPA results shown in Figure 3a exhibit the up-regulation of the above mentioned cytokines in brains of SFV-infected and Mn-pretreated (419 mg kg) mice. In EMCV-infected mice, a moderate increase in IL-6, and IL-1-α & β was also seen in brains from Cd (0.13 mg kg) pretreated mice (Figure 3b). Similar observations were noted following pretreatment of mice

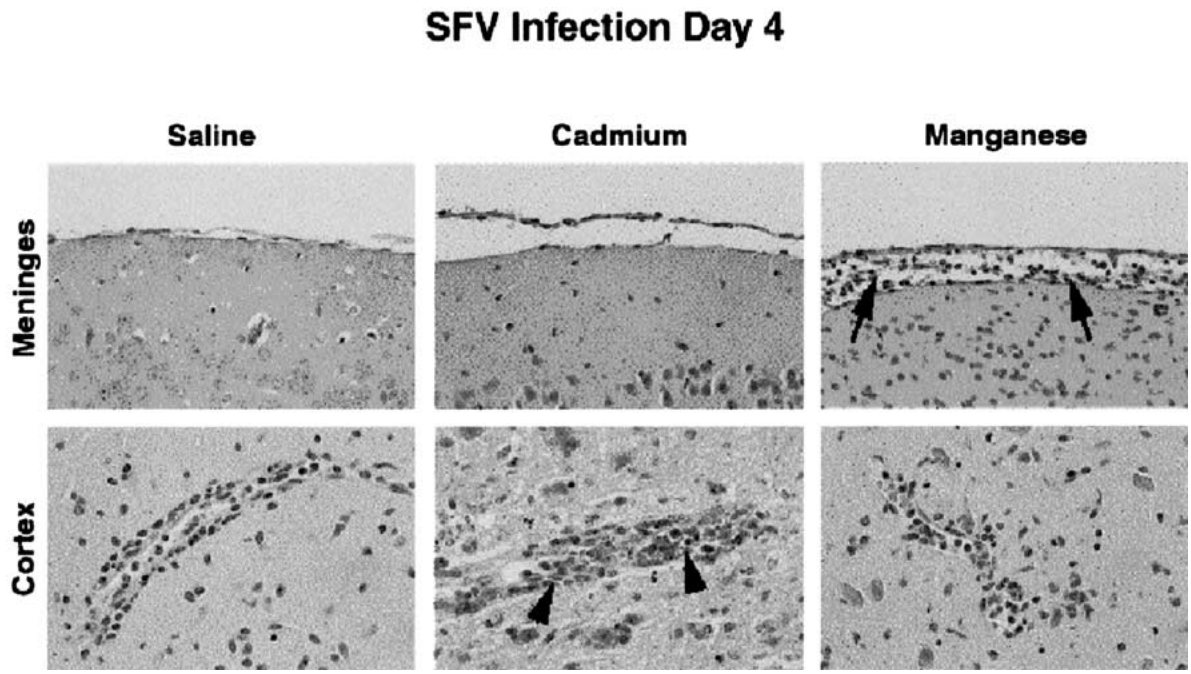

Fig. 4. Histopathological evidence of tissue pathology in brain tissue of Cd and Mn pretreated SFV infected mice. Enhanced perivascular inflammation is seen in cortex region (arrow heads) in sections from Cd (0.13 mg kg, p.o) group animals on day 4. Significant meningitis is seen (arrows) in section from Mn (419 mg kg, p.o) pretreated mice brain as compared to SFV alone. H & E staining of paraffin embedded brain tissue sections of meninges and cortex regions at magnification 40× and 60×, respectively.

with Mn at 419 mg kg (data not shown). Cd & Mn *per se* had no effect on the mRNA levels of cytokine investigated. These patterns were reproducible in repeated experiments.

Histopathological studies

Histopathological evaluation shows that all the infected animals showed features of encephalomyelitis. There was no pathology on day 3 and only a mild vascular inflammation was noted on day 4, in brain tissue of control animals (i.e., vehicle treated and virus infected mice). Perivascular inflammation and encephalitis (day 4) appeared earlier in metal pretreated SFV infected mice (Figure 4). Furthermore, Mn pre-treatment in mice at 419 mg kg dose caused significant meningitis (Figure 4). In EMCV and metal pretreated mice, the perivascular inflammation was significant at day 2 post infection and perichimal inflammatory neuronal damage was significant on day 3. Mn pretreatment caused greater meningitis while encephalitis was maximized in Cd treated (0.26 mg kg) animals (Figure 5). Overall, the intensity of inflammation and neuronal destruction was higher in the tissues of animals treated with Cd & Mn when compared with untreated infected mice.

Discussion

All living organisms are constantly combating a variety of infectious microbes. The invasion of pathogenic microorganisms may create a potentially-life threatening situation in the host if they are not eliminated by host immune mechanisms. Exposure to a variety of environmental pollutants may subsequently affect the susceptibility to disease as well as the course and complications of the disease process (Ilback *et al.* 1992). Exposure to several environmental contaminants can result in immunosuppression leading to decreased resistance to infections in experimental animals and humans (Krzystynika *et al.* 1995). This is particularly true for systemic exposure to toxic metals (Lawrence 1984). Although, the emission of Cd and several other metals are being controlled more efficiently, some of these metals remain of great significance as environmental pollutants (Gainer 1977; Ilback 1992). Exon *et al.* (1979) reported that Cd treated mice were more susceptible to EMCV infection.

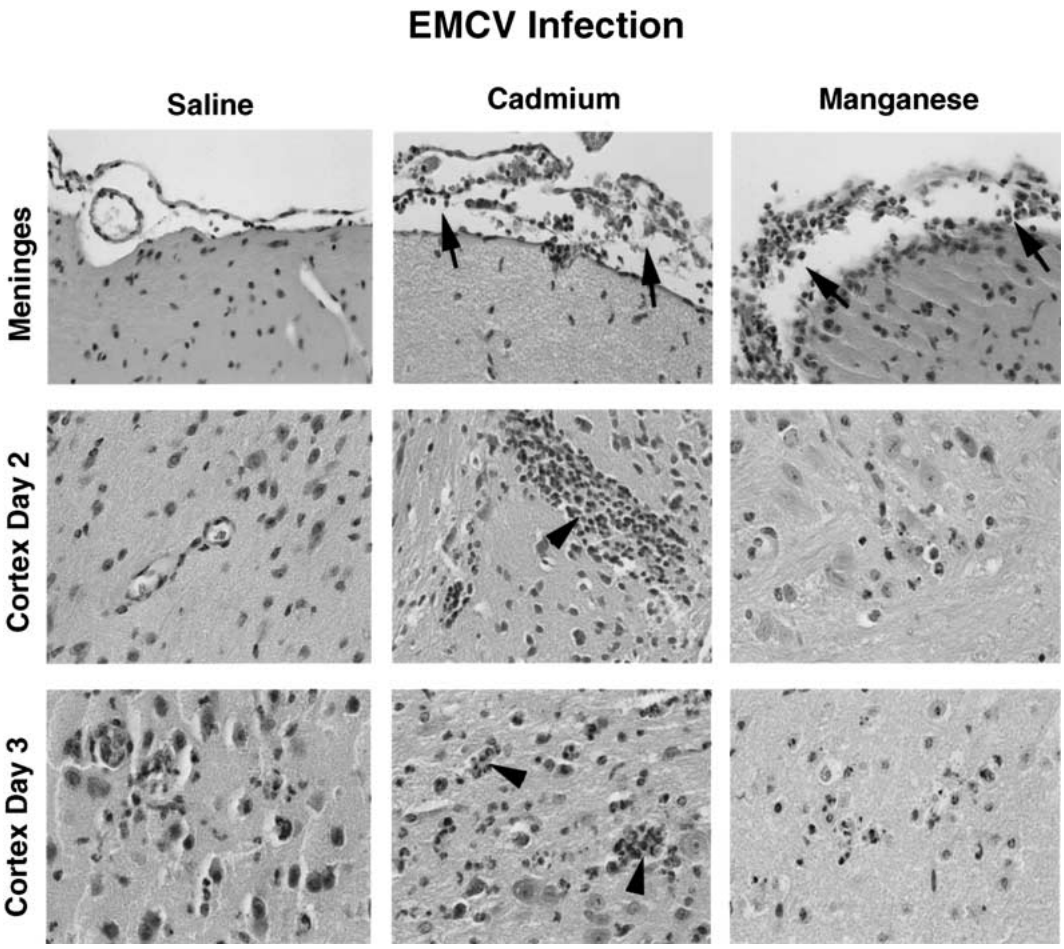

Fig. 5. Represents H & E staining in brain tissue sections exhibiting tissue pathology in Cd (0.13 mg kg, p.o) and Mn (419 mg kg, p.o) pretreated EMCV infected mice of meninges and cortex regions at magnification 40× and 60×, respectively. Significant meningitis is seen in (arrows) section from Cd as well as Mn pretreated mice in meninges area as compared to EMCV alone or the saline treated group. Enhanced perivascular inflammation is seen in cortex region (arrow heads) in sections from Cd group animals on day 2 as compared to the saline treated group (EMCV control). Not much difference is seen in cortex region of Mn treated group.

The studies on the adverse effects of Mn on the immune system including host susceptibility to pathogens are obscure, although Mn exerts its chronic deleterious effects on neurochemical functions. Manganese ranks third among heavy metals that possess immunosuppressive properties (Lawrence 1981). Specifically, heavy metals have been ranked according to immunosuppressive properties as follows: Hg > Cu > Mn > Co > Cd > Cr (Lawrence 1981). Our results demonstrated for the first time, that Mn significantly increased the susceptibility of mice to EMCV, SFV, and VEE.

The earlier appearance of virus-induced symptoms, increased mortality and morbidity, greater tissue pathology significantly elevated viral titers, clearly demonstrate the early viral presence in brain, following treatment with Cd and Mn. We also noted a concomitant up-regulation of inflammatory cytokines at the mRNA levels. Interestingly, these effects were not restricted to any one virus, but were observed following infection with different virus such as SFV, EMCV, or VEE.

Cytokines play an important role in SFV encephalitis. SFV has previously been used as a tool to study mechanisms in neuro-immunology. Various interleukins including IL-1α, IL-2, IL-4 and IL-6 have been shown to be up-regulated in the CNS of SFV-infected mice (Potvin *et al.* 1997; Morris *et al.* 1997). In our studies, following SFV or EMCV infection, we observed an augmentation of mainly IL-1Ra,

IL-12p40 and IL-1 in brains, at the mRNA levels. Treatment of mice with Cd and Mn further enhanced the mRNA levels of these cytokines. Cd and Mn by itself had no effect on cytokine up-regulation.

IL-1Ra levels are increased by a number of viruses. IL-1Ra has been reported to be up-regulation in monocytes/macrophages by cytomegalovirus (Kline *et al.* 1994), in uninfected monocytes by Tat gene of HIV-1 (Rautonen 1994), and in neutrophils by Epstein-Barr virus (EBV) (Roberge *et al.* 1996). Elevated IL-1Ra/IL-1β ratios, have been reported in serum of hepatitis patients (Sekiyama *et al.* 1994). Recently, significant production of IL-1Ra levels by human monocytes, that exceeded production of IL-1α and IL-1β, was seen in HIV infection. Additionally, higher IL-1Ra concentrations were reported in the cerebrospinal fluid of HIV-infected patients. We propose, that in our studies, the up-regulation of IL-1Ra in mice treated with Cd and Mn may have been responsible for disrupting immune responses in the early stages of SFV infection. This could have led to early onset of the virus in tissue, an accelerated dissemination and ultimately have the potential for greater virus replication and increased severity of disease. Our investigations reveal that Cd and Mn pretreatment increased mortality, viral titer, tissue pathology and up-regulated cytokines like IL-1Ra at mRNA levels. These alterations may affect host defense system of mice. However, additional research is required to determine the total effects, sites and mechanism of interaction of the pollutant with the pathogen within the host.

Inducers of inflammatory cytokines, like lipopolysaccharide (LPS) (Xioa *et al.* 2001), and certain inflammatory cytokines (Tsao *et al.* 2001) have been shown to alter the blood brain barrier (BBB) permeability. Some recent reports also demonstrate that inflammatory cytokines modulate the expression of certain matrix metalloproteinases (MMP), in particular, MMP-9. MMPs have been implicated for weakening of the basement membrane and may contribute to the disruption of BBB (Lukes *et al.* 1999) in certain CNS disorders, like multiple sclerosis. Disruption of BBB may facilitate the early entry of virus in brain tissue and account for the increased viral titers in our study. Currently, we are investigating the effect of these metals on BBB permeability that may help the virus to gain an early entry.

Widespread environmental contamination by these metals, and their potential for simultaneous exposure and infection of humans and animals are clearly indicative that further in depth studies not only of these metals, but contaminants in general, are needed to understand the total effect of environmental pollution on human health.

Acknowledgements

This work was supported by a grant (G174 HA) from the Naval Medical Research, the Army Medical Research and Material Command grant (G174 KY) and Development Command and US-INDIA Foreign Currency Fund from US Department of State to USUHS. The authors are grateful to Dr P.K. Seth, Director, Industrial Toxicological Research Center, Lucknow, India, for fruitful suggestions for this study. The opinions or assertions contained herein are the private views of the authors and should not be construed as official or necessarily reflecting the views of the Uniformed Services University of the Health Sciences or the Department of Defense, USA.

References

Alonso E, Cambra K, Martinez T. 2001 Lead and cadmium exposure from contaminated soil among residents of a farm area near an industrial site. *Arch Environ Health* **56**, 278–282.

ATSDR: Toxicological Profile for Cadmium. (Update) – 99/07. US Dept. of Health and Human Services, Public Heath Service, Atlanta, GA: Agency for Toxic Substances and Disease Registry 1999.

ATSDR: Toxicological Profile for Manganese. (Update) – 2000/10. US Dept. of Health and Human Services, Public Heath Service, Atlanta, GA: Agency for Toxic Substances and Disease Registry 2000.

Beliles RP. 1994 The Metals. In: Clayton GD & Clayton FE, eds. *Patty's Industrial Hygiene and Toxicology*, Vol. 11, Part C, 4th Edn. New York: John Wiley and Sons Inc.; 1879–1967.

Beliles RP. 1994 The Metals. In: Clayton GD & Clayton. FE, eds. *Patty's Industrial Hygiene and Toxicology*, Vol. 11, Part C, 4th Edn, New York, John Wileys Sons Inc.; 2106–2124.

Bernier J, Brousseau P, Krzytyniak K, Tryphonas H, Fournier M. 1995 Immunotoxicology of heavy metals in relation to great lakes. *Env Health Persp* **103** (Suppl. 9), 23–34.

Burchiel SW. 1999 The effects of environmental and other chemicals on the human immune system: the emergence of immunotoxicology. *Clin Immunol* **90**, 285–286.

Burns LA, Meade BJ, Munson AE. 1996 Toxic responses of the immune system. In: Klassen CD, Amdur MO, Doull J, eds. *Casarett and Daull's Toxicology, The Basic Science of Poisons*, 5th edn., New York: McGrawHill Publishers; 355–402.

Chaumard C, Forestier F, Quero AM. 1991 Influence of inhaled cadmium on the immune response to influenza virus. *Arch Environ Health* **46**, 50–56.

Daniels MJ, Menache MG, Burleson GR, Graham JA, Selgrade MJK. 1987 Effects of $NiCl_2$ and $CdCl_2$ on susceptibility to murine cytomegalovirus and virus augmented natural killer cell and interferon responses. *Fund Appl Toxicol* **8**, 443–453.

Davis NL, Willis LV, Smith JF and Johnston RE. 1989 *In vitro* synthesis of infectious venezuelan equine encephalitis virus RNA from a cDNA clone: analysis of a variable deletion mutant. *Virology* **171**, 189–204.

Exon JH, Koller LD, Kerkvliet NI. 1979 Lead-Cadmium interaction: Effects on viral induced mortality and tissue residues in mice. *Arch Environ Health* **34**, 469–475.

Exon JH, Koller LD, Kerkvliet NJ. 1986 Tissue residues, pathology and viral induced mortality in mice chronically exposed to different cadmium salts. *J Exptl Pathol Toxicol Oncol (JEPTO)* **7**, 109–114.

Fawl RL, Gesser RM, Valyi-Nagi T, Fraser NW. 1996 Reactivation of herpes simplex virus from latently injected mice after administration of cadmium is mouse-strain-dependent. *J Gen Virol* **77**, 2781–2786.

Gainer JH. 1977 Effects of Heavy Metals and of deficiency of zinc on mortality rates in mice infected with Encephalomyocarditis virus. *Am J Vet Res* **38**, 869–872.

Glynn AW, Lind Y, Funseth E, Ilback NG. 1998 The intestinal absorption of cadmium increases during a common viral infection (coxsackie virusB3) in mice. *Chem-Biol Interac* **113**, 79–89.

Grieder FB & Nguyen HT. 1996 Virulent and attenuated mutant Venezuelan equine encephalitis virus shows marked differences in replication in infection in murine macrophages. *Microbiol Pathol* **21**, 85–95.

Ilback N-G, Fohlman J, Friman G, Glynn AW. 1992 Altered distribution of ^{109}cadmium in mice during viral infection. *Toxicology* **71**, 193–202.

Kline JN, Geist LJ, Monick MM, Stinski, MF, Hunninghake GW. 1994 Regulation of expression of the IL-1 receptor antagonist (IL-1Ra) gene by products of the human cytomegalovirus immediate early genes. *J Immunol* **152**, 2351–2357.

Krzystyniak K, Tryphonas H, Fournier M. 1995 Approaches to the evaluation of chemical-induced immunotoxicity. *Environ Health Persp* **103**, 17–22.

Lawrence DA. 1984 Immunotoxicity of Heavy Metals. In: Dean JH, Luster MI, Munson AE, Amos H., eds. *Immunotoxicology and Immunopharmacology*. New York: Raven Press; 341–353.

Lawrence DA. 1981 Heavy metals modulation of lymphocyte activities. *Toxicol Appl Pharmacol* **57**, 439–451.

Lukes A, Mun-Bryce S, Lukes M, Rosenberg GA. 1999 Extracellular matrix degradation by metalloproteinases and central nervous system diseases. *Mol Neurobiol* **19**, 267–284.

Maheshwari RK, Srikantan V, Bhartiya D. 1991 Chloroquine enhances replication of Semliki Forest virus and encephalomyocarditis virus in mice. *J Virol* **65**, 992–995.

Minami A, Takeda A, Nishibaba D, Takefuta S, Oku N. 2001 Cadmium toxicity in synaptic neurotransmission in the brain. *Brain Res* **894**, 336–339.

Morris MM, Dyson H, Baker D, Harbige LS, Fazakerley JK, Amor S. 1997 Characterization of the cellular and cytokine response in the central nervous system following Semliki Forest virus infection. *J Neuroimmunol* **74**, 185–197.

Pelletier L & Druet P. 1995 Immunotoxicology of Metals. In: Goyer RA & Cherian MG, eds. *Toxicology of Metals, Biochemical Aspects.* (Handbook of Exp Pharm **115**, 77–92). New York: Springer-Verlag.

Potvin F, Petitclerc E, Marceau F, Poubelle PE. 1997 Mechanisms of action of antimalarials in inflammation: induction of apoptosis in human endothelial cells. *J Immunol* **158**, 1872–1879.

Rautonen N, Rautonen J, Martin NL, Wara DW. 1994 HIV-1 Tat induces cytokine synthesis by uninfected mononuclear cells. *AIDS* **8**, 1504–1506.

Roberge CJ, Poubelle PE, Beaulieu AD, Heitz D, Gosselin J. 1996 The IL-1 and IL-1 receptor antagonist (IL-1Ra) response of human neutrophils to EBV stimulation. Preponderance of IL-1Ra detection. *J Immunol* **156**, 4884–4891.

Sekiyama KD, Yoshiba M, Thomson AW. 1994 Circulating proinflammatory cytokines (IL-1a, TNF-a and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol* **98**, 71–77.

Srisuchart B, Taylor MJ, Sharma RP. 1987 Alteration of humoral and cellular immunity in manganese chloride treated mice. *J Toxicol Environ Health* **22**, 91–99.

Thomas PT, Ratajczak HV, Aranyi C, Gibbons R, Fenters JD. 1985 Evaluation of host resistance and immune function in cadmium exposed mice. *Toxicol Appl Pharmacol* **80**, 440–456.

Tsao N, Hsu HP, Wu CM, Liu CC, Lei HY. 2001 Tumour necrosis factor-alpha causes an increase in blood-brain barrier permeability during sepsis. *J Med Microbiol* **50**, 812–821.

Xaio H, Banks WA, Niehoff ML, Morley JE. 2001 Effect of LPS on the permeability of the blood-brain barrier to insulin. *Brain Res* **896**, 36–42.