Environmentally Relevant Metal and Transition Metal Ions Enhance FcεRI-Mediated Mast Cell Activation

Aurelia Walczak-Drzewiecka,[1] Janina Wyczółkowska,[1] and Jaroslaw Dastych[2]

[1]Department of Biogenic Amines, Polish Academy of Sciences, Lodz, Poland; [2]Laboratory of Molecular Immunology, International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland

Upon contact with allergen, sensitized mast cells release highly active proinflammatory mediators. Allergen-mediated mast cell activation is an important mechanism in the pathogenesis of atopic asthma. Asthmatic patients are especially susceptible to air pollution. Epidemiologic studies found a positive correlation between severity of symptoms among asthmatic patients and the level of particulate matter (PM) in the air. Among the constituents of PM are metals and transition metals, which could mediate some of its adverse effects on human health. We sought to determine the effect of metal and transition metal ions on allergen-mediated mast cell activation. We observed that several metal and transition metal ions activated mast cells and enhanced allergen-mediated mast cell activation. Thus, Al^{3+}, Cd^{2+}, and Sr^{2+} induced release of granule-associated *N*-acetyl-β-D-hexosaminidase, and Al^{3+} and Ni^{2+} enhanced antigen-mediated release. Metal and transition metal ions also induced significant secretion of interleukin (IL)-4 and increased antigen-mediated IL-4 secretion in mast cells. These effects of metal and transition metal ions on mast cells were observed at concentrations that do not result in direct cytotoxicity and might be relevant for environmental exposure. Thus, metals and transition metals could increase the level of allergen-mediated mast cell activation, which might be one of the mechanisms mediating exacerbation of allergen-driven asthma symptoms by air pollution. *Key words:* **allergy, asthma, mast cell, IL-4, metals, PM, particulate matter, transition metals.** *Environ Health Perspect* **111:708–713 (2003). doi:10.1289/ehp.5960 available via *http://dx.doi.org/* [Online 21 January 2003]**

Environmental or occupational exposures to metal and transition metal compounds have been linked to adverse health effects in humans, including dysfunctions of the immune system such as allergic and autoimmune diseases (Bigazzi 1999; Domingo 1994; Kanerva et al. 2000; Norseth 1988; Rondeau et al. 2000; Stejskal and Stejskal 1999). One of the possible sources of exposure to metal and transition metal ions in a nonoccupational environment is the particulate matter (PM) present in the air. The mass of particles of a certain diameter [PM ≤ 10 μm (PM_{10}) or PM ≤ 2.5 μm ($PM_{2.5}$)] is a standard measure for level of air pollution (Donaldson and MacNee 2001). Among the constituents of PM_{10} and $PM_{2.5}$ are metals and transition metals such as nickel, lead, ferrite, ferrous, chromium, manganese, and aluminum (Balachandran et al. 2000; Hrsak et al. 2000; Ozkaynak et al. 1996). A correlation between some biological effects of residual oil fly ash observed *in vitro* and its metal and transition metal content has been reported (Lambert et al. 2000; Samet et al. 1997).

An increase in prevalence of allergic diseases, including allergic asthma, among populations of several industrialized countries has been reported (Holgate 1999). The influence of environmental factors is the most likely explanation for this increase, and air pollution has been considered one of these factors (Heinrich et al. 1999). Two types of possible links between air pollution and allergic asthma have been sought. One hypothesis proposes that exposure to air pollution increases the probability of developing allergic asthma (Donaldson et al. 2000; Heinrich et al. 1999). This causative link cannot be ruled out, but several epidemiologic studies did not find a correlation between the number of allergic asthma patients and the level of exposure to air pollution in a given population (Donaldson et al. 2000). However, several independent epidemiologic studies support a positive correlation between severity of symptoms among asthmatic patients and PM_{10} concentration (Donaldson et al. 2000; Gavett and Koren 2001). Thus, air pollution seems to precipitate and worsen asthma attacks among people with already existing disease. It is not clear which component of airborne pollutants constitutes the greatest health risk for asthmatic patients, but metal ions are considered one of the likely culprits (Costa and Dreher 1997).

IgE-mediated allergic inflammation is the central mechanism involved in the pathogenesis of atopic asthma (Busse and Lemanske 2001). Allergen-mediated activation of immune cells results in release of a number of inflammatory mediators. This in turn leads to the pathological changes in lung tissues, which finally result in airflow obstruction (Maddox and Schwartz 2002). Mast cells are critical effector cells in IgE-mediated allergic inflammation (Hart 2001; Taylor and Metcalfe 2001; Williams and Galli 2000). They accumulate in tissues exposed to the outside environment and are capable of releasing large numbers of highly active mediators such as histamine, serotonin, heparin, prostaglandins, leukotrienes, neutral proteases, and a number of cytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-4 (Metcalfe et al. 1997). Antigen-mediated mast cell activation depends on aggregation of high-affinity receptors for IgE (FcεRI), which in turn initiates phosphorylation of multiple mast cell proteins on tyrosine (Benhamou et al. 1990; Hamawy et al. 1995; Kent et al. 1994). This signaling cascade activates exocytosis and upregulates expression of multiple genes, including cytokines such as TNF-α and IL-4 (Brown and Hural 1997; Gordon et al. 1990; Metcalfe et al. 1997).

Heavy-metal ions Hg^{2+} and Ag^{2+} induce mediator release and enhance allergen-mediated mediator release from mast cells *in vitro* (Dastych et al. 1999; Suzuki et al. 2001). We decided to expand this observation and investigate effects of several other metal and transition metal ions on allergen-mediated mast cell activation. In this article, we will show that several metal ions activate mast cells *in vitro* to release granule contents and produce IL-4. Furthermore, some metal ions enhance IgE-mediated mast cell degranulation and IL-4 expression. We observed this effect on mast cell function at concentrations that do not result in a significant level of direct cytotoxicity and might be relevant for environmental exposure. The enhancement of allergen-mediated mast cell activation by metal and transition metal ions could be one mechanism that allows for exacerbation of allergen-driven asthma symptoms by air pollution.

Materials and Methods

Materials. Dulbecco's modified Eagle's medium (DMEM), RPMI-1640, fetal calf serum (FCS), HEPES, L-glutamine, 2-mercaptoethanol, penicillin/streptomycin, bovine serum albumin (BSA), dinitrophenyl conjugated human serum albumin (DNP-HSA), *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside, and neutral red were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Address correspondence to J. Dastych, International Institute of Molecular and Cell Biology in Warsaw, Ks. Trojdena 4, 02-102 Warsaw, Poland. Telephone: (48-22) 668 5095. Fax: (48-22) 668 5288. E-mail: jdastych@iimcb.gov.pl

This work was supported in part by grant 4 P05A 051 18 from the State Committee for Scientific Research in Poland. The authors thank A. Ślusarczyk and T. Michoń for their excellent technical support.

The authors declare they have no conflict of interest.

Received 20 August 2002; accepted 16 January 2003.

We purchased antimurine IL-4 monoclonal antibody (mAb) clone 11B11 from Hazleton (Hazleton, PA, USA); biotin conjugated anti–IL-4 mAb clone BVD6-24G2 from Pharmingen (San Diego, CA, USA); horseradish peroxidase (HRP)-conjugated avidin from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA); recombinant murine IL-4 from PeproTech (Rocky Hill, NJ, USA); and analytical-grade nickel sulfate, aluminum chloride, strontium chloride, cadmium chloride, and lead nitrate from POCH (Gliwice, Poland). Murine monoclonal dinitrophenyl (DNP)-specific IgE was obtained from the culture of hybridoma Hi-DNP-ε-26.82.

Cell culture. C1.MC/C57.1 (C57) (Tsai 1996) mouse mast cells were cultured in DMEM supplemented with 10% heat-inactivated FCS, 4 mM L-glutamine, 25 mM HEPES, 50 μM 2-mercaptoethanol, and 100 μg/mL penicillin/streptomycin (complete DMEM). Cells were cultured at 37°C in a CO_2 incubator (5% CO_2).

Sensitization and stimulation of mast cells. Mast cells were collected by centrifugation; the cell number was adjusted to 10^6 cells/mL by suspending the cell pellet in complete DMEM. The cell suspension was mixed with anti-DNP IgE containing supernatant in dilution 1:5 (vol:vol), and cells were incubated 2 hr in a CO_2 incubator (5% CO_2; 37°C). Cells were then washed with medium three times and resuspended in RPMI-1640 without phenol red for the *N*-acetyl-β-D-hexosaminidase activity release assay or in complete DMEM for the cytokine secretion assay.

Neutral red uptake assay. Mast cells were incubated with increasing concentrations of metal ions for 4 hr at 37°C, washed twice with centrifugation, then incubated with 50 μg/mL neutral red for 3 hr. After this incubation, cells were washed and centrifuged, and supernatants were removed. Acetic acid was added to the cell suspensions to a final concentration of 1%, and cell lysates were transferred to a 96-well plate. The plates were read in an enzyme-linked immunosorbent assay (ELISA) reader at 550 nm. To verify that the neutral red uptake assay can detect the cytotoxic effect of metal salts, the cytotoxicity of increasing concentrations of $CdCl_2$ was assessed using parallel neutral red uptake and trypan blue exclusion tests. Statistically significant cytotoxic effects (decrease in neutral red uptake and increase in number of trypan blue–positive cells) were first observed at 10^{-4} M Cd^{2+}. A 6% decrease in the number of trypan blue–negative cells was associated with a 50% decrease in optical density (OD) 550 nm.

Mediator release assay. The percentage of *N*-acetyl-β-D-hexosaminidase activity released into supernatants was assayed as described by Dastych et al. (1999) and Schwartz et al. (1979). Briefly, 4×10^4 mast cells suspended in RPMI-1640 without phenol red were placed in individual wells on 96-well plates. Increasing concentrations of metal ions dissolved in media with or without indicated concentrations of antigen (DNP-HSA) dissolved in media or media alone were added to selected wells for a final volume of 100 μL. After 15-min incubation at 37°C, the reaction was stopped by centrifugation at 4°C. We added 20 μL of 1% Triton X-100 to some of the wells to obtain cell lysate (total). We then transferred 50 μL of supernatant from control or assay wells or 60 μL of cell lysate into corresponding wells on a second 96-well plate. We then added 10 μL of 1% Triton X-100 to the wells containing supernatant or control media (blank) to adjust the volume and Triton X-100 concentration. This was followed by the addition of 60 μL 0.08 mM citric buffer, pH 4.5, containing 8 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside as a chromogenic substrate for *N*-acetyl-β-D-hexosaminidase, and the plate was incubated for 1.5 hr at 37°C. The reaction was stopped by the addition of 200 μL 0.2 M glycine. The OD at 405 nm was determined with an ELISA reader, and the percentage of the total enzymatic

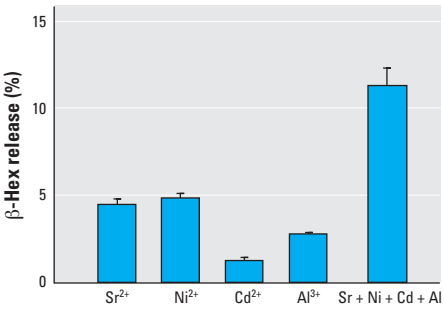

Figure 1. Effect of metal and transition metal ions on mediator release in nonsensitized mast cells. Mouse C57 mast cells were incubated with 10^{-7} M $SrCl_2$, $NiSO_4$, $CdCl_2$, $AlCl_3$, or a mixture containing 10^{-7} M of each for 10 min. The percentage of granule-associated *N*-acetyl-β-D-hexosaminidase (β-Hex) activity released into supernatant was determined, and the spontaneous release (9.8 ± 1.3) was subtracted. Bars represent mean ± SEM from three independent experiments, each performed in triplicate.

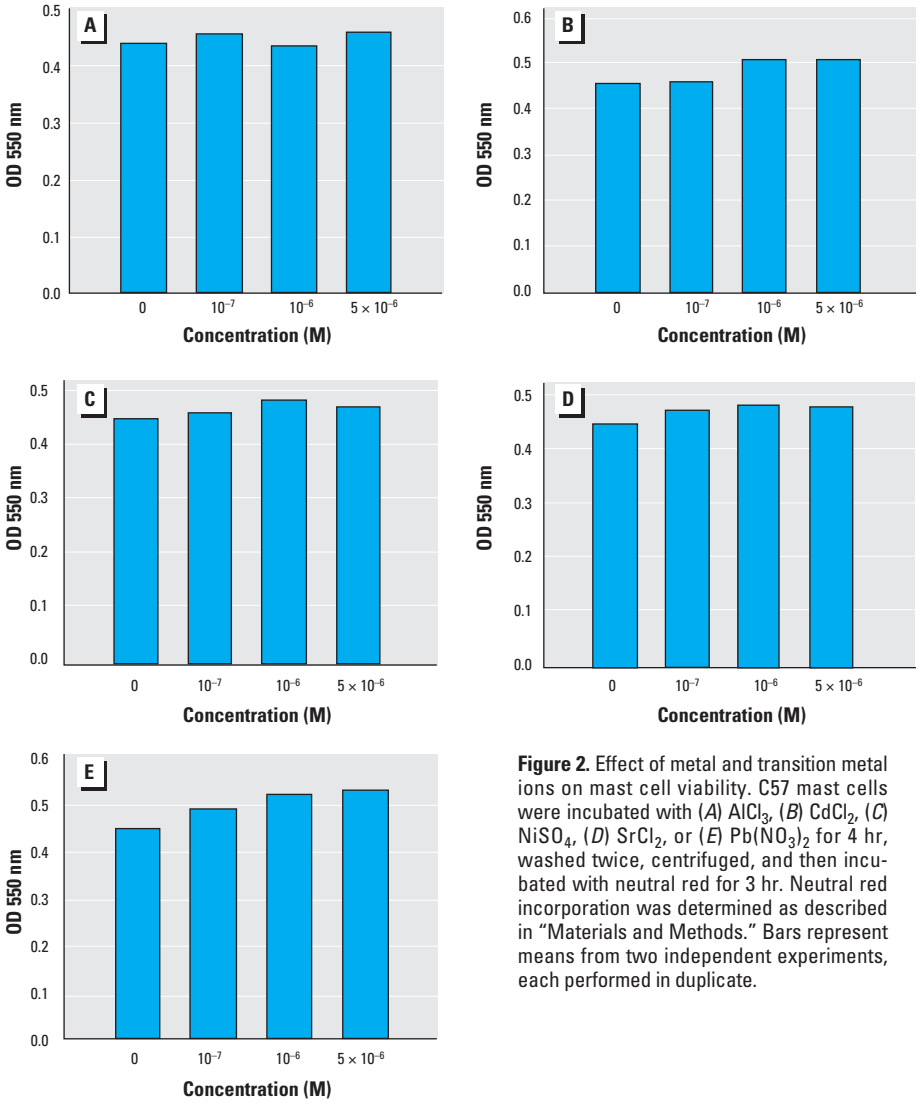

Figure 2. Effect of metal and transition metal ions on mast cell viability. C57 mast cells were incubated with (*A*) $AlCl_3$, (*B*) $CdCl_2$, (*C*) $NiSO_4$, (*D*) $SrCl_2$, or (*E*) $Pb(NO_3)_2$ for 4 hr, washed twice, centrifuged, and then incubated with neutral red for 3 hr. Neutral red incorporation was determined as described in "Materials and Methods." Bars represent means from two independent experiments, each performed in duplicate.

activity released into the supernatant was calculated by using the following formula:

$$\text{Release} = (\text{supernatant} - \text{blank}) \div (\text{total} - \text{blank}) \times 100\%.$$

We tested the possible effect of the presence of $NiSO_4$, $AlCl_3$, $SrCl_2$, $CdCl_2$, and $Pb(NO_3)_2$ in the assay buffer on the enzymatic activity of *N*-acetyl-β-D-hexosaminidase. None of the tested compounds affected *N*-acetyl-β-D-hexosaminidase enzymatic activity in mast cell lysate at concentrations ≤ 5×10^{-6} M.

Cytokine secretion assay. To measure the amount of IL-4 protein secreted into the supernatant, we suspended mast cells in complete DMEM at a density of 10^6 cells/mL. Increasing concentrations of metal ions, antigen (DNP-HSA), or a combination of both were added in a final volume of 0.3 mL, and cells were incubated at 37°C for 4 hr. After incubation, cell suspensions were centrifuged at 600 × *g* for 10 min, and supernatants were collected and stored at –20°C. The ELISA for IL-4 was based on a standard protocol provided by Pharmingen. Briefly, 96-well plates were coated overnight with 11B11 anti–mIL-4 mAb (2 µg/mL). Plates were blocked with 3% BSA dissolved in phosphate-buffered saline (PBS) for 3 hr at room temperature. Wells were rinsed with PBS containing 0.1% Tween-20, samples or standards of rmIL-4 were added, and plates were incubated overnight at 4°C. Each plate was then rinsed with PBS containing 0.1% Tween-20 and incubated 45 min at room temperature with biotinylated anti–IL-4 mAb. This was followed by incubation with HRP-conjugated avidin for 30 min (250 ng/mL) and with *o*-phenylenediamine as a chromogenic substrate for additional 20 min. We added 3 M H_2SO_4 to stop the reaction; plates were read in an ELISA reader at 450 nm.

Western blotting. Cells were collected by centrifugation and lysed in buffer containing 50 mM Tris-HCl (pH 8.0), 1% nonionic detergent IGEPAL CA-630 (Sigma Chemical Company), 20 mM ethylenediaminetetraacetic acid, 150 mmol/L sodium chloride, 1 mmol/L magnesium chloride, 10 mM tetrasodium pyrophosphate, 100 mM sodium fluoride, 2 mM sodium orthovanadate, 10 µg/mL leupeptin, 10 µg/mL aprotinin, and 1 mM phenylmethylsulfonyl fluoride. After 30-min incubation at 4°C, lysates were centrifuged at 10,000 × *g* for 15 min at 4°C. Supernatants were mixed with loading buffer containing sodium dodecylsulfate (SDS) and 2-mercaptoethanol, boiled for 5 min, and separated with SDS-polyacrylamide gel electrophoresis (4–12% gradient gels). Proteins were electrotransferred to nitrocellulose and immunoblotted with the HRP-conjugated PY20 antiphosphotyrosine antibody, and specific bands were detected with chemiluminescence (SuperSignal Substrate; Pierce Biotechnology Inc, Rockford, IL, USA). Next, the nitrocellulose membrane was stripped and reblotted with antiactin antibody followed by secondary HRP-conjugated antibody, and specific bands were detected with chemiluminescence. Optical densities associated with specific bands were measured using Quantity One software (BioRad Laboratories, Hercules, CA, USA).

Statistical analysis. Statistical significance of observed differences was determined using one-way analysis of variance followed by Dunnett test.

Results

The level of mast cell degranulation was determined by the percentage of release of granule-associated *N*-acetyl-β-D-hexosaminidase activity into supernatant (Dastych et al. 1997). The majority of *N*-acetyl-β-D-hexosaminidase in mast cells is stored in mast cell granules (Schwartz and Austen 1980) and is released upon mast cell activation in parallel with other preformed mediators such as β-glucuronidase, β-D-galactosidase, mast cell-specific proteases, chondroitin sulfate E proteoglycan, and histamine (Razin et al. 1983; Schwartz and Austen 1980; Schwartz et al. 1979, 1981). The release of *N*-acetyl-β-D-hexosaminidase is associated with the extrusion of exocytotic granules, which can be visualized with specific staining (Demo et al. 1999). To determine if some metal and transition metal ions expected to be present in PM of ambient air could cause mast cell degranulation, mouse mast cells C57.1 were exposed to 10^{-7} M $SrCl_2$, $NiSO_4$, $CdCl_2$, or $AlCl_3$, or to the mixture containing 10^{-7} M of each compound. As shown in Figure 1, incubation with such concentrations of $NiSO_4$, $AlCl_3$, $SrCl_2$, or $CdCl_2$ resulted in a low (2–5%) release of granule-associated *N*-acetyl-β-D-hexosaminidase in nonsensitized mast cells. Interestingly, a mixture of these compounds induced a much higher release (11%), which suggests that the effects of exposure to metal and transition metal ions at these concentrations could be additive. We next

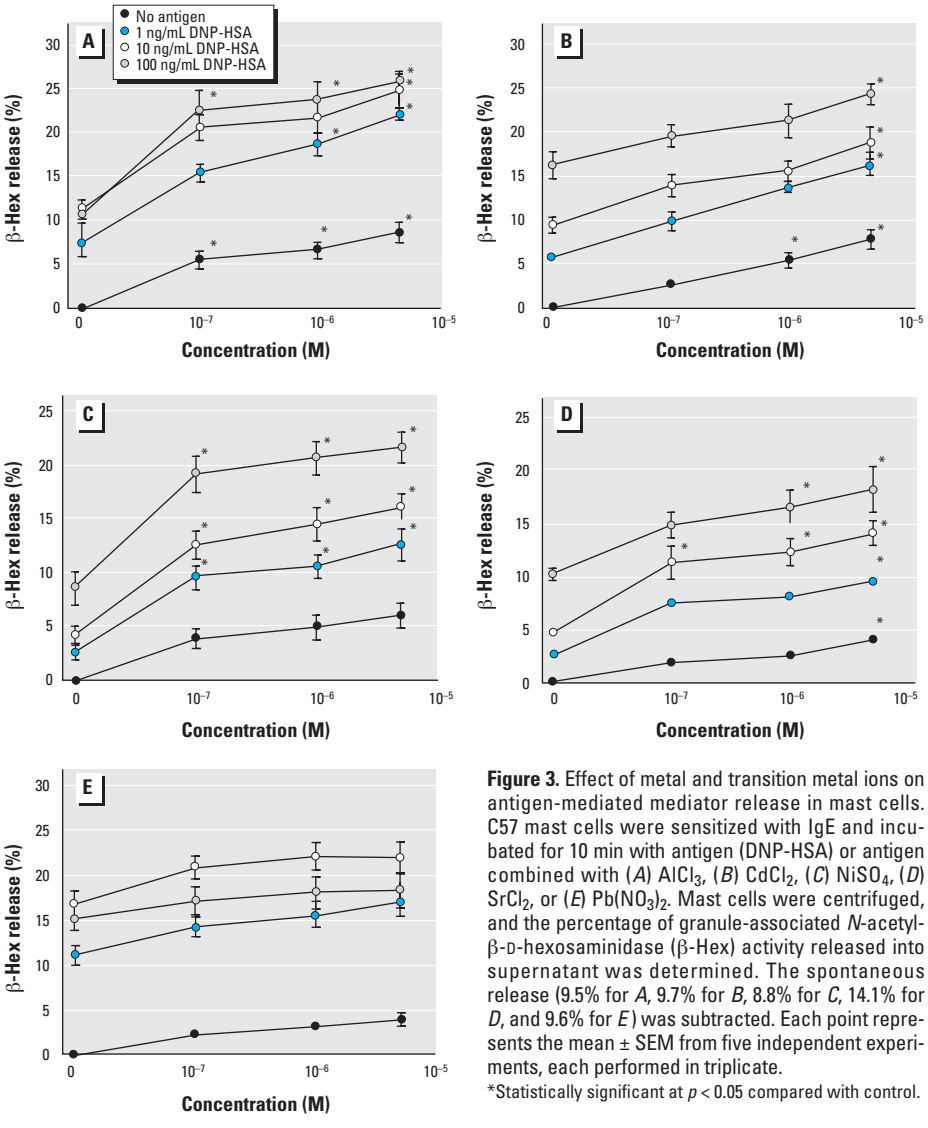

Figure 3. Effect of metal and transition metal ions on antigen-mediated mediator release in mast cells. C57 mast cells were sensitized with IgE and incubated for 10 min with antigen (DNP-HSA) or antigen combined with (*A*) $AlCl_3$, (*B*) $CdCl_2$, (*C*) $NiSO_4$, (*D*) $SrCl_2$, or (*E*) $Pb(NO_3)_2$. Mast cells were centrifuged, and the percentage of granule-associated *N*-acetyl-β-D-hexosaminidase (β-Hex) activity released into supernatant was determined. The spontaneous release (9.5% for *A*, 9.7% for *B*, 8.8% for *C*, 14.1% for *D*, and 9.6% for *E*) was subtracted. Each point represents the mean ± SEM from five independent experiments, each performed in triplicate.
*Statistically significant at $p < 0.05$ compared with control.

decided to systematically test the effect of increasing concentrations of $NiSO_4$, $AlCl_3$, $SrCl_2$, $CdCl_2$, or $Pb(NO_3)_2$ present in incubation media on mast cell function. First, the direct cytotoxic effects of increasing concentrations of these compounds have been determined. As shown in Figure 2, incubation of C57 mast cells for 4 hr in medium containing 10^{-7}–5×10^{-6} M $NiSO_4$, $AlCl_3$, $SrCl_2$, $CdCl_2$, or $Pb(NO_3)_2$ did not result in a decrease in neutral red uptake compared with control. Thus, exposure of mast cells to metal and transition metal salts at concentrations and incubation time employed did not result in a decrease in cell viability.

Mast cells sensitized *in vitro* with IgE respond to antigen by degranulation and cytokine secretion. To determine how the presence of noncytotoxic concentrations of metal ions affects the antigen-mediated degranulation, we incubated sensitized mast cells with concentrations of tested metal ions or antigen (DNP-HSA) or a combination of both. As shown in Figure 3, incubation of mast cells in the presence of some metal and transition metal ions resulted in release of a significantly higher percentage of *N*-acetyl-β-D-hexosaminidase compared with spontaneous release. The maximum *N*-acetyl-β-D-hexosaminidase release of about 8.5% was observed with 5×10^{-6} M Al^{3+} and Cd^{2+}. A lower but significant release (5%) was observed with 5×10^{-6} M Sr^{2+}, but not with Ni^{2+} and Pb^{2+}. Interestingly, the percentages of released *N*-acetyl-β-D-hexosaminidase observed with Sr^{2+} and Al^{3+} in sensitized mast cells (Figure 3) differed from those observed in nonsensitized cells (Figure 1). Metal ions in combination with optimal concentrations of antigen resulted in greater *N*-acetyl-β-D-hexosaminidase release compared with antigen alone. For example, mast cells challenged with antigen (100 ng/mL) and 10^{-7} M $AlCl_3$ released 23% of *N*-acetyl-β-D-hexosaminidase, whereas mast cells incubated with antigen alone released 11% of *N*-acetyl-β-D-hexosaminidase. Similar effects were observed with 10^{-7} M $NiSO_4$. For these two transition metal ions, the combination of antigen and ions resulted in a release of the amount of mediator higher than the sum of the amounts released with each of these stimuli acting separately. Cd^{2+}, Sr^{2+}, and Pb^{2+} also enhanced the antigen-mediated mediator release, but to a lesser extent. For Ni^{2+}, Al^{3+}, Sr^{2+}, and Cd^{2+} ions, there was a direct relation between the concentration and the effect on antigen-mediated *N*-acetyl-β-D-hexosaminidase release. Thus, noncytotoxic concentrations of metal and transition metal ions stimulated mediator release and enhanced antigen-mediated mediator release in mast cells.

Mast cell activation is regulated by a complex signal transduction process that is initiated by phosphorylation of multiple mast cell proteins on tyrosine residues (Benhamou et al. 1990; Hamawy et al. 1995; Kent et al. 1994). To determine if transition metal and metal ions affect this signal transduction process, mast cells were activated with the optimal dose of antigen in the absence or presence of $NiSO_4$, $AlCl_3$, or $Pb(NO_3)_2$, and mast cell proteins were analyzed with Western blot using antiphosphotyrosine specific antibodies. As expected, addition of antigen induced phosphorylation of multiple proteins in C57 mast cells, resulting in the increased intensity of several electrophoretic bands and the appearance of additional bands, which were not observed in resting mast cells (Figure 4). The presence of Ni^{2+}, Pb^{2+}, and Al^{3+} in incubation media resulted in different intensity of several electrophoretic bands compared with mast cells activated with antigen in the absence of these metal ions. Ni^{2+} and Pb^{2+} mediated an increase in the amount of several phosphorylated proteins at certain time points. In contrast, Al^{3+} mediated a decrease in the level of phosphorylation of multiple proteins compared with control. To assess quantitative differences in the level of protein phosphorylation, we analyzed densitometrically three protein bands, approximately 33, 37, and 56 kD in molecular weight, that were upregulated after antigen challenge in all Western blot experiments. We then normalized resultant optical densities to actin to control for differences in loading. As shown in Figure 4, all three protein bands from mast cells activated with antigen in the presence of Ni^{2+} were phosphorylated to a greater extent and with different kinetics compared with control. Addition of Pb^{2+} resulted in increased phosphorylation of 33 and 37 kD bands, whereas the 56 kD band was phosphorylated to an extent similar to control. The presence of Al^{3+} in incubation media resulted in a decrease in the level of antigen-mediated phosphorylation of 56 and 37 kD bands. Interestingly, despite the overall inhibition of protein phosphorylation observed with this transition metal ion, the 33 kD band shows a greater level of phosphorylation than those observed in control cells.

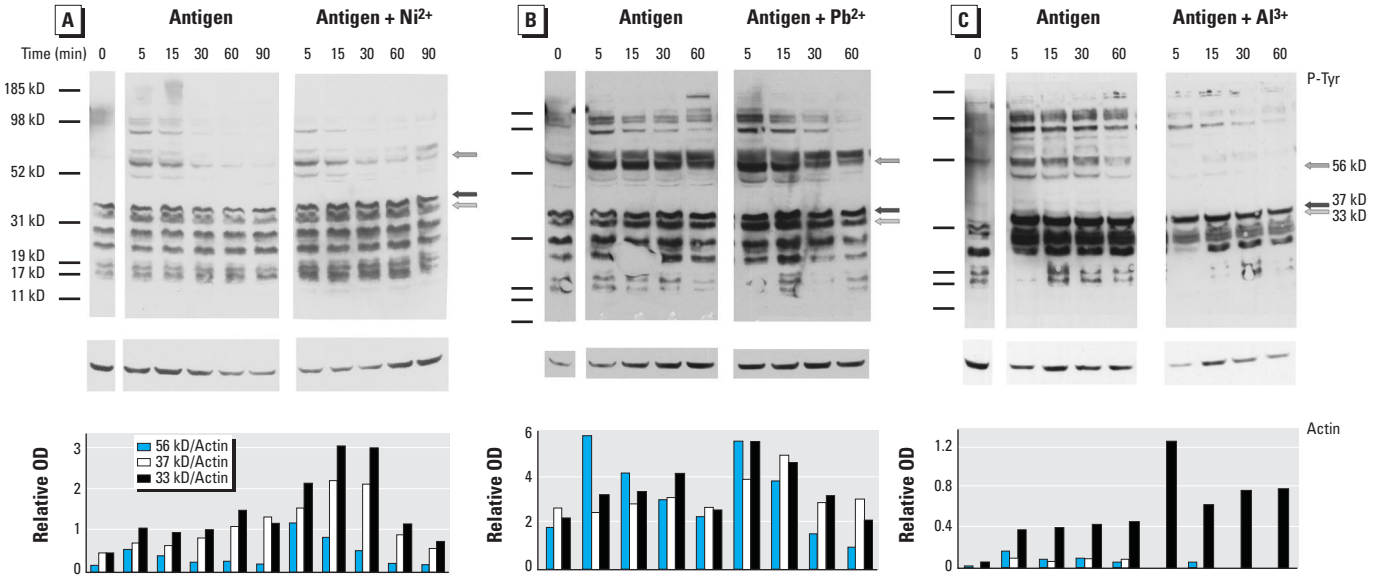

Figure 4. Effect of transition metal and metal ions on antigen-induced protein phosphorylation in mast cells. C57 mast cells were sensitized with IgE and incubated with media alone (time 0) or with antigen (50 ng/mL DNP-HSA) or a combination of antigen and (*A*) 10^{-7} M $NiSO_4$, (*B*) 10^{-7} M $Pb(NO_3)_2$, or (*C*) 10^{-6} M $AlCl_3$. Protein phosphorylation was analyzed using Western blots and HRP-conjugated PY20 antiphosphotyrosine antibody (P-Tyr); nitrocellulose membranes were stripped and reblotted with antiactin antibody (actin). Optical densities of selected phosphorylated bands were determined with densitometry and normalized to optical densities of corresponding actin bands. See "Materials and Methods" for details. Each result represents two similar experiments.

Mast cells challenged with antigen express and secrete to supernatant several proinflammatory cytokines including IL-4. To determine if metal ions change the level of expression of IL-4 in antigen-activated mast cells, we sensitized mast cells with IgE and challenged them with antigen in the presence or absence of increasing concentrations of $NiSO_4$, $AlCl_3$, $SrCl_2$, $CdCl_2$, or $Pb(NO_3)_2$. As shown in Figure 5, the addition of antigen resulted in accumulation of IL-4 in the supernatant, with maximum accumulation observed at an optimal concentration of 10–100 ng/mL antigen. Higher antigen concentrations resulted in significantly lower IL-4 secretion (data not shown). Incubation of C57 mast cells with $NiSO_4$, $AlCl_3$, and $CdCl_2$ at the lowest concentration tested (10^{-7} M) resulted in a significant secretion of IL-4 into the supernatant compared with control. For example, mast cells incubated with 10^{-7} M $NiSO_4$ secreted 200 pg/mL IL-4 compared with 80 pg/mL in control. In contrast, incubation of cells with Sr^{2+} and Pb^{2+} did not result in significant IL-4 secretion. Pb^{2+}, however, enhanced the amount of IL-4 secreted by mast cells stimulated with optimal concentration of antigen by 210%. Similarly, 10^{-7} M Al^{3+} increased the amount of secreted IL-4 by 200%. Interestingly, incubation of mast cells with higher concentrations (10^{-6} M) of Al^{3+} or Pb^{2+} either did not change or inhibited antigen-driven IL-4 secretion in mast cells. Incubation of mast cells with Sr^{2+} and Cd^{2+} also increased antigen-mediated IL-4 secretion above the level observed with antigen alone. In contrast, Ni^{2+} did not increase the amount of secreted IL-4 above the level observed with optimal dose of antigen alone. Thus, at certain concentrations, all tested metal and transition metal ions either induced IL-4 secretion or enhanced antigen-driven IL-4 secretion from mast cells.

Discussion

In this article we have shown that several metal and transition metal ions induced mediator release and enhanced antigen-mediated mediator release in mast cells (Figures 1 and 3). Mediator release did not result from direct cytotoxicity, as it occurred at ion concentrations that did not cause cell death (Figure 2). Some effects of transition metal and metal ions on the level of tyrosine phosphorylation of proteins in antigen-activated mast cells were observed (Figure 4). This is consistent with the hypothesis that these ions affect antigen-mediated signal transduction processes. Furthermore, mast cell degranulation was associated with IL-4 secretion (Figure 5), which requires *de novo* transcription and translation (Dastych et al. 1999). All these observations are consistent with the hypothesis that metal and transition metal compounds, at certain concentrations, are capable of activating mast cells and enhancing antigen-mediated activation.

Metal and transition metal compounds can affect functions of multiple cell types (Ghio et al. 1998; Klein et al. 1994; McCabe and Lawrence 1990). There are, however, some unique characteristics of mast cells that affect how they respond to metal ions and how such responses affect human health. Mast cells are present in large numbers in tissues exposed to the outside environment (Metcalfe et al. 1997). It has been previously shown in animal models that mast cells mediate proinflammatory and immunomodulatory effects of such environmental factors as the midrange spectrum of ultraviolet light (Ikai et al. 1985), ozone (Kleeberger et al. 2001), and heavy metals (Kiely et al. 1997). Several mast cell-derived mediators possess strong immunomodulatory activities (Taylor and Metcalfe 2001). For example, IL-4 is a prototypic immunomodulatory cytokine expressed only in few cell types, which supports the Th2 type of immune response and IgE production (Brown and Hural 1997). There is evidence that metals and transition metals stimulate Th2-driven immune responses (Heo et al. 1997; McCabe and Lawrence 1991; Probst et al. 1995; Szepietowski et al. 1997) and induce IgE production (Cirla 1994; Lutz et al. 1999; Murdoch et al. 1986; Prouvost-Danon et al. 1981; Revoltella and Ovary 1969) in humans and in experimental animals. Thus, mast cell activation could be involved in metal- and transition metal-mediated immunomodulation.

The effects of metal and transition metal ions on mast cells were observed at concentrations relevant for occupational and environmental exposure. For example, the concentration of Ni^{2+} (10^{-7} M) that induced IL-4 secretion and enhanced antigen-mediated mast cell degranulation (Figures 3 and 4) is similar to that found in the serum of stainless steel welders (Angerer and Lehnert 1990). Although serum concentrations of Ni^{2+} in the general population are lower (10^{-9}–10^{-8} M), there is evidence that environmental exposure from air pollution could increase the internal dose of Ni^{2+} (Andersen and Svenes 1999; Nixon et al. 1989; Smith-Sivertsen et al. 1997). Unlike the

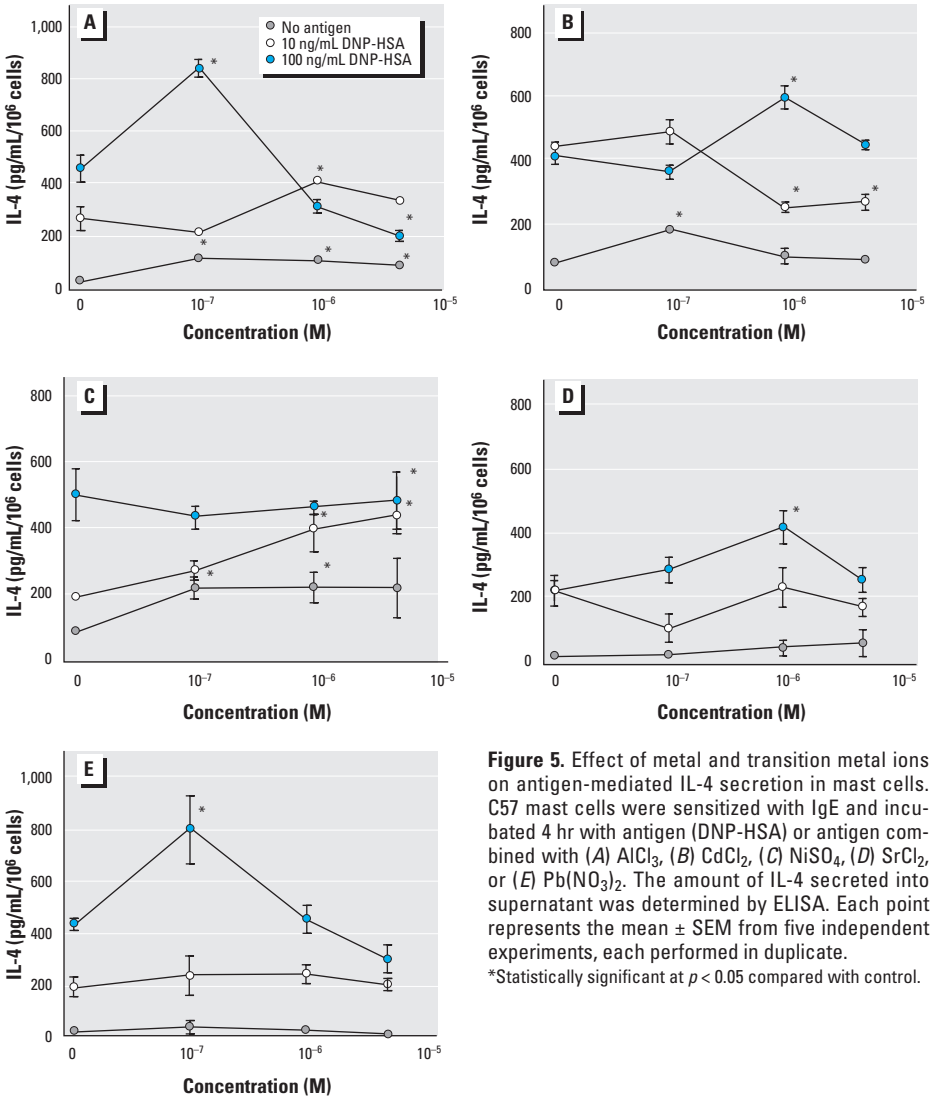

Figure 5. Effect of metal and transition metal ions on antigen-mediated IL-4 secretion in mast cells. C57 mast cells were sensitized with IgE and incubated 4 hr with antigen (DNP-HSA) or antigen combined with (*A*) $AlCl_3$, (*B*) $CdCl_2$, (*C*) $NiSO_4$, (*D*) $SrCl_2$, or (*E*) $Pb(NO_3)_2$. The amount of IL-4 secreted into supernatant was determined by ELISA. Each point represents the mean ± SEM from five independent experiments, each performed in duplicate.
*Statistically significant at $p < 0.05$ compared with control.

experimental setting, environmental sources such as PM contain a mixture of multiple metal and transition metal salts. We observed that the mixture of Ni^{2+}, Al^{3+}, Cd^{2+}, and Sr^{2+} induced mast cell degranulation at a level suggesting that the effects of these metal ions on mast cell function could be additive (Figure 1). Thus, it seems possible that metal ions derived from PM could reach the concentrations necessary to induce or enhance mast cell activation.

Asthmatic patients are especially susceptible to air pollution (Donaldson et al. 2000; Gavett and Koren 2001), and mast cell activation is an important part of the pathogenic process underlying an asthma attack (Busse and Lemanske 2001). We observed that several metal ions enhanced antigen-mediated mast cell activation (Figures 3 and 5), which resulted in the release of mediators in amounts exceeding the maximum amount observed with antigen alone. This observation is consistent with the hypothesis that bioavailable metals and transition metals from PM in ambient air increase the level of antigen-mediated mast cell activation and could worsen asthma symptoms. It would, however, require concomitant exposure of asthmatic patients to both allergen and metal compounds. Such a possibility is supported by data demonstrating the presence of multiple allergens being adsorbed on the surface of inhalable particles (Ormstad 2000). Thus, the enhancement of antigen-mediated mast cell activation with low concentrations of metals and transition metals should be considered one of the mechanisms explaining special susceptibility of asthmatic patients to air pollution.

Immune response is modulated by environmental factors that enhance or suppress responses of immune cells to antigen. Several metal and transition metal ions mediate immunomodulation in experimental animals (Bigazzi 1999; Kiely et al. 1997; Lambert et al. 2000). The presence of metallic compounds in PM from ambient air raises questions about the possible impact of air pollution on the immune system. To address these concerns, the cellular and molecular mechanisms of metal ion-mediated immunomodulation must be better understood. Metal and transition metal ion-mediated mast cell activation resulting in release of multiple proinflammatory and immunomodulatory mediators could be part of such immunomodulatory processes.

References

Andersen I, Svenes K. 1999. Establishing normal values for nickel in human lung disease. J Environ Monit 1:553–555.

Angerer J, Lehnert G. 1990. Occupational chronic exposure to metals. II: Nickel exposure of stainless steel welders—biological monitoring. Int Arch Occup Environ Health 62:7–10.

Balachandran S, Meena BR, Khillare PS. 2000. Particle size distribution and its elemental composition in the ambient air of Delhi. Environ Int 26:49–54.

Benhamou M, Gutkind JS, Robbins KC, Siraganian RP. 1990. Tyrosine phosphorylation coupled to IgE receptor-mediated signal transduction and histamine release. Proc Natl Acad Sci USA 87:5327–5330.

Bigazzi PE. 1999. Metals and kidney autoimmunity. Environ Health Perspect 107(suppl 5):753–765.

Brown MA, Hural J. 1997. Functions of IL-4 and control of its expression. Crit Rev Immunol 17:1–32.

Busse WW, Lemanske RF Jr. 2001. Asthma. N Engl J Med 344:350–362.

Cirla AM. 1994. Cobalt-related asthma: clinical and immunological aspects. Sci Total Environ 150:85–94.

Costa DL, Dreher KL. 1997. Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models. Environ Health Perspect 105(suppl 5):1053–1060.

Dastych J, Hardison MC, Metcalfe DD. 1997. Aggregation of low affinity IgG receptors induces mast cell adherence to fibronectin: requirement for the common FcR gamma-chain. J Immunol 158:1803–1809.

Dastych J, Walczak-Drzewiecka A, Wyczolkowska J, Metcalfe DD. 1999. Murine mast cells exposed to mercuric chloride release granule-associated *N*-acetyl-beta-D-hexosaminidase and secrete IL-4 and TNF-alpha. J Allergy Clin Immunol 103:1108–1114.

Demo SD, Masuda E, Rossi AB, Throndset BT, Gerard AL, Chan EH, et al. 1999. Quantitative measurement of mast cell degranulation using a novel flow cytometric annexin-V binding assay. Cytometry 36:340–348.

Domingo JL. 1994. Metal-induced developmental toxicity in mammals: a review. J Toxicol Environ Health 42:123–141.

Donaldson K, Gilmour MI, MacNee W. 2000. Asthma and PM_{10}. Respir Res 1:12–15.

Donaldson K, MacNee W. 2001. Potential mechanisms of adverse pulmonary and cardiovascular effects of particulate air pollution (PM_{10}). Int J Hyg Environ Health 203:411–415.

Gavett SH, Koren HS. 2001. The role of particulate matter in exacerbation of atopic asthma. Int Arch Allergy Immunol 124:109–112.

Ghio AJ, Carter JD, Samet JM, Reed W, Quay J, Dailey LA, et al. 1998. Metal-dependent expression of ferritin and lactoferrin by respiratory epithelial cells. Am J Physiol 274:L728–L736.

Gordon JR, Burd PR, Galli SJ. 1990. Mast cells as a source of multifunctional cytokines. Immunol Today 11:458–464.

Hamawy MM, Mergenhagen SE, Siraganian RP. 1995. Protein tyrosine phosphorylation as a mechanism of signalling in mast cells and basophils. Cell Signal 7:535–544.

Hart PH. 2001. Regulation of the inflammatory response in asthma by mast cell products. Immunol Cell Biol 79:149–153.

Heinrich J, Hoelscher B, Wjst M, Ritz B, Cyrys J, Wichmann H. 1999. Respiratory diseases and allergies in two polluted areas in East Germany. Environ Health Perspect 107:53–62.

Heo Y, Lee WT, Lawrence DA. 1997. In vivo the environmental pollutants lead and mercury induce oligoclonal T cell responses skewed toward type-2 reactivities. Cell Immunol 179:185–195.

Holgate ST. 1999. The epidemic of allergy and asthma. Nature 402:B2–B4.

Hrsak J, Sega K, Balagovic I. 2000. Lead, manganese, and cadmium content in PM_{10} and $PM_{2.5}$ particle fractions—a pilot study. Arh Hig Rada Toksikol 51:243–247.

Ikai K, Danno K, Horio T, Narumiya S. 1985. Effect of ultraviolet irradiation on mast cell-deficient W/Wv mice. J Invest Dermatol 85:82–84.

Kanerva L, Jolanki R, Estlander T, Alanko K, Savela A. 2000. Incidence rates of occupational allergic contact dermatitis caused by metals. Am J Contact Dermat 11:155–160.

Kent UM, Mao SY, Wofsy C, Goldstein B, Ross S, Metzger H. 1994. Dynamics of signal transduction after aggregation of cell-surface receptors: studies on the type I receptor for IgE. Proc Natl Acad Sci USA 91:3087–3091.

Kiely PD, Pecht I, Oliveira DB. 1997. Mercuric chloride-induced vasculitis in the Brown Norway rat: alpha beta T cell-dependent and -independent phases: role of the mast cell. J Immunol 159:5100–5106.

Kleeberger SR, Ohtsuka Y, Zhang LY, Longphre M. 2001. Airway responses to chronic ozone exposure are partially mediated through mast cells. J Appl Physiol 90:713–723.

Klein CL, Kohler H, Kirkpatrick CJ. 1994. Increased adhesion and activation of polymorphonuclear neutrophil granulocytes to endothelial cells under heavy metal exposure in vitro. Pathobiology 62:90–98.

Lambert AL, Dong W, Selgrade MK, Gilmour MI. 2000. Enhanced allergic sensitization by residual oil fly ash particles is mediated by soluble metal constituents. Toxicol Appl Pharmacol 165:84–93.

Lutz PM, Wilson TJ, Ireland J, Jones AL, Gorman JS, Gale NL, et al. 1999. Elevated immunoglobulin E (IgE) levels in children with exposure to environmental lead. Toxicology 134:63–78.

Maddox L, Schwartz DA. 2002. The pathophysiology of asthma. Annu Rev Med 53:477–498.

McCabe MJ Jr, Lawrence DA. 1990. The heavy metal lead exhibits B cell-stimulatory factor activity by enhancing B cell Ia expression and differentiation. J Immunol 145:671–677.

———. 1991. Lead, a major environmental pollutant, is immunomodulatory by its differential effects on $CD4^+$ T cells subsets. Toxicol Appl Pharmacol 111:13–23.

Metcalfe DD, Baram D, Mekori YA. 1997. Mast cells. Physiol Rev 77:1033–1079.

Murdoch RD, Pepys J, Hughes EG. 1986. IgE antibody responses to platinum group metals: a large scale refinery survey. Br J Ind Med 43:37–43.

Nixon DE, Moyer TP, Squillace DP, McCarthy JT. 1989. Determination of serum nickel by graphite furnace atomic absorption spectrometry with Zeeman-effect background correction: values in a normal population and a population undergoing dialysis. Analyst 114:1671–1674.

Norseth T. 1988. Metal carcinogenesis. Ann NY Acad Sci 534:377–386.

Ormstad H. 2000. Suspended particulate matter in indoor air: adjuvants and allergen carriers. Toxicology 152:53–68.

Ozkaynak H, Xue J, Spengler J, Wallace L, Pellizzari E, Jenkins P. 1996. Personal exposure to airborne particles and metals: results from the Particle TEAM study in Riverside, California. J Expo Anal Environ Epidemiol 6:57–78.

Probst P, Kuntzlin D, Fleischer B. 1995. TH2-type infiltrating T cells in nickel-induced contact dermatitis. Cell Immunol 165:134–140.

Prouvost-Danon A, Abadie A, Sapin C, Bazin H, Druet P. 1981. Induction of IgE synthesis and potentiation of anti-ovalbumin IgE antibody response by HgCl2 in the rat. J Immunol 126:699–792.

Razin E, Mencia-Huerta JM, Stevens RL, Lewis RA, Liu FT, Corey E, et al. 1983. IgE-mediated release of leukotriene C4, chondroitin sulfate E proteoglycan, beta-hexosaminidase, and histamine from cultured bone marrow-derived mouse mast cells. J Exp Med 157:189–201.

Revoltella R, Ovary Z. 1969. Preferential production of rabbit reaginic antibodies. Int Arch Allergy Appl Immunol 36:282–289.

Rondeau V, Commenges D, Jacqmin-Gadda H, Dartigues JF. 2000. Relation between aluminum concentrations in drinking water and Alzheimer's disease: an 8-year follow-up study. Am J Epidemiol 152:59–66.

Samet JM, Stonehuerner J, Reed W, Devlin RB, Dailey LA, Kennedy TP, et al. 1997. Disruption of protein tyrosine phosphate homeostasis in bronchial epithelial cells exposed to oil fly ash. Am J Physiol 272:L426–L432.

Schwartz LB, Austen KF. 1980. Enzymes of the mast cell granule. J Invest Dermatol 74:349–353.

Schwartz LB, Austen KF, Wasserman SI. 1979. Immunologic release of beta-hexosaminidase and beta-glucuronidase from purified rat serosal mast cells. J Immunol 123:1445–1450.

Schwartz LB, Lewis RA, Seldin D, Austen KF. 1981. Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. J Immunol 126:1290–1294.

Smith-Sivertsen T, Lund E, Thomassen Y, Norseth T. 1997. Human nickel exposure in an area polluted by nickel refining: the Sor-Varanger study. Arch Environ Health 52:464–471.

Stejskal J, Stejskal VD. 1999. The role of metals in autoimmunity and the link to neuroendocrinology. Neuroendocrinol Lett 20:351–364.

Suzuki Y, Yoshimaru T, Yamashita K, Matsui T, Yamaki M, Shimizu K. 2001. Exposure of RBL-2H3 mast cells to Ag(+) induces cell degranulation and mediator release. Biochem Biophys Res Commun 283:707–714.

Szepietowski JC, McKenzie RC, Keohane SG, Aldridge RD, Hunter JA. 1997. Atopic and non-atopic individuals react to nickel challenge in a similar way. A study of the cytokine profile in nickel-induced contact dermatitis. Br J Dermatol 137:195–200.

Taylor ML, Metcalfe DD. 2001. Mast cells in allergy and host defense. Allergy Asthma Proc 22:115–119.

Tsai M HJ, Arm JP, London C, Gurish M, Galli SJ. 1996. The C1.MC/C57.1 (C57) mouse mast cell line is of BALB/c origin and is tumorigenic in BALB/c mice [Abstract]. FASEB J 10:A1218.

Williams CM, Galli SJ. 2000. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. J Allergy Clin Immunol 105:847–859.