Impact of acute exposure to WTC dust on ciliated and goblet cells in lungs of rats

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Abstract

Clinical studies and the World Trade Center (WTC) Health Registry have revealed increases in the incidence of chronic (non-cancer) lung disorders among first responders (FR) who were at Ground Zero during the initial 72 h after the collapse. Our previous analyses of rats exposed to building-derived WTC dusts using exposure scenarios/levels that mimicked FR mouth-breathing showed that a single WTC dust exposure led to changes in expression of genes whose products could be involved in the lung ailments, but few other significant pathologies. We concluded that rather than acting as direct inducers of many of the FR health effects, it was more likely inhaled WTC dusts instead may have impacted on toxicities induced by other rescue-related co-pollutants present in Ground Zero air. To allow for such effects to occur, we hypothesized that the alkaline WTC dusts induced damage to the normal ability of the lungs to clear inhaled particles. To validate this, rats were exposed on two consecutive days (2 h/d, by intratracheal inhalation) to WTC dust (collected 12–13 September 2001) and examined over a 1-yr period thereafter for changes in the presence of ciliated cells in the airways and hyperplastic goblet cells in the lungs. WTC dust levels in the lungs were assessed in parallel to verify that any changes in levels of these cells corresponded with decreases in host ability to clear the particles themselves. Image analyses of the rat lungs revealed a significant decrease in ciliated cells and increase in hyperplastic goblet cells due to the single series of WTC dust exposures. The study also showed there was only a nominal non-significant decrease (6–11%) in WTC dust burden over a 1-yr period after the final exposure. These results provide support for our current hypothesis that exposure to WTC dusts caused changes in airway morphology/cell composition; such changes could, in turn, have led to potential alterations in the clearance/toxicities of other pollutants inhaled at Ground Zero in the critical initial 72-h period.

Keywords

Ciliated cell; clearance; dust; goblet cell; World Trade Center

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Introduction

In the years since the 9/11 disaster, chronic health problems continue to become evident among firefighters and rescue personnel (i.e. First Responders; FR) who were at Ground Zero for repeated/prolonged periods during the initial 72 h (Gibbs et al., 2010, 2011; Weakley et al., 2011; Webber et al., 2009). Evidence from clinical studies and data accumulated from the World Trade Center (WTC) Health Registry have noted increases in the incidence of chronic lung disorders, such as certain forms of granulomatous diseases, persistent airway hyper-reactivity [AHR], and asthma, as well as a greater risk for development of atherosclerosis/heart disease (Caplan-Shaw et al., 2011; Crowley et al., 2011; Guidotti et al., 2011; Jordan et al., 2011a, b, c; Nolan et al., 2012; Schenck et al., 2014; Weiden et al., 2013). Attempts to understand etiologies of these health problems initially focused on the effects from dusts derived from collapse of the buildings. However, underlying reasons for the development of the chronic health disorders in FR remain undefined.

The air at Ground Zero in the initial 72 h following the collapse was comprised of a mixture of fine, coarse and (predominantly) alkaline supercoarse particles (>95% of mass was 10–53 μm [MMAD = 22 μm]) bearing a wide variety of toxic metals and organic agents (Gavett, 2003; Gavett et al., 2003; Maciejczyk et al., 2005; McGee et al., 2003). Apart from building-derived materials (collectively termed WTC dusts), other pollutants were also omnipresent in the air on those earliest days, i.e. metal cutting fume particles (CFP, by-products of steel cutters aiding in rescue) and diesel exhaust particles (DEP, from heavy trucks and cranes involved in rescue). In general, particle densities onsite during those first 72 h were initially quite high. Estimates for 11–13 September 2001 were hundreds of mg/m^3; these levels then "dropped" to mg – hundreds of $\mu g/m^3$ after $\approx$3 wk (CDC, 2002; Geyh, 2004 [personal communication]; Geyh et al., 2005; Maciejczyk et al., 2005). As a result, it was deemed that arrival time and respirator use\non-use at Ground Zero during the initial 72 h were critical factors for the development of health problems (primarily pulmonary) in the FR (Antao et al., 2011; Feldman et al., 2004; Gibbs et al., 2010, 2011; Prezant, 2012 [personal communication]).

Under normal circumstances, inhaled particles have a relatively limited period to induce toxicity. However, until entrained particles are cleared from the lungs, many of their constituents are capable of inducing local/systemic toxicities (Cohen, 2004, 2005). Because inhaled alkaline agents can damage respiratory epithelium and give rise to increased epithelial cell death/airway denudation, altered cilia beat frequency and/or ciliostasis (Bui et al., 1998; Clary-Meinesz et al., 1998; Holma et al., 1977), it is possible that entrained supercoarse WTC particles could have caused altered clearance activities in lungs of the exposed FR. If in fact, damage to ciliated (and non-ciliated) pulmonary cells by the inhaled alkaline WTC dusts could have led to altered clearance mechanisms *in situ*, this would provide a novel mechanism to explain the increased incidence of pulmonary health problems seen among exposed FR. Specifically, if WTC dust facilitated the retention of CFP/DEP co-pollutants from Ground Zero air, their respective constituents would have more time to exert toxicities. In this case, pathologies could then develop that ultimately manifest into chronic anomalies, including (cardio)pulmonary diseases. In fact, these particular co-pollutants are

known to induce by themselves many of the health problems increasingly being documented among exposed FR (Antonini et al., 2004; Chen et al., 2010; Erdely et al., 2011; Hansen et al., 2007; Huang et al., 2010; Ibfelt et al., 2010; Kim et al., 2011, 2012; Lillienberg et al., 2008; Maes et al., 2010; Minami et al., 1999; Quan et al., 2010; Riedl et al., 2012; Toren et al., 2007).

This paper reports the results of studies undertaken to establish whether or not exposure to the alkaline WTC dusts (under conditions designed to specifically mimic exposures to the dusts faced by mouth-breathing FR during the initial 72-h period at Ground Zero) could cause sufficient damage to ciliated cells in the lungs such that the ability of the host (here, rats) to clear the WTC dust particles themselves (or potentially other co-pollutant particles).

Materials and methods

WTC dusts

WTC dusts were collected at representative sites on/around the Main Pile at Ground Zero during September 12–13, 2001. All samples were stored in airtight containers in the dark at room temperature to minimize potential light, heat or ambient gas-induced changes in physicochemical properties. The parent WTC dusts were sieved to yield all particles of diameters 53 μm (i.e. WTC_{53-}) for use in exposures (WTC_{53-} referred to hereafter as "WTC dust"). Details about the preparation of the WTC_{53-} materials, as well as physicochemical properties of this fraction were previously reported (Cohen et al., 2014; Gavett et al., 2003; Maciejczyk et al., 2005; McGee et al., 2003; Vaughan et al., 2014).

Experimental design

Male F344 rats (8-wk-old), purchased from Harlan Labs (Frederick, MD), were placed in polycarbonate cages with corncob bedding in a facility maintained at 23 °C with a 30–50% relative humidity and 12-h interval light/dark cycle. Food (Purina lab chow) and tap water were provided *ad libitum*. All rats were acclimated 1 wk prior to use. All animal procedures were conducted under an animal protocol approved by New York University Institutional Animal Care and Use Committee (IACUC).

For the study, sets of rats were exposed for 2-h periods on two consecutive days to WTC_{53-} dusts while under isoflurane anesthesia (ISO; IsoFlo, Abbott Laboratories, North Chicago, IL) in O_2 carrier gas (2.5% final concentration after mixing with house air); two controls were also used, i.e. rats exposed to ISO only (2.5% in carrier O_2 gas) or naïve hosts. An atmosphere of ≈33 mg WTC dust/m^3 was used; this dose was chosen as a conservative estimate to model the rat exposure to correspond to one likely to have occurred in mouth-breathing FR exposed to Ground Zero levels of ≈250 mg WTC dust/m^3 during 11–13 September 2001 (Asgharian, personal communication; Lorber et al., 2007). This dust level (as well as that used in all past exposure studies) was determined using the Multiple Path Particle Deposition Model program (MPPDep Version 1.11, CIIT, Research Triangle Park, NC; RIVM, Bilthoven, the Netherlands). For this WTC dust concentration, a conversion was made to generate rat equivalents (based on dose/surface area of trachea, bronchial and alveolar regions of respiratory tracts of humans and rats; Asgharian, personal

communication). Using equations outlined in Jarabek et al. (2005), and since these are polydisperse atmospheres, this meant an atmospheres bearing 33 mg WTC dust/m³ (of aerodynamic diameter 53 μm) would need to be generated for each 2-h rat exposure to correspond to exposures FR underwent facing atmospheres of 250 mg WTC dust/m³ during continuous 4-h periods on/around Ground Zero. This 4-h value was deemed representative of a "reference person" exposure by the Mayor's WTC Medical Working Group (personal communication).

All rats were exposed to the WTC$_{53-}$ dusts or ISO alone in an intra-tracheal inhalation (ITIH) integrated system (Vaughan et al., 2014) that allowed the WTC particles, including those with $d_a > 2.5$ μm, to circumvent the rat nasal region so that they were introduced (and deposited) in the lungs in a manner to mimic mouth-breathing FR. Protocols for all steps of the exposures (i.e. anesthesizing, intubation, exposure) have been described in detail in both Vaughan et al. (2014) and Cohen et al. (2014). At 2 h, and at various timepoints, post-final exposure, rats (dust, ISO or naïve) from each treatment group were euthanized by injection with Sleepaway® (500 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA). The lungs (along with heart and vasculature) were first removed *en bloc* and then perfused with PBS. The right lung was then removed, weighed and then placed at −80 °C for later use in dust/ metal burden analyses. The left lung was then perfused with 2% glutaraldehyde, the trachea and major bronchi were then removed and the lung sample was then placed in glutaraldehyde until processed for analyses of airway goblet and ciliated cells.

Histologic analyses of ciliated cells and goblet cells in the lungs of rats

A transverse section of the left lobe of the lung was taken at the 5th axial airway generation (G5). Using a microtome, a 5-μm section was then cut and placed on a slide and the slide was then put in a 37 °C oven overnight to bond the tissue. The slide was then removed from the oven and placed at room temperature for 48 h. Thereafter, the slides were stained with Alcian blue-periodic acid Schiff (ABPAS) solution, cover-slipped and morphometric analyses then performed from photomicrograph images. Specifically, several photographs were taken of the slide using a Canon Powershot SD550 camera fitted with a 40 × objective. Using Image J software (NIH, Bethesda, MD), cells were counted at three random 0.1-mm lengths along the airways; the three measurements were then averaged to produce a score for total hyperplastic goblet (ABPAS$^+$) cells/100-μm basement membrane for each rat (Figure 1).

Other sections were prepared from portions of the left lung for analyses of intact goblet cell numbers. Specifically, 5-μm consecutive serial sections were cut from the G5 region of the lung and placed on slides. After being in a 37 °C oven overnight to bond the tissue, the slides were placed at room temperature for 48 h, then stained with hematoxylin/eosin (H&E) for subsequent morphometric analyses (see above for details). Cells were counted at three random 0.1-mm lengths along the airways; the three measurements were then averaged to produce a score for total for total ciliated cell levels in the upper airways/100-μm basement membrane for each rat.

Measures of WTC dust burden/retention

Procedures used by our NIEHS Center Analytical Core were applied to determine tissue levels of aluminum (Al) and titanium (Ti). These metals were selected as potential markers of WTC dust exposure in that unlike many other metals that might be found in the lungs, these could be attributable only to WTC dust (Maciejczyk et al., 2005), and because we previously showed they were in fact good marker of the dust (Cohen et al., 2014; Vaughan et al., 2014). Soluble and insoluble forms of all metals were measured in the tissues. Each tissue sample was dissolved by adding a 7:3 mixture of hydrochloric acid and hydrofluoric acid and then nitric acid, and then with heating to 95 °C in an oven for 2 h. Samples were then cooled and quenched by the addition of a boric acid solution. After appropriate dilution with water, metals were analyzed using inductively coupled plasma mass spectroscopy (ICPMS; Elan DRC II, Perkin Elmer, Norwalk, CT). Sensitivities of the instruments were ≈1 ppt for Al and 0.1 ppt for Ti. All reagents used in these analyses were reagent grade and acids were Fisher Optima Grade; materials used to generate standard curves (5-point calibration along with standard blank to assure accurate base-line) for analyses of each metal were NIST traceable. All dilutions (for reagents and AAS standards) were made up fresh in ultrapure (18.2 MΩ cm at 25 °C) water. To assure no metal contamination occurred, all glassware was washed in micro-soap solution, rinsed in ultrapure water, soaked in 20% HNO_3 overnight and then rinsed in ultrapure water.

From the two sets of values, the amount of WTC dust that was delivered/deposited in the lungs as a result of the two daily exposures, and present at each of the post-final exposure timepoints, was calculated. Values for the total amounts of Ti and Al in the lungs were each divided by their corresponding relative amount of each metal in the samples of "intact" dust that was analyzed in parallel. From this calculation (i.e. × μg Al/[Y μg Al/mg WTC dust]), the total amount of dust (i.e. mg dust, in each case as a function of individual metal) was then calculable.

Statistics

All data were analyzed by one-way analysis of variance (ANOVA) with the exposure group (naïve, ISO only or WTC dust/ISO) as the main factor. Prior to performing ANOVA, all data were tested to assure assumptions of normality and homogeneity of variance were met. Data were also screened for outliers *via* Dixon and Grubbs analyses (Taylor, 1990). Statistical significance in all cases was considered at $p < 0.05$. Statistical analyses were performed using Graphpad Prism Software, v5.0 (Graphpad Software Inc., San Diego, CA).

Results

WTC dust generation

Based on filter measurements taken before and immediately after each 2 h exposure (i.e. no concurrent measures due to impact on flow delivery to rats), the rats in the Day 7, 120, and 360 groups were exposed to the WTC dusts at average levels of, respectively, 35.45 (± 4.21; SE), 32.53 (± 2.30) and 29.96 (± 1.56) mg/m^3. The mass median aerodynamic diameter (MMAD) of the WTC dust for this study was confirmed (*via* horizontal elutriation prior to animal exposures) to be 23 μm ($\sigma_g = 1.45$). As the WTC dusts used for each 2-h exposure

were comprised of all particles with aerodynamic diameters 53 μm, this would mean that the atmospheres introduced into the lungs of the rats would nominally have been comprised of >95% particles with sizes 10–53 μm, 1.1% in the 2.5–10 μm range and ≈3.5% were of <2.5 μm diameters.

Because the average exposure levels associated with the differing rat groups did not significantly differ from one another, the average dust level across all exposures (i.e. 32.22 [± 1.48, SE] mg/m^3) performed here would approximate one of ≈250 mg dust/m^3 likely to have been experienced by a "reference" FR and others at Ground Zero over a 4-h period during the initial 72 h after the buildings collapsed (Mayor's WTC Medical Working Group; personal communication).

Effects of exposures on ciliated cell levels in airways

The effect of the WTC dusts on the integrity of ciliated cells in the airways was assessed; the data show that these cells were significantly impacted in the post-exposure period. Specifically, levels of ciliated cells (per rat; three sets of counts/100-μm basement membrane, at three sites/rat section analyzed) in the lungs of rats exposed on two consecutive days (2 h/d) to 33 mg dust/m^3 were significantly decreased over the period from Day 7 to Day 120 and then to Day 360 post-exposure (Figure 2). At Day 7, the WTC rats (n = 9–15/set) yielded a mean (±SE) of 10.6 [± 0.7] ciliated cells/100-μm basement membrane, while rats that received only anesthetic (ISO) or only air (n = 9–15/set) values were 14.9 [± 1.2] and 14.4 [± 1.3] cells/100-μm basement membrane. These corresponded to changes of 27–29% due to the dust exposure. At Day 120, values for the WTC, ISO and air rats were, respectively, 9.1 [± 0.8], 14.7 [± 0.6] and 14.3 [± 1.8] cells/100-μm basement membrane; these corresponded to changes of 31–38% due to the dust exposure. Lastly, at Day 360, values for the WTC, ISO and air rats were, respectively, 8.7 [± 0.8], 15.4 [± 1.2] and 14.3 [± 1.1] cells/100-μm basement membrane; these corresponded to changes of 39–43% due to the dust exposure. The data also reflect that the damage was progressive, with ciliated cell levels in the lungs of the WTC dust-exposed rats decreasing by >17% over the Day 7 to Day 360 timeframe, albeit that the decreases were greater in the period from Day 7 to Day 120 (decrease of 13.4%) than from Day 120 to Day 360 (decrease of 4.6%).

Effects of exposure on presence of hyperplastic goblet cells in the lungs

Goblet cell hyperplasia in the bronchial epithelium was assessed from ABPAS$^+$ cell counts in the sections (three sets of counts/100-μm basement membrane, at three sites/rat section analyzed). Image analyses were performed on stained sections much like those generated in earlier studies of rats that were euthanized 24 h after their second and final exposure to a dose of 100 mg dust/m^3 (Figure 2A) or to ISO anesthesia (or air) (Figure 2B). In those studies, the WTC rats (n = 11) yielded a very high level of hyperplastic goblet cells/100-μm basement membrane (i.e. mean [±SE] = 115.2 [±20.0]) while rats that received air only had a mean of 6.0 [±0.4] cells/100-μm membrane.

The analyses here of the lungs of rats exposed to 33 mg dust/m^3 revealed a significant increase in goblet cell levels associated with WTC dust exposures over the period from Day 7 to Day 120 and then to Day 360 post-exposure (Figure 2C). Specifically, at Day 7, the

WTC rats (n = 5/set) yielded a mean (± SE) of 15.2 (± 5.0) hyperplastic goblet cells/100-μm membrane, while for rats that received only ISO or air (n = 9–15/set), the value (means combined due to non-significant differences between the two groups) was 1.2 [± 0.6] cells/ 100-μm membrane. This corresponded to an ≈13-fold increase due to dust exposure. At Day 120, values for the WTC and pooled control rats were, respectively, 8.3 [± 1.7] and 1.5 [± 0.7] cells/100-μm basement membrane; this corresponded to a ≈5.5-fold increase due to dust. Lastly, at Day 360, values for the WTC and pooled control rats were, respectively, 8.8 [± 1.2] and 1.8 [± 0.8] cells/100-μm basement membrane, a ≈5-fold increase. The data also reflect that the induced damage was initially high but plateaued over the 360-d timeframe (change from Day 7 to Day 120 of ≈42%).

These results were in keeping with earlier data from a study wherein rats were exposed to 100 mg WTC dust/m^3. In that case, as a result of a single 2-h exposure to the dusts, rats (n = 10) presented with a mean [± SE] of 115 [± 20] cells/100-μm basement membrane, while rats that received only anesthetic (ISO) or only air (n = 4 each) values were 22 [± 20] and 6 [± 4] cells/100-μm basement membrane.

WTC dust retention

The results shown in Figure 3 (in terms of mg dust/g lung tissue [wet weight]) illustrate that in lungs of rats exposed to 2-h rounds of 33 mg WTC dust/m^3 on two consecutive days, there was only a nominal non-significant decrease in dust burden from Day 0 to Day 360 post-final exposure. These outcomes were equally demonstrable if either measured Al (net 11.6% decrease; Figure 3A) or Ti (net 6.2% decrease; Figure 3B) content was used to calculate actual lung WTC dust burdens.

Discussion

When both 100-story towers of the WTC collapsed, the successive collisions of the concrete floor slabs as they cascaded down crushed the concrete, wallboard and insulation fibers present into a finely divided mixed powder with aerodynamic diameters ranging up from 2.5 μm. In fact, the WTC dusts present in the initial 72 h after the collapse contained had most of its overall matter in the coarse thoracic size range ($PM_{2.5–10}$), with the greatest mass fractions in the $PM_{10–50}$ and $PM_{>50}$ ranges. Apart from differences in aerodynamic size, these various fractions also varied in chemical (specifically, metal) compositions (Maciejczyk et al., 2005). Further, these fractions also differed in pH, with PM in the larger size ranges being alkaline (Lioy & Gochfeld, 2002; Maciejczyk et al., 2005). Since the coarse/supercoarse materials comprised the vast (>90%) of the materials in the WTC dusts, it can be assumed that the materials taken into the lungs of mouth-breathing FR were overtly alkaline, if not caustic. As a result, deposition of these particles in the upper respiratory tract and tracheobronchial airways presented a unique challenge to host ability to clear their airways – without eliciting further adverse effects (such as erosion/death of epithelial cells) apart from the direct damage to the cells by the dusts.

Reports in the literature about potential effects of inhaled alkaline/caustic agents are limited. Most are reports of human exposures associated with accidental acute and, in some cases chronic, occupational exposures to agents like sodium hydroxide mists (used in cleaning) or

detergents (i.e. including those containing sodium metasilicate/dichloroisocyanurate, ≈pH 13) sometimes used in disinfection in homes\medical settings (Goverdhan & Gaston, 2003; Hannu et al., 2012; Rubin et al., 1992; Wolkoff et al., 1998). There are also reports of children accidentally inhaling some of these products (Bertinelli et al., 2006; Turner & Robinson, 2005). In most papers, the major pathologies – apart from reports of inflammation/edema/scarring, etc. – seemed to be induction of reactive airway dysfunction syndrome (type of asthma without latency period that is induced by irritating vapors/fumes) or obstructive airway diseases (Bardana, 1999; Dries & Endorf, 2013; McKay, 2014; Saetti et al., 2003). There is little information specifically on the impact of the inhalation exposures on the airway epithelium or cilia in general.

As noted earlier, the limited information on the effects of alkaline agents on airway cells has indicated that inhalation of such agents could cause epithelial cell death/airway denudation, altered cilia beat frequency and/or ciliostasis (Bui et al., 1998; Clary-Meinesz et al., 1998; Holma et al., 1977). Indeed, our earlier *in vitro* studies provided findings that portended such outcomes *in situ* as a result of WTC dust exposure (Xu et al., 2011). Those studies showed that the supercoarse WTC dust was highly toxic to cultured human BEAS-2B airway epithelial cells after a single 2-h dust exposure. Further, these outcomes were characterized by changes in cell repair enzymes that were consistent with necrosis (rather than apoptosis) as a potential mode of dust-induced damage to/death among epithelial cells lining the airways.

Normally, it would be expected that damage induced by the WTC dusts should have been repaired over the post-exposure period. Indeed, findings from earlier studies from our laboratory (Wang et al., 2010) indicated the WTC dusts affected cultured epithelial cell abilities to produce some cytokines (i.e. increased IL-6, IL-10) important in repair of cell damage. Similarly, *in vivo* exposure studies in rats showed that a single set of 2-h exposures to the dusts led to strongly increased expression of the *IL-6* gene in the lungs within 24 h (Cohen et al., 2014). It is known that IL-6 helps to promote regeneration of airway ciliated cells from basal cells (Tadokoro et al., 2014). Cohen et al. (2014) also showed increased expression of *JAK2* in the lungs of dust-exposed rats (*JAK1* expression not assessed). As noted by Heinrich et al. (1998) and Mitzel et al. (2014), JAK2 is essential to airway epithelial cell responses to IL-6. Thus, it seems conditions *vis-à-vis* IL-6/JAK2 were initially triggered in the lungs that should, if they persisted over time or continued to be initiated due to the prolonged presence of the WTC dusts, have allowed WTC dust-damaged cells to regenerate over time.

Still, the fact that ciliated epithelium cell levels remain reduced even up to a year after the single set of WTC dust exposures suggested to us that the particles somehow triggered important regulators of repair. At this point, we can only speculate that somehow the WTC dusts were able to impact on STAT3 activity in the cells, as STAT3 activation is required for bronchiolar/alveolar region repair after damage (Crosby & Waters, 2010; Hokuto et al., 2004; Kida et al., 2008). Our ongoing analyses of the WTC dust-exposed rats' lung tissues isolated at several timepoints in a 1-yr post-exposure period should provide the needed clarity as to whether dysregulation of inducible STAT3-related repair processes was one

means by which WTC dust particles led to prolonged states of ciliated airway cell damage seen here.

With respect to the increased levels of hyperplastic goblet cells in the lungs of the WTC dust-exposed rats, our previous findings do not readily provide a clear mechanism to explain this outcome. It is tempting to speculate that the WTC dusts might have been able to impact on levels of IL-13 in the lungs, as an increased presence of this cytokine is known lead to greater presence of goblet cells in airways (Haswell et al., 2010; Tanabe et al., 2011; Thavagnanam et al., 2011). IL-13 causes this effect in part through induced increases in MCP-1 expression (Zeki et al., 2012) that, in turn, results in induction of goblet cell hyperplasia/increases in mucin production (Monzon et al., 2011; Rose & Voynow, 2006). Again, at this point, this is only one speculative pathway of toxicity. We await the results of our ongoing analyses of lung tissues isolated at several timepoints in the 1-yr post-exposure period to verify if, in fact, there are increases in IL-13 (and MCP-1) in the lungs of the WTC dust-exposed rats and if these levels remain elevated over the course of this 1-yr period.

The findings here that the damage persisted, and in the case of the loss of ciliated cells even worsened, indicate that prolonged retention of the supercoarse WTC dust particles (due to reduced ability to clear) likely provided their many constituents extended periods in which to induce local toxicities. In a self-perpetuating cycle, the retained dust particles would affect cells that would have developed to replace the initially-damaged ones and, with fewer cells present, the dust would be retained even longer to then affect next generation of replacement cells and so on. If this were to have been the case in the lungs of the exposed FR as well, this would provide a novel mechanism to explain their ongoing increases in incidence of pulmonary health problems.

By this, firstly, if the WTC dusts *themselves* were/are causative of many of the documented lung disorders, their prolonged retention would permit their constituents to continuously induce local toxicities. To establish this possibility, ongoing rat studies seek to ascertain if the presence of dusts in the lungs at later timepoints correlate with changes that had only been noted acutely post-exposure, i.e. changes in expression of genes associated with oxidative stress, immune function, cell cycle, etc. (Cohen et al., 2014). The analyses will also let us ascertain if the single sets of exposures (and so a presence of fixed amount of WTC dust in lungs) could also then give rise to *increasing* levels of histopathologic/ biochemical changes *in situ*, reflecting ongoing detrimental effects due to a continuous inability for the particles to be cleared.

Secondly, if the WTC dusts continuously affected the presence/function of cells required for clearing particles from the lungs, this then could also have potentially facilitated retention of metal cutting fume particles (CFP, by-products of steel cutters aiding in rescue) and diesel exhaust particles (DEP, from heavy trucks and cranes involved in rescue) co-pollutants that were also present at high levels in Ground Zero air. As noted earlier, in the exposed FR, pathologies could then develop that manifest into chronic anomalies, including pulmonary diseases known to be induced by DEP and/or CFP (Antonini et al., 2004; Erdely et al., 2011; Hansen et al., 2007; Kim et al., 2011; Lillienberg et al., 2008; Maes et al., 2010; Quan et al., 2010; Riedl et al., 2012). Upcoming studies in rats will determine if co-inhalation of these

agents with the WTC dusts leads to their increased retention and concurrent/subsequent increases in lung gene dysregulation/histopathologic/biochemical changes *in situ* at levels above those seen in rats exposed to CFP or DEP alone.

Acknowledgments

References

Antao VC, Pallos LL, Shim YK, et al. Respiratory protective equipment, mask use, and respiratory outcomes among World Trade Center rescue and recovery workers. Am J Ind Med. 2011; 54:897–905. [PubMed: 21932428]

Antonini JM, Taylor MD, Zimmer AT, Roberts JR. Pulmonary responses to welding fumes: role of metal constituents. J Toxicol Environ Health. 2004; 67:233–249.

Bardana EJ Jr. Reactive airways dysfunction syndrome (RADS): guidelines for diagnosis and treatment and insight into likely prognosis. Ann Allergy Asthma Immunol. 1999; 83:583–586. [PubMed: 10619325]

Bertinelli A, Hamill J, Mahadevan M, Miles F. Serious injuries from dishwasher powder ingestions in small children. J Paediatr Child Care. 2006; 42:129–133.

Bui QQ, Clark CR, Naas DJ, et al. A subacute inhalation exposure evaluation of a scrubbing solution used in petroleum refineries. J Toxicol Environ Health. 1998; 54:49–62.

Caplan-Shaw CE, Yee H, Rogers L, et al. Lung pathologic findings in a local residential and working community exposed to World Trade Center dust, gas, and fumes. J Occup Environ Med. 2011; 53:981–991. [PubMed: 21860325]

CDC (Centers for Disease Control). Occupational exposures to air contaminants at the World Trade Center disaster site – New York, September–October, 2001. MMWR. 2001; 51:453–456.

Chen LC, Quan C, Hwang JS, et al. Atherosclerosis lesion progression during inhalation exposure to environmental tobacco smoke: a comparison to concentrated ambient air fine particles exposure. Inhal Toxicol. 2010; 22:449–459. [PubMed: 20235771]

Clary-Meinesz C, Mouroux J, Cosson J, et al. Influence of external pH on ciliary beat frequency in human bronchi and bronchioles. Eur Respir J. 1998; 11:330–333. [PubMed: 9551733]

Cohen MD. Pulmonary immunotoxicology of select metals: aluminum, arsenic, cadmium, chromium, copper, manganese, nickel, vanadium, and zinc. J Immunotoxicol. 2004; 1:39–69. [PubMed: 18958639]

Cohen, MD. Pulmonary immunotoxicology. In: Gardner, D., editor. Toxicology of the lung. 4th ed.. Vol. Chapter 9. Boca Raton (FL): Taylor and Francis/CRC Press; 2005. p. 351-420.

Cohen MD, Vaughan JM, Garrett BJ, et al. Acute high-level exposure to WTC particles alters expression of genes associated with oxidative stress and immune function in the lung. J Immunotoxicol. 2014; 12:140–153. 2015. [PubMed: 24911330]

Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. Am J Physiol. 2010; 298:L715–L731.

Crowley LE, Herbert R, Moline JM, et al. Sarcoid like granulomatous pulmonary disease in World Trade Center disaster responders. Am J Ind Med. 2011; 54:175–184. [PubMed: 21298693]

Dries DJ, Endorf FW. Inhalation injury: epidemiology, pathology, treatment strategies. Scand J Trauma Resusc Emerg Med. 2013; 21:31. [PubMed: 23597126]

Erdely A, Salmen-Muniz R, Liston A, et al. Relationship between pulmonary and systemic markers of exposure to multiple types of welding particulate matter. Toxicology. 2011; 287:153–159. [PubMed: 21708214]

Feldman DM, Baron SL, Bernard BP, et al. Symptoms, respirator use, and pulmonary function changes among New York City firefighters responding to the World Trade Center disaster. Chest. 2004; 125:1256–1264. [PubMed: 15078732]

Gavett SH. World Trade Center fine particulate matter – chemistry and toxic respiratory effects: an overview. Environ Health Perspect. 2003; 111:971. [PubMed: 12782500]

Gavett SH, Haykal-Coates N, Highfill JW, et al. World Trade Center fine particulate matter causes respiratory tract hyper-responsiveness in mice. Environ Health Perspect. 2003; 111:981–991. [PubMed: 12782502]

Geyh AS, Chillrud S, Williams D, et al. Assessing truck driver exposure at the World Trade Center disaster site: personal and area monitoring for particulate matter and volatile organic compounds during October 2001 and April 2002. J Occup Environ Hyg. 2005; 2:179–193. [PubMed: 15764541]

Gibbs, L.; Farley, T.; Aldrich, TK., et al. World Trade Center Medical Working Group. 2010 Annual Report on 9/11 Health. 2010. Available from: www.nyc.gov/9-11HealthInfo.

Gibbs L, Farley T, Aldrich TK, et al. World Trade Center Medical Working Group. 2011 Annual Report on 9/11 Health. 2011 Available from: www.nyc.gov/9-11HealthInfo.

Goverdhan S, Gaston H. Sanichlor-induced atopic dermatitis and asthma in ophthalmologists. Eye. 2003; 17:108–109. [PubMed: 12579187]

Guidotti TL, Prezant D, de la Hoz RE, Miller A. The evolving spectrum of pulmonary disease in responders to the World Trade Center tragedy. Am J Ind Med. 2011; 54:649–660. [PubMed: 23236631]

Hannu TJ, Riihimäki VE, Piirilä PL. Reactive airways dysfunction syndrome from acute inhalation of dishwasher detergent powders. Can Respir J. 2012; 19:e25–e28. [PubMed: 22679618]

Hansen CS, Sheykhzade M, Moller P, et al. Diesel exhaust particles induce endothelial dysfunction in apoE$^{-/-}$ mice. Toxicol Appl Pharmacol. 2007; 219:24–32. [PubMed: 17234226]

Haswell LE, Hewitt K, Thorne D, et al. Cigarette smoke total particulate matter increases mucous secreting cell numbers *in vitro*: a potential model of goblet cell hyperplasia. Toxicol In Vitro. 2010; 24:981–987. [PubMed: 20060463]

Heinrich PC, Behrmann I, Muller-Newen G, et al. IL-6-type cytokine signaling through the gp130/Jak/STAT pathway. Biochem J. 1998; 334:297–314. [PubMed: 9716487]

Hokuto I, Ikegami M, Yoshida M, et al. Stat-3 is required for pulmonary homeostasis during hyperoxia. J Clin Invest. 2004; 113:28–37. [PubMed: 14702106]

Holma B, Lindegren M, Andersen JM. pH effects on ciliomotility and morphology of respiratory mucosa. Arch Environ Health. 1977; 32:216–226. [PubMed: 20855]

Huang CH, Lin LY, Tsai MS, et al. Acute cardiac dysfunction after short-term diesel exhaust particles exposure. Toxicol Lett. 2010; 192:349–355. [PubMed: 19913602]

Ibfelt E, Bonde JP, Hansen J. Exposure to metal welding fume particles and risk for cardiovascular disease in Denmark: a prospective cohort study. Occup Environ Med. 2010; 67:772–777. [PubMed: 20581417]

Jarabek AM, Asgharian B, Miller FJ. Dosimetric adjustments for interspecies extrapolation of inhaled poorly-soluble particles (PSP). Inhal Toxicol. 2005; 17:317–334. [PubMed: 16020031]

Jordan HT, Brackbill RM, Cone JE, et al. Mortality among survivors of the Sept 11, 2001, World Trade Center disaster: results from the World Trade Center Health Registry cohort. Lancet. 2011a; 378:879–887. [PubMed: 21890052]

Jordan HT, Miller-Archie SA, Cone JE, et al. Heart disease among adults exposed to the September 11, 2001 World Trade Center disaster: results from the World Trade Center Health Registry. Prevent Med. 2011b; 53:370–376.

Jordan HT, Stellman SD, Prezant D, et al. Sarcoidosis diagnosed after September 11, 2001, among adults exposed to the World Trade Center disaster. J Occup Environ Med. 2011c; 53:966–974. [PubMed: 21860326]

Kida H, Mucenski ML, Thitoff AR, et al. GP130-STAT3 regulates epithelial cell migration and is required for repair of the bronchiolar epithelium. Am J Pathol. 2008; 172:1542–1554. [PubMed: 18467707]

Kim J, Natarajan S, Vaickus LJ, et al. Diesel exhaust particulates exacerbate asthma-like inflammation by increasing CXC chemokines. Am J Pathol. 2011; 179:2730–2739. [PubMed: 21967814]

Kim JB, Kim C, Choi E, et al. Particulate air pollution induces arrhythmia via oxidative stress and calcium calmodulin kinase II activation. Toxicol Appl Pharmacol. 2012; 259:66–73. [PubMed: 22197715]

Lillienberg L, Zock JP, Kromhout H, et al. A population-based study on welding exposures at work and respiratory symptoms. Ann Occup Hyg. 2008; 52:107–115. [PubMed: 18216372]

Lioy PJ, Gochfeld M. Lessons learned on environmental, occupational, and residential exposures from attack on the World Trade Center. Am J Ind Med. 2002; 42:560–565. [PubMed: 12439887]

Lorber M, Gibb H, Grant L, et al. Assessment of inhalation exposures and potential health risks to the general population that resulted from the collapse of the World Trade Center towers. Risk Anal. 2007; 27:1203–1221. [PubMed: 18076491]

Maciejczyk, PB.; Zeisler, RL.; Hwang, JS., et al. Urban aerosols and their impacts. Vol. 919. Washington, DC: American Chemical Society; 2005. Characterization of size-fractionated World Trade Center dust and estimation of relative dust contribution to ambient particulate concentrations; p. 114-131.

Maes T, Provoost S, Lanckacker EA, et al. Mouse models to unravel the role of inhaled pollutants on allergic sensitization and airway inflammation. Resp Res. 2010; 11:7.

McGee JK, Chen LC, Cohen MD, et al. Chemical analysis of World Trade Center fine particulate matter for use in toxicologic assessment. Environ Health Perspect. 2003; 111:972–980. [PubMed: 12782501]

McKay CA Jr. Toxin-induced respiratory distress. Emerg Med Clin N Am. 2014; 32:127–147.

Minami M, Endo T, Hamaue N, et al. Electrocardiographic changes induced by diesel exhaust particles (DEP) in guinea pigs. Res Commun Mol Pathol Pharmacol. 1999; 105:67–76. [PubMed: 10850370]

Mitzel DN, Jaramillo RJ, Stout-Delgado H, et al. Human metapneumovirus inhibits IL-6-induced JAK/ STAT3 signaling cascade in airway epithelium. J Gen Virol. 2014; 95:26–37. [PubMed: 24114793]

Monzon ME, Forteza RM, Casalino-Matsuda SM. MCP-1/CCR2B-dependent loop up-regulates MUC5AC and MUC5B in human airway epithelium. Am J Physiol. 2011; 300:L204–L215.

Nolan A, Naveed B, Comfort AL, et al. Inflammatory biomarkers predict airflow obstruction after exposure to World Trade Center dust. Chest. 2012; 142:412–418. [PubMed: 21998260]

Quan C, Sun Q, Lippmann M, Chen LC. Comparative effects of inhaled diesel exhaust and ambient fine particles on inflammation, atherosclerosis, and vascular dysfunction. Inhal Toxicol. 2010; 22:738–753. [PubMed: 20462391]

Riedl MA, Diaz-Sanchez D, Linn WS, et al. Allergic inflammation in the human lower respiratory tract affected by exposure to diesel exhaust. Res Rep Health Effects Inst. 2012; 165:5–43.

Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol Rev. 2006; 86:245–278. [PubMed: 16371599]

Rubin AE, Bentur L, Bentur Y. Obstructive airway disease associated with occupational sodium hydroxide inhalation. Br J Ind Med. 1992; 49:213–214. [PubMed: 1554619]

Saetti R, Silvestrini M, Cutrone C, et al. Endoscopic treatment of upper airway and digestive tract lesions caused by caustic agents. Ann Otol Rhinol Laryngol. 2003; 112:29–36. [PubMed: 12537055]

Schenck EJ, Echevaria GC, Girvin FG, et al. Enlarged pulmonary artery is predicted by vascular injury biomarkers and is associated with WTC lung injury in exposed fire fighters: a case-control study. BMJ Open. 2014; 4:e005575.

Tadokoro T, Wang Y, Barak LS, et al. IL-6/STAT3 promotes regeneration of airway ciliated cells from basal stem cells. Proc Natl Acad Sci USA. 2014; 111:E3641–E3649. [PubMed: 25136113]

Tanabe T, Kanoh S, Tsushima K, et al. Clarithromycin inhibits IL-13-induced goblet cell hyperplasia in human airway cells. Am J Respir Cell Mol Biol. 2011; 45:1075–1083. [PubMed: 21642590]

Taylor, JK., editor. Statistical techniques for data analysis. Chelsea, MI: Lewis Publishers; 1990.

Thavagnanam S, Parker JC, McBrien ME, et al. Effects of IL-13 on mucociliary differentiation of pediatric asthmatic bronchial epithelial cells. Ped Res. 2011; 69:95–100.

Toren K, Bergdahl IA, Nilsson T, Jarvholm B. Occupational exposure to particulate air pollution and mortality due to ischemic heart disease and cerebrovascular disease. Occup Environ Med. 2007; 64:515–519. [PubMed: 17303673]

Turner A, Robinson P. Respiratory and gastrointestinal complications of caustic ingestion in children. Emerg Med J. 2005; 22:359–361. [PubMed: 15843706]

Vaughan JM, Garrett BJ, Prophete C, et al. A novel system to generate WTC dust particles for inhalation exposures. J Exposure Sci Environ Epidemiol. 2014; 24:105–112.

Wang S, Prophete C, Soukup JM, et al. Roles of MAPK pathway activation during cytokine induction in BEAS-2B cells exposed to World Trade Center (WTC) dust. J Immunotoxicol. 2010; 7:298–307. [PubMed: 20731619]

Weakley J, Webber MP, Gustave J, et al. Trends in respiratory diagnoses and symptoms of firefighters exposed to World Trade Center disaster: 2005–2010. Prev Med. 2011; 53:364–369. [PubMed: 21930151]

Webber MP, Gustave J, Lee R, et al. Trends in respiratory symptoms of firefighters exposed to the World Trade Center disaster: 2001–2005. Environ Health Perspect. 2009; 117:975–980. [PubMed: 19590693]

Weiden MD, Naveed B, Kwon S, et al. Cardiovascular biomarkers predict susceptibility to lung injury in World Trade Center dust-exposed firefighters. Eur Respir J. 2013; 41:1023–1030. [PubMed: 22903969]

Wolkoff P, Schneider T, Kildesø J, et al. Risk in cleaning: chemical and physical exposure. Sci Total Environ. 1998; 215:135–156. [PubMed: 9599458]

Xu A, Prophete C, Chen LC, et al. Interactive effect of cigarette smoke extract and World Trade Center dust particles on airway cell cytotoxicity. J Toxicol Environ Health. 2011; 74:887–902.

Zeki AA, Thai P, Kenyon NJ, Wu R. Differential effects of simvastatin on IL-13-induced cytokine gene expression in primary mouse tracheal epithelial cells. Resp Res. 2012; 13:38.
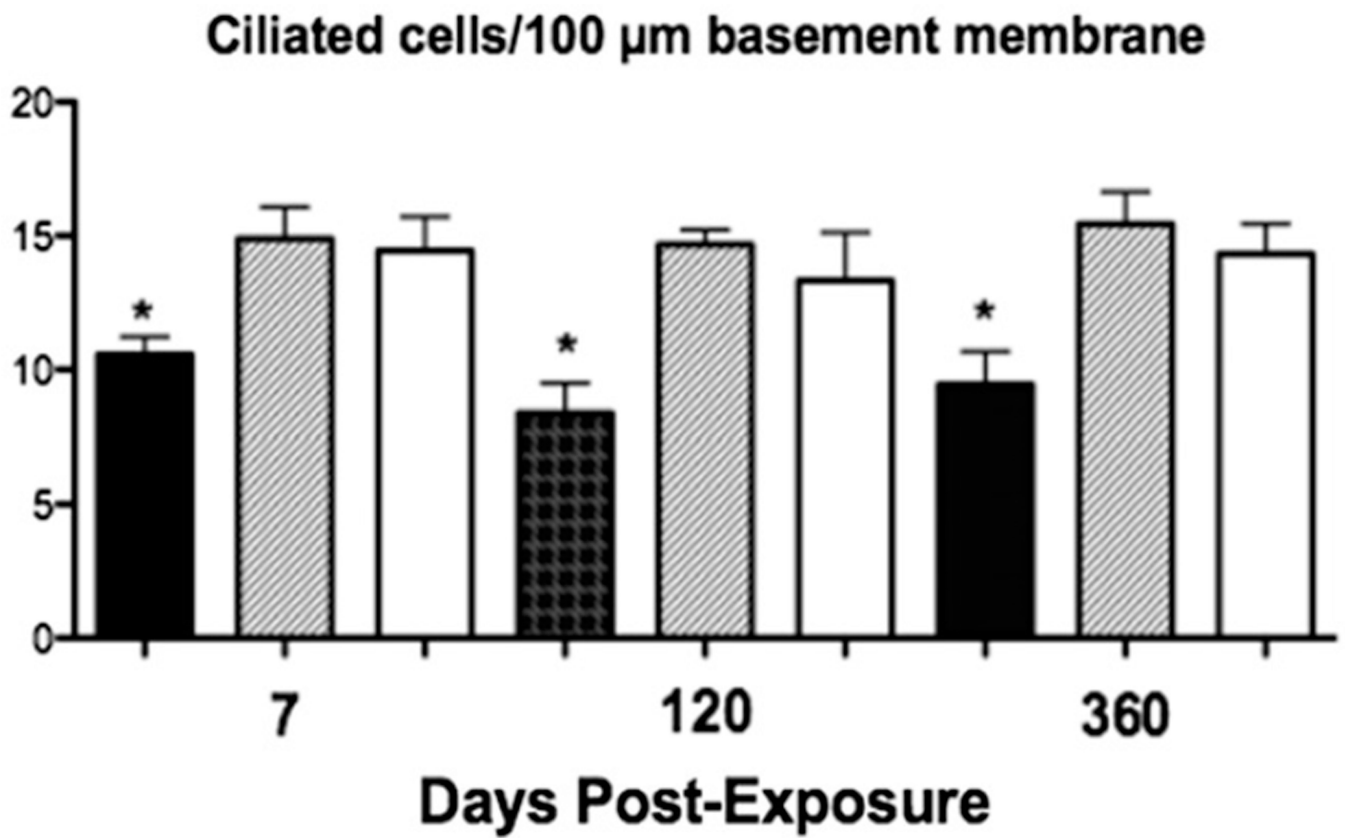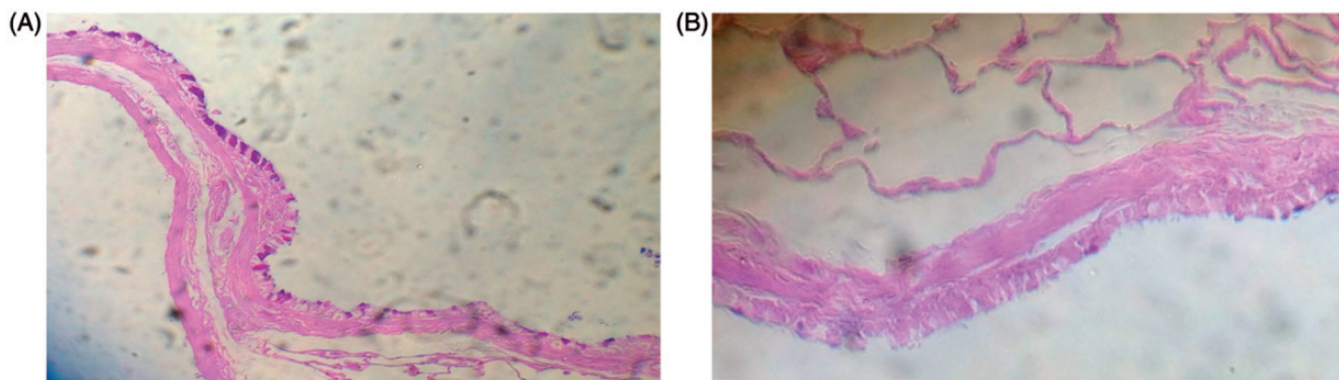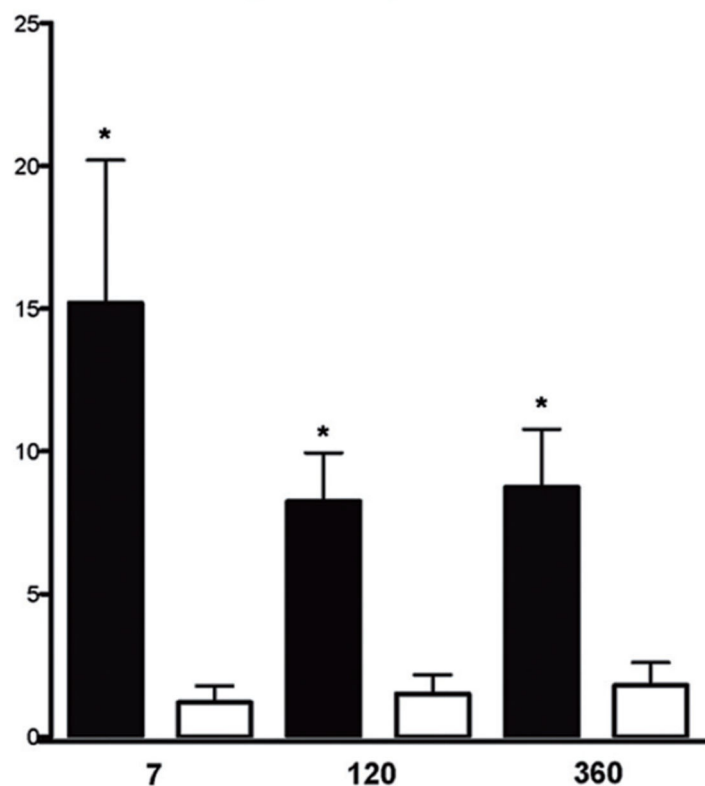
Ciliated cells/100 μm basement membrane

Figure 1.
Ciliated cell levels at the 5th axial airway generation (G5) (counts/100-μm basement membrane); (L–R within each time set) WTC, ISO, Air/Naïve. Values are mean ± SE, $n = 9$/set. *$p < 0.05$ versus both ISO and Air/Naïve values at given timepoint.

(C) Mucin-containing cells/100 µm basement membrane

Figure 2.
(A) Representative image (from an earlier study) of a *strongly affected* rat airway 24 h after host exposure to ≈100 mg WTC dust/m^3 on two consecutive days (2 h/d). Lung tissues were stained with ABPAS. (B) Representative image (from an earlier study) of rat airway 24 h after host exposure to ISO (or air; results were comparable for both groups) on two consecutive days (2 h/d). (C) Hyperplastic goblet cell levels in lung sections (counts/100-µm basement membrane) of rats exposed in the current study to ≈33 mg WTC dust/m^3 on two consecutive days (2 h/d); (L, R within each time set) WTC and pooled control outcomes (ISO and Air rats). Values shown are mean ± SE, n = 5–10/set. *$p < 0.05$ versus pooled control value at given timepoint.
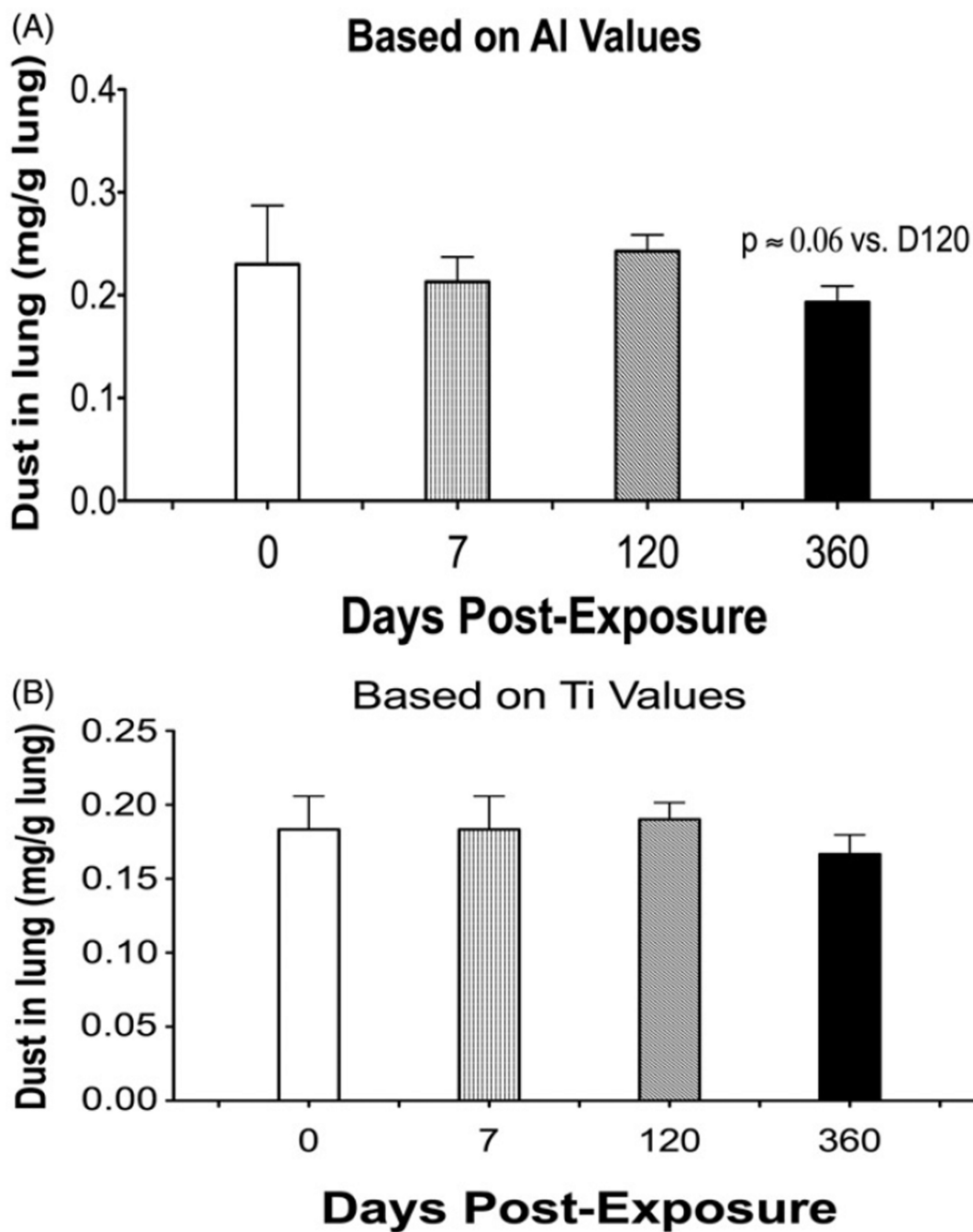
Figure 3.
(A) Lung burdens of WTC dust based on values of Al in the lungs of rats at the indicated post-final exposure timepoints. (B) Lung burdens of WTC dust based on values of Ti in the lungs. All values for ISO and Air/Naïve hosts were all below the level of detection in the ICPMS system. Values are mean (± SD) $n = 4$–5/set.