Induced Sputum Assessment in New York City Firefighters Exposed to World Trade Center Dust

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New York City Firefighters (FDNY-FFs) were exposed to particulate matter and combustion/pyrolysis products during and after the World Trade Center (WTC) collapse. Ten months after the collapse, induced sputum (IS) samples were obtained from 39 highly exposed FDNY-FFs (caught in the dust cloud during the collapse on 11 September 2001) and compared to controls to determine whether a unique pattern of inflammation and particulate matter deposition, compatible with WTC dust, was present. Control subjects were 12 Tel-Aviv, Israel, firefighters (TA-FFs) and 8 Israeli healthcare workers who were not exposed to WTC dust. All controls volunteered for this study, had never smoked, and did not have respiratory illness. IS was processed by conventional methods. Retrieved cells were differentially counted, and metalloproteinase-9 (MMP-9), particle size distribution (PSD), and mineral composition were measured. Differential cell counts of FDNY-FF IS differed from those of health care worker controls ($p < 0.05$) but not from those of TA-FFs. Percentages of neutrophils and eosinophils increased with greater intensity of WTC exposure ($< 10$ workdays or $\geq 10$ workdays; neutrophils $p = 0.046$; eosinophils $p = 0.038$). MMP-9 levels positively correlated to neutrophil counts ($p = 0.002$; $r = 0.449$). Particles were larger and more irregularly shaped in FDNY-FFs ($1-50$ µm; zinc, mercury, gold, tin, silver) than in TA-FFs ($1-10$ µm; silica, clays). PSD was similar to that of WTC dust samples. In conclusion, IS from highly exposed FDNY-FFs demonstrated inflammation, PSD, and particle composition that was different from nonexposed controls and consistent with WTC dust exposure. Key words: firefighters, inflammation, inhalation exposure, particulates, sputum, World Trade Center. Environ Health Perspect 112:1564–1569 (2004). doi:10.1289/ehp.7233 available via http://dx.doi.org/ [Online 22 September 2004]
matter was assessed by size distribution, mineral composition, and by comparison to settled dust samples collected at the WTC site.

**Methods**

**Study population.** Ten months after the WTC collapse, we studied 39 male FDNY-FFs who worked at the WTC, 12 male firefighters who lived in Tel-Aviv, Israel (TA-FF), and 8 male Israeli hospital workers, all apparently free of respiratory disease. Firefighters were recruited from those undergoing medical monitoring during June 2002. The only inclusion criterion was that firefighters must have worked in the WTC dust cloud the morning of 11 September 2001. Current or past tobacco smokers were excluded. IS induction was voluntary, and all were informed about the ongoing research. For FDNY-FFs, cumulative exposure was assessed by size distribution, and by comparison to settled dust samples collected at the WTC site.

**IS induction and processing.** IS was obtained at the FDNY Bureau of Health Services as previously described (Fireman et al. 1999a, 1999b; Lerman et al. 2003b). Before pretreatment with a short acting beta-2 agonist, 3% saline was administered by nebulizer (U1 Ultrasonic Nebulizer; Omron HealthCare, Henfield, West Sussex, UK) for up to 20 min while subjects were encouraged to cough and expectorate sputum into a sterile container. Samples were stored at 4°C and processed within 3 hr. All portions with little or no squamous epithelial cells (rich nonsquamous epithelial cell fraction considered to originate from the lower respiratory tract, hereafter referred to as “plugs”) were collected using the selection plug method and processed as previously described (Fireman et al. 1999b; Pin et al. 1992; Popov et al. 1994). Briefly, plugs were selected and treated with dithiothreitol (DTT; Sputalysin; Calbiochem Corp., San Diego, CA, USA). The cell suspension was filtered through a 52-µm nylon mesh (BNSH Thompson, Scarborough, Ontario, Canada) and the effect of DTT was stopped by diluting the suspension with phosphate-buffered solution to a volume equal to the sputum plus DTT. After centrifugation, the supernatants were deep frozen for consecutive measurement of MMP-9. The pellets were resuspended and cytospinned (Shandon Southern Instruments, Sewickley, PA, USA), and the slides were stained with Giemsa. We counted 200 nonsquamous cells; the results were expressed as a percentage of the total nonsquamous count. Each slide was read by two independent readers.

**Differential cell counts.** Samples from the cellular fraction were resuspended and processed to cytospin slides, centrifuged, air-dried, and stained with Giemsa. Differential cell counts were measured by scanning cytospin slides with high power (>500 magnification). We counted 200 nonsquamous cells and expressed cell differentials as percentages of total nonsquamous cell counts.

**Measurement of MMP-9 ELISA.** We determined the absolute value of MMP-9 in IS supernatants using an ELISA commercial kit (R&D Systems Inc., Minneapolis, MN, USA). MMP-9 values expressed total levels of active and pro-MMP-9 (0.1–25 ng/mL). We performed spike experiments using 10 and 20 ng/mL pure MMP-9 protein to assess the efficacy of recovery. We found that only 10–12% of protein was equally denatured in the pure protein and in all IS samples by DTT. No other metabolites were measured in the study.

**Particle examination.** After separation of the plugs and viscous materials, all fractions of IS were preserved in 10% formalin and stored at 4°C until analysis of mineral particles. We performed spike experiments using 10 and 20 µg/mL of the pure protein and in all IS samples by DTT. No other metabolites were measured in the study.

**Particle examination.** After separation of the plugs and viscous materials, all fractions of IS were preserved in 10% formalin and stored at 4°C until analysis of mineral particles. We used the samples containing both extracellular and intracellular particles for the SEM analysis; samples were treated with 14% formamide solution and filtered onto a 0.8-µm carbon-coated Nuclepore filter (Millipore Filter Corp., Bedford, MA, USA). Particles with a diameter > 0.4 µm were analyzed by a JEOL 840 SEM (JEOL Ltd., Hertfordshire, UK) equipped with a Link 10,000 energy-dispersive system (EDS; Link Oxford Analytical Instruments, Oxford, UK). The spectrometer of the EDS system separated the elements according to energy rather than wavelength. In addition, we used a petrographic microscope to identify minerals (Fireman et al. 1999a, 1999b). We assessed the size and shape of the particles from the rich cell fraction of the processed plugs with a Cis-100 Analyzer and the analyzer’s video channel (Ankersmid, Yokneam, Israel) (Fireman et al. 1999b) using a PSD method in the range of 0.5–3,600 based on the time of transition (Aharonson et al. 1986; Cohen et al. 1999; Pin et al. 1992) theory where the duration of interaction between the particles and the detector is measured.

**Results.**

**Table 1. IS differential cell counts and MMP-9 levels.**

<table>
<thead>
<tr>
<th></th>
<th>Macrophages (%)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Eosinophils (%)</th>
<th>MMP-9 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDNY-FF</td>
<td>34.3 ± 16.2</td>
<td>50.7 ± 17</td>
<td>12 ± 5.8</td>
<td>2.8 ± 4</td>
<td>2.2 ± 2.6</td>
</tr>
<tr>
<td>TA-FF</td>
<td>35 ± 18.9</td>
<td>44 ± 22.9</td>
<td>15.6 ± 9.6</td>
<td>5.4 ± 8.9</td>
<td>1.2 ± 0.99</td>
</tr>
<tr>
<td>Control</td>
<td>64.8 ± 7.9**</td>
<td>29.1 ± 8.9**</td>
<td>5.7 ± 2.2**</td>
<td>0.2 ± 0.4**</td>
<td>0.3 ± 0.09**</td>
</tr>
</tbody>
</table>

* * * p = 0.057 between levels in FDNY-FFs versus TA-FFs. **p < 0.05 between cells in FDNY-FFs and TA-FFs versus controls.

**Table 2. Differential counts, MMP-9 levels, and particle size distribution in FDNY-FFs analyzed according to cumulative exposure.**

<table>
<thead>
<tr>
<th></th>
<th>Duty (years)</th>
<th>Macrophages (%)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Eosinophils (%)</th>
<th>MMP-9 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 days</td>
<td>15.6 ± 8.3</td>
<td>31.1 ± 13.7</td>
<td>44.2 ± 16.5</td>
<td>11.6 ± 7.0</td>
<td>15 ± 1.9</td>
<td>32.3 ± 13.7</td>
</tr>
<tr>
<td>≥ 10 days</td>
<td>17.6 ± 8.6</td>
<td>38.4 ± 16.5</td>
<td>55.7 ± 15.2</td>
<td>12.9 ± 6.8</td>
<td>4.4 ± 5.2</td>
<td>38 ± 6 ± 17</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.78</td>
<td>0.18</td>
<td>0.05</td>
<td>0.59</td>
<td>0.04</td>
<td>0.48</td>
</tr>
</tbody>
</table>

**Figure 1.** Correlation between concentrations of Ln MMP-9 and the percentage of neutrophils (A) and the percentage of macrophages (B) in induced sputum from all samples (n = 56). Values are expressed as the percentage of 200 cells as described in “Methods.”
beam and particle provides a direct measurement of each particle’s size. A helium-neon laser beam interfered with the intracellular particles, and the results were recorded. We performed dynamic shape characterization using image analysis techniques. Measurements were performed on 2 drops of a suspension of sputum cells (10⁶ cells/mL) introduced into a quartz cuvette containing stirred water for IS samples and water with glycerol (1:1) for the WTC settled dust sample collected 7 days postcollapse on Cortlandt Street, one block east of the WTC. Each result was an average of three consecutive measurements.

Statistical analysis. Demographic comparisons between groups were performed by parametric or nonparametric (Kruskal-Wallis) analysis of variance (ANOVA). FDNY-FFs were analyzed according to cumulative exposure days using a continuous scale and dichotomized based on the median (< 10 days vs. 10 days). Groups were compared by independent t-test, Mann-Whitney test, and chi-square test. In addition, group means of all percent cells and particle size, adjusted for MMP9, were compared by a one-way analysis of covariance with the natural logarithm of MMP-9 as covariate. Natural logarithm transformation was applied to MMP-9 because of its skewed distribution (Ln MMP-9). The association between percent parameters was evaluated by Pearson correlation coefficients. For all tests, p-values < 0.05 were considered statistically significant. The data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows software, Version 11.0 (SPSS, Chicago, IL, USA).

Table 3. Particle analysis in IS of FDNY-FFs and TA-FFs.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>FDNY-FF</th>
<th>TA-FF</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of particles &gt; 2 µm</td>
<td>34.01 ± 15.5</td>
<td>10.4 ± 5.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Percent of particles &gt; 5 µm</td>
<td>8.1 ± 15.3</td>
<td>1.6 ± 1.25</td>
<td>0.0001</td>
</tr>
<tr>
<td>Percent of particles &gt; 5% particles &gt; 2 µm</td>
<td>88.6% (31/35)</td>
<td>83.4% (11/13)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Percent of particles &gt; 5% particles &gt; 5 µm</td>
<td>67.7% (30/45)</td>
<td>0.0% (0/12)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Percentage of total particles present in the sample. Values are means ± standard deviation.

Results

Demographic characteristics. We found no significant differences in age between the FDNY-FFs and the TA-FFs (37.4 vs. 36.6 years; p = 0.109, respectively) or their respective firefighting work tenure (17.5 vs. 13.5 years; p = 0.399, respectively). The average age of the nonfirefighter controls was 41.6 years. All 39 FDNY-FFs were caught in the WTC dust cloud during the morning of 11 September 2001, representing the highest acute exposure group as defined by arrival time (Banauch et al. 2003; Feldman et al. 2004). The period of cumulative WTC work exposure for the FDNY-FF group varied from 1 to 75 days (mean of 20.2 days), with all but 2 of the FDNY-FFs working at the WTC for ≥ 2 days. We found no differences in age or FDNY work years when we separated the group based on the median of cumulative WTC work-site exposure time: < 10 days (n = 23) or 10 days (n = 16).

Differential cell counts and MMP-9 levels in sputum samples. We performed IS differential cell counts in 36 of the 39 FDNY-FFs, 12 of 12 TA-FFs, and 8 of 8 controls. The firefighter groups were significantly different from the nonfirefighter controls, but we found no significant differences between the firefighter groups (Table 1). Differential counts for neutrophils and eosinophils increased with cumulative WTC workday exposure intensity (dichotomized to < 10 or 10 WTC workdays) (Table 2).

We measured MMP-9 levels in IS supernatants retrieved from sample preparations of 25 of 39 FDNY-FFs, 12 of 12 TA-FFs, and 8 of 8 controls. There was a trend for higher levels in the FDNY-FFs vs. the TA-FFs (2.23 ng/mL versus 1.21 ng/mL; p = 0.057), and the combined levels for both groups were significantly higher than for the controls (0.30 ng/mL; p = 0.0001). Independent of exposure group, the levels of MMP-9 were positively correlated to the percentage of neutrophils (r = 0.449, p = 0.002; Figure 1A) and negatively correlated to the percentage of macrophages (r = 0.488, p = 0.001; Figure 1B).

Particle size distribution. We determined PSD in 35 of 39 FDNY-FF samples (4 samples were eliminated because of contamination before measurement) and 12 of 12 TA-FF samples. Table 3 shows that more of the FDNY-FF samples contained a higher percentage of particles > 2 µm (p = 0.0001) and > 5 µm (p = 0.0001) compared with the TA-FF samples. We found no significant differences in PSD measurements when they were correlated to cumulative WTC exposure (tested by a continuous or a dichotomized 10-day analysis). Most of the particles showed an irregular shape (Figure 2B,D).

We also demonstrated compatibility between the PSD of the settled raw dust samples from Cortlandt Street (located one block east of the WTC) collected 7 days after the collapse with measurements from the IS of FDNY-FFs (n = 39; Figure 3). Both PSD curves showed similar patterns for particles > 4.37 µm in diameter, but the curve for the FDNY-FF IS showed a left shift compared to the settled raw dust material, indicating a higher proportion of small particles in the lung than in bulk samples collected from settled...
dust. This would be consistent with the fact that the largest dust particles do not efficiently penetrate past the nasopharyngeal region. An inversion between both curves can be observed where they intersect at 7.19 µm.

Chemical and mineralogic analysis of particles. Chemical and mineralogic analyses were performed on IS samples from 4 FDNY-FFs and 2 TA-FFs randomly chosen from high quality specimens. Chemical analysis of the FDNY-FF samples revealed many elements, for example, titanium, zinc, mercury, gold, tin, and nickel (Table 4), which were present as metal alloys or metal oxides in abundant large particle sizes (range 1–50 µm). The shape varied from irregular (multiple angles) to spherical. In the TA-FF samples, a few smaller particles (1–10 µm) of silica and clays were found that are more typical of normal Tel-Aviv soil than of pollution. Intracellular particles are shown in Figure 4. Figure 5 shows X-ray spectrums of representative particles identifying them as zinc, copper, silver, and mercury.

Discussion

In FDNY-FFs—all highly exposed to WTC dust and combustion/pyrolysis products during the morning of the collapse and nearly all with additional cumulative workday exposures to WTC dust—IS analysis were significantly different from controls, in inflammation (percentages of neutrophils and eosinophils that increased with exposure intensity; increased MMP-9) and particulate matter deposition (a shift in PSD toward larger size particles and chemical/mineral analysis consistent with WTC dust).

It is not surprising to find abnormalities in lung particulate matter and inflammation months after exposure. After chronic occupational exposures, IS has shown increased eosinophil counts in sensitized asthmatics (Maestrelli et al. 1994; Quirce et al. 2001) and in asbestos (Paris et al. 2002), radon (Marek et al. 2001) and uranium (Marek et al. 1994; Skloot et al. 2004) after exposures to WTC dust. This would be consistent with the fact that the largest dust particles do not efficiently penetrate past the nasopharyngeal region. An inversion between both curves can be observed where they intersect at 7.19 µm.

Chemical and mineralogic analysis of particles. Chemical and mineralogic analyses were performed on IS samples from 4 FDNY-FFs and 2 TA-FFs randomly chosen from high quality specimens. Chemical analysis of the FDNY-FF samples revealed many elements, for example, titanium, zinc, mercury, gold, tin, and nickel (Table 4), which were present as metal alloys or metal oxides in abundant large particle sizes (range 1–50 µm). The shape varied from irregular (multiple angles) to spherical. In the TA-FF samples, a few smaller particles (1–10 µm) of silica and clays were found that are more typical of normal Tel-Aviv soil than of pollution. Intracellular particles are shown in Figure 4. Figure 5 shows X-ray spectrums of representative particles identifying them as zinc, copper, silver, and mercury.

Table 4. Mineralogic analysis of IS particles from FDNY-FFs and TA-FFs.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Particle size (µm)</th>
<th>Frequent elements</th>
<th>Type of particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDNY-FF 16</td>
<td>1.5–50</td>
<td>Si, SiCa, SiFe</td>
<td>Silica</td>
</tr>
<tr>
<td>FDNY-FF 3</td>
<td>1.5–50</td>
<td>Zn, Ca</td>
<td>Zinc oxide</td>
</tr>
<tr>
<td>FDNY-FF 26</td>
<td>1.0–100</td>
<td>Si, SiCa, SiFe</td>
<td>Silicon</td>
</tr>
<tr>
<td>FDNY-FF 35</td>
<td>1.0–50</td>
<td>Ca, MgSiCa, AlSiCa, AlSi</td>
<td>Clays</td>
</tr>
<tr>
<td>TA-FF 10</td>
<td>0.8–7.0</td>
<td>Fe, Si</td>
<td>Stainless steel</td>
</tr>
<tr>
<td>TA-FF 12</td>
<td>3–10</td>
<td>Fe, Ca, FeCr</td>
<td>Ferric oxide</td>
</tr>
</tbody>
</table>

Analysis was performed as described in “Methods.”
in FDNY-FF IS, consistent with exposures to aerosolized building debris and dust and smoke generated by the collapse and fires (Lioy et al. 2002). Mineral particles were seen in macrophages and epithelial cells. The presence of these particles in epithelial cells illustrates the high concentration of respirable particulate matter, overwhelming normal nasopharyngeal filtering, mucociliary clearance, and alveolar macrophage defense systems (Churg 1996). Although asbestos fibers were found in the BAL from the FDNY-FFs with eosinophilic pneumonitis after WTC exposure (Rom et al. 2002), we did not find asbestos fibers in our FDNY-FF IS samples.

Despite our finding significant correlations between inflammation and cumulative WTC exposure and significant differences in PSD between FDNY-FFs and TA-FFs, we could not detect a significant effect of cumulative WTC exposure on PSD. This may be related to other physical factors at the WTC site, such as differences in work location, minute ventilation (related to subject size, physical fitness, experience, and work tasks), specific work task–related exposures, and the use of respiratory protection (minimal during week one and variable thereafter) (CDC 2002b). It may also reflect potential limitations of scoring cumulative exposure only in terms of workdays, unweighted for the above differences and other variables such as environmental conditions. Additionally, selection bias (all FDNY-FF subjects volunteered for this study) may have influenced our current ability to detect the effect of cumulative exposure. However, the strengths of this study outweigh these limitations and include the following: a) all FDNY-FFs had significant WTC dust exposures because they were caught in the dust cloud during the collapse and then continued to work at the WTC site for days thereafter; b) IS provided an assessment of persistent inflammation and cumulative particle deposition because the enormity of the disaster prevented us from collecting IS until 10 months after the collapse; c) IS assessments were not biased toward acute, transient inflammation and particle deposition because at least 1 month had elapsed between collecting IS and the last workday at the WTC site; and d) controls were truly unexposed because they were recruited from outside this region—a necessity because by this time nearly every FDNY-FF and most other rescue workers and many Manhattan residents had reported some level of exposure.

We believe the differences between FDNY-FFs, TA-FFs, and controls demonstrate a unique exposure following the WTC collapse (Tables 3 and 4). This conclusion is supported by similarities between quantitative and qualitative analyses of IS sputum samples and dust samples collected from settled material one block east of the WTC. These dust samples demonstrate a complex mixture of coarse particles and fibers consisting of relatively larger particles (> 90% of dust particle mass was > 10 µm in diameter) (Lioy et al. 2002). Thus, the fact that IS samples from FDNY-FFs had higher amounts of particles > 2.0 µm in diameter than samples from TA-FFs was due to the nature and composition of the sources that dominated FDNY-FF exposure patterns. In 87% of FDNY-FFs, > 20% of the particles found in IS were > 2.0 µm in diameter compared with only 8% of those from TA-FFs. SEM also showed relatively larger and more irregularly shaped particles in FDNY-FFs compared with TA-FFs. Once inhaled, particles > 2.0 µm in diameter are most commonly deposited in the upper airways, causing significant irritation because of their alkaline, caustic nature (Lioy et al. 2002); this explains the increased incidence of upper airway symptoms (nasal congestion/drip, throat irritation, cough, and gastroesophageal reflux) described in highly and moderately exposed FDNY-FFs (Banauch et al. 2003; Feldman et al. 2004; Prezant et al. 2002).

Although helpful, measurements obtained from settled dust samples, which are often enriched with larger particles, are not entirely representative of the particle types or size distribution of aerosolized, potentially respirable dust during the height of exposure. In fact, the majority of particles in FDNY-FF IS samples were < 2.0 µm in diameter; 74% of FDNY-FFs had > 60% of PSD < 2.0 µm in diameter (Figure 2). Compared with settled WTC dust samples, FDNY-FF IS samples demonstrated a distinct leftward shift of the average curve of all PSD measurements toward smaller particles. Particles 2.0 µm in diameter are commonly deposited in the smaller airways. This may explain why post-WTC FDNY-FFs (Banauch et al. 2003; Prezant et al. 2002) have shown an increased incidence of bronchial hyperreactivity, reactive airways dysfunction syndrome, and asthma. Six months after the WTC collapse, methacholine challenge testing demonstrated that highly exposed FDNY rescue workers were 6.8 times more likely to have bronchial hyperreactivity than moderately exposed and unexposed FDNY controls (Banauch et al. 2003).

Our findings support both the practical utility and scientific usefulness of IS as a noninvasive method for screening and follow-up monitoring of populations exposed to high concentrations of aerosolized particulates following a disaster—natural or man-made. IS is superior to BAL because it is noninvasive and collection can occur at nearly any field location. In contrast to traditional blood and urine biomonitoring, IS directly samples the lung.
the specific target organ of interest following an inhalation exposure. For example, blood and urine samples were collected 1 month after the collapse in a different group of FDNY-FFs, and only a few of the 110 chemicals measured showed significant, yet small, differences when WTC-exposed FDNY-FFs were compared with nonexposed FDNY-FFs (Edelman et al. 2003). In contrast, IS directly assesses respiratory particle matter and pulmonary inflammation, thereby serving an important complementary role to traditional biomonitoring techniques.

In conclusion, IS from FDNY-FFs caught in the WTC dust cloud during the morning of the collapse showed an influx of inflammatory cells, percentages of neutrophils and eosinophils that increased with exposure intensity, increased MMP-9 levels, a shift in PSD toward larger-size particles, and chemical/mineral analyses consistent with exposure to building debris, smoke, and dust generated by the attack on the WTC. If future population studies demonstrate that IS measures of inflammation or PSD correlate with health outcomes, then IS evaluations would be a valuable addition to medical screening/monitoring programs following inhalation exposures.

REFERENCES


