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RAPID DECLINE IN SPUTUM IL-10 CONCENTRATION FOLLOWING OCCUPATIONAL SMOKE EXPOSURE

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*The acute effects of smoke exposure on inflammatory mediators such as interleukin-10 (IL-10), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) are not well understood. Our study was designed to measure sputum concentrations of these cytokines in firefighters following low-level smoke exposure. At baseline, participating firefighters underwent blood collection, pulmonary function testing, and sputum induction through inhalation of nebulized hypertonic saline. Study participants later performed overhaul of a structural fire, during which time they wore cartridge respirators and were monitored for smoke exposure. Overhaul involves searching for and extinguishing hidden sources of combustion. One hour following overhaul, blood, pulmonary function data, and induced sputum were again collected. IL-10, IL-8, and TNF-α concentrations were measured by enzyme-linked immunosorbent assay (ELISA) in sputum supernatant. In 17 firefighters, baseline sputum IL-10 concentrations were 57.0 ± 56.8 pg/L, and declined to 16.9 ± 27.2 pg/L following overhaul (*p = .02*). No significant changes were observed in sputum IL-8 and TNF-α concentrations. Forced vital capacity (FVC) declined significantly in study participants following overhaul. Serum concentrations of Clara-cell protein and surfactant-associated protein A increased significantly following overhaul, indicating increased lung permeability. IL-10 concentrations appear to be exquisitely sensitive to smoke, and studies of IL-10 in sputum should control for recent exposure. Reduced suppression of inflammation by IL-10 may be a mechanism by which low-level smoke exposure causes lung injury.*

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Smoke inhalation leads to a complex series of changes in the lung resulting in an inflammatory response (Traber & Herndon, 1990). Firefighters have been shown to have acute changes in lung function following smoke exposure (Sherman et al., 1989; Brandt-Rauf et al., 1989; Large et al., 1990; Musk et al., 1979) and may suffer long-term consequences of repeated exposures (Burgess et al., 1999). A particular phase of firefighting, called overhaul, follows extinguishment of visible flames and involves searching for and putting out hidden sources of combustion. During this phase, firefighters often do not wear respiratory protection due to the lack of visible smoke, but elevated concentrations of a number of products of combustion may still be present (Bolstad-Johnson et al., 2000).

Alterations in lung function following chronic smoke exposure have been correlated with altered cytokine production and increased pulmonary inflammation. Tumor necrosis factor alpha (TNF-α) and interleukin-8 (IL-8) are proinflammatory cytokines produced by macrophages and are known to play a role in recruiting neutrophils. These cytokines have been measured in induced sputum from nonsmokers, healthy smokers, and chronic obstructive pulmonary disease (COPD) patients (Keatings et al., 1996). Sputum TNF-α is increased in smokers with COPD, while IL-8 is significantly increased in smokers, and in COPD patients at levels four times higher than in healthy smokers. Concentrations of TNF-α and IL-8 are correlated with the percentage of neutrophils in BAL from smokers and COPD patients (Soler et al., 1999).

Interleukin-10 (IL-10) is thought to play an important role in limiting the degree of inflammation in the lungs through suppression of release of inflammatory cytokines (de Waal Malefyt & Moore, 1998). In addition IL-10 plays an important role in immunity (Hagenbaugh et al., 1997) and autoimmunity (Bettelli et al., 1998; Cua et al., 1999). Levels of IL-10 in induced sputum are significantly depressed with chronic cigarette smoke exposure in COPD patients (Takanashi et al., 1999).

Exposure to smoke occurs both occupationally and environmentally as discrete acute exposures, with variable time intervals between exposures. To understand the pathophysiology of smoke exposure it is important to study both acute and chronic effects. The acute effects of smoke exposure on inflammatory mediators in the lung are not well known. This study was designed to evaluate acute changes in induced sputum concentrations of IL-10, IL-8, and TNF-α following low-level occupational smoke exposure in firefighters.

METHODS

This research was approved by the University of Arizona Human Subjects Institutional Review Board. As part of a larger study of smoke exposure during overhaul, 17 City of Phoenix Fire Department firefighters were evaluated with sputum TNF-α, IL-8, and IL-10 measurements. After obtain-

ing informed consent, a baseline evaluation was completed including a self-administered questionnaire. Prior to sputum induction, collection of venous blood for serum pneumoproteins and pulmonary function testing were completed as previously described (Burgess et al., 2001).

Sputum was induced by inhalation of 3% hypertonic saline administered with an ultrasonic nebulizer set at maximal output (DeVilbiss UltraNeb 99HD, Somerset, PA) through an adult face mask. Subjects inhaled the saline for 30 min, or until 5 ml sputum was obtained. Sputum samples were stored in sterile conical vials at 4°C and transported to the lab for analysis within 2 h. Samples were processed according to previously described protocols (Von Essen et al., 1998). Each sample was combined with 10 ml of 10% dithiothreitol solution, prepared by mixing 10 ml dithiothreitol (Sputolysin; Calbiochem, San Diego, CA), 90 ml HEPES buffered solution (140 m*M* NaCl, 5 m*M* KCl, 1.5 m*M* $CaCl_2$, 5 m*M* glucose, and 10 m*M* HEPES), and 0.5 ml penicillin/streptomycin (Sigma). Samples were placed in a shaking water bath for 15 min, then vortex mixed. Each sample was filtered through sterile nylon mesh to remove any remaining mucous or particulate matter. Samples were centrifuged at 1500 rpm for 15 min. Two milliliters of supernatant were removed and frozen at –80°C until time of cytokine analysis. Cytokine concentrations were analyzed using commercially available assays (R & D, Minneapolis, MN).

With the exception of training fires, only actual residential or commercial structural fires were selected for inclusion in the study. The fire must have involved at least two rooms of the structure. No subjects were allowed to participate if they had been exposed less than 24 h previously to visible smoke. When possible, all subjects were asked to participate in overhaul only, and not the prior entry/ventilation or extinguishment. Sampling started when firefighters entered the structure for overhaul following self-contained breathing apparatus (SCBA) removal. Firefighters left their face pieces in place with multipurpose cartridges (Scott Aviation model 642-MPC-P100, Monroe, NC) on the face piece inserted over a T-piece adaptor. These cartridges protect against particulate, organic vapor, ammonia, methylamine, SO_2, formaldehyde, chlorine, chlorine dioxide, hydrogen chloride (HCl), and hydrogen fluoride exposure. Overhaul did not begin until CO levels were below 150 ppm as measured by direct-read instruments. Overhaul was conducted for a minimum of 25 min in all incidents. One hour following cessation of overhaul, repeat testing was performed in the same order, with the addition of blood collected in a heparinized syringe for venous carboxyhemoglobin (COHb) analysis.

The methods for exposure monitoring during overhaul, including limits of detection, have previously been described (Bolstad-Johnson et al., 2000). National Dräger (Pittsburgh, PA) Miniwarn four-gas direct-read meters (configured to detect CO, NO_2, SO_2, and CH_4) and Pac III single-gas meters (configured for HCN) were used to collect continuous exposure data. Sorbent tube samples were collected for aldehydes (acetalde-

hyde, acrolein, benzaldehyde, formaldehyde, glutaraldehyde, isovaleraldehyde), benzene, hydrochloric acid, hydrogen cyanide, and sulfuric acid. Respirable dust was collected using personal cyclone samplers (Mine Safety Appliance, Pittsburgh, PA).

Baseline and overhaul forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) measurements were compared using a paired *t*-test. Baseline and overhaul sputum cytokine concentrations were compared using the Wilcoxon matched-pairs signed-rank test, since they were not normally distributed. Combustion products present above the detectable limit for at least 10 subjects were compared with changes in IL-10 concentration using Pearson's correlation coefficient. Significant associations at the $p < .05$ level were reported.

RESULTS

A description of the firefighter population is provided in Table 1. All subjects were male, with the majority being Hispanic. Only one subject was a current smoker. Concentrations of products of combustion measured during overhaul for the 17 firefighters with IL-10 measurements are listed in Table 2. These concentrations were in general below American Conference of Governmental Industrial Hygienists (ACGIH) occupational exposure limits, with the exception of carbon monoxide, formaldehyde, sulfuric acid, benzene, and respirable dust (ACGIH, 2000). Acrolein, benzaldehyde, glutaraldehyde, and isovaleraldehyde concentrations were negligible, exceeding detectable limits in at most one firefighter.

Results of cytokine assays are reported in Table 3. Of the 17 firefighters with baseline and overhaul concentrations of IL-10, 16 had IL-8 concentrations measured and 15 had TNF-α concentrations measured. IL-10 concentrations declined by 70% following overhaul ($p = .02$). The mean decrease in IL-10 concentration was 40.1 ± 64.1 pg/L, and the distribution is illustrated in Figure 1. Of the 17 firefighters evaluated, sputum IL-10 following overhaul declined in 11, did not change in 3, and increased in 3. No significant changes were observed in IL-8 and TNF-α concentrations, although there was an approximate twofold increase in IL-8 concentration following overhaul.

TABLE 1. Firefighter participants ($n = 17$)

Age (yr)	37.9 ± 8.7
Male gender	17 (100%)
Race/ethnicity	
Hispanic	9 (53%)
White	7 (41%)
Black	1 (6%)
Current smoker	1 (6%)
Ever smoker	6 (35%)

TABLE 2. Smoke exposure during overhaul

Analyte	*n*	>LOD[a]	Mean ± SD[b]	ACGIH limit[c]
Carbon monoxide (ppm)	10	10	27.5 ± 22.8	TLV-TWA 25 ppm
Carboxyhemoglobin (%)	17	17	0.99 ± 0.93	BEI 3.5%
Nitrogen dioxide (ppm)	10	10	0.004 ± 0.006	TLV-TWA 3 ppm
Sulfur dioxide (ppm)	10	10	1.11 ± 0.83	TLV-TWA 2 ppm
Hydrogen cyanide (ppm)	10	10	0.83 ± 0.87	TLV-C 4.7 ppm
Formaldehyde (ppm)	12	12	0.25 ± 0.26	TLV-C 0.3 ppm
Acetaldehyde (ppm)	12	11	0.41 ± 0.54	TLV-C 25 ppm
Hydrochloric acid (ppm)	12	6	0.93 ± 0.74	TLV-C 5 ppm
Sulfuric acid (ppm)	12	7	3.85 ± 4.28	TLV-STEL 3 mg/m^3
Benzene (ppm)	14	6	0.53 ± 0.26	TLV-TWA 0.5 ppm
Respirable dust (mg/m^3)	13	8	5.26 ± 6.34	TLV-TWA 3 mg/m^3

[a]Number of samples exceeding limit of detection (LOD).
[b]Mean and standard deviations calculated using only concentrations >LOD.
[c]American Conference of Governmental Hygienists (ACGIH) threshold limit values (TLV) include: TLV-TWA, an 8-h time-weighted average over a workday; BEI, a biological exposure limit measured for carboxyhemoglobin in blood; TLV-C, a ceiling concentration not to be exceeded; and TLV-STEL, a short-term exposure limit not to be exceeded over a 15-min averaging period (ACGIH, 2000).

Mean FVC (baseline 5.42 ± 0.59 L, overhaul 5.28 ± 0.68 L, $p = .02$) declined significantly with smoke exposure, while decrease in FEV_1 (baseline 4.26 ± 0.43 L, overhaul 4.19 ± 0.52 L, $p = .12$) was not statistically significant. Serum Clara-cell protein increased (baseline 10.2 ± 2.9 μg/L, overhaul 15.5 ± 4.6 μg/L, $p < .01$), as did serum surfactant-associated protein A (baseline 269 ± 130 μg/L, overhaul 334 ± 161 μg/L, $p = .03$), indicating increased lung permeability following smoke exposure. Change in IL-10 was not correlated with concentrations of carboxyhemoglobin, individual products of combustion, decline in FEV_1 or FVC, or increase in serum pneumoproteins.

DISCUSSION

This study observed a marked decrease in sputum IL-10 concentrations following low-level occupational smoke exposure, while no significant changes occurred in IL-8 and TNF-α concentrations. While the cartridge respirators worn by the firefighter subjects may have provided some

TABLE 3. Sputum cytokine concentrations for firefighters before and after overhaul

Cytokine	*n*	Baseline	Overhaul	*p*[a]
IL-10 (pg/L)	17	57.0 ± 56.8	16.9 ± 27.2	.020
IL-8 (pg/L)	16	492 ± 778	905 ± 987	.379
TNF-α (pg/L)	15	10.9 ± 13.2	11.0 ± 13.0	.733

[a]Wilcoxon matched-pairs signed-ranks test.

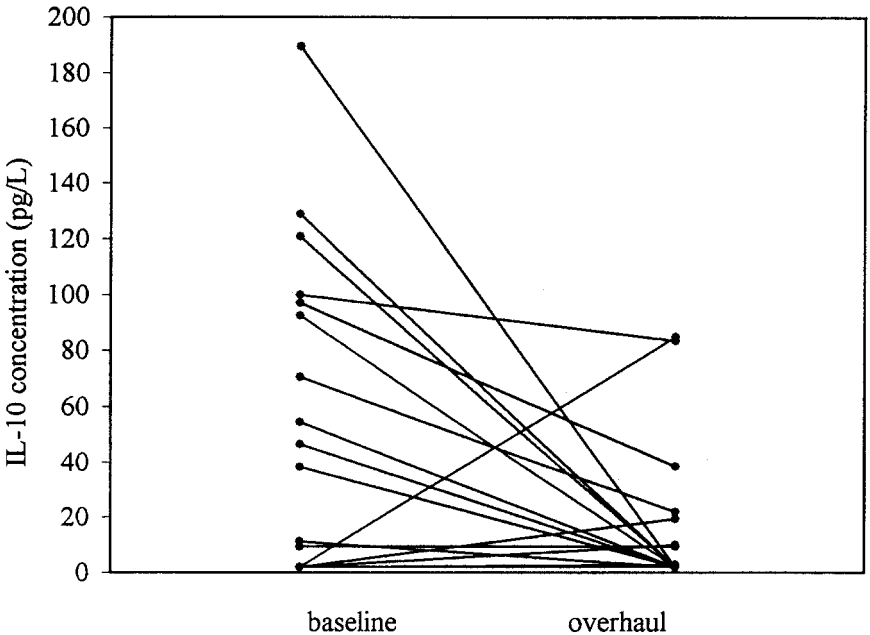

FIGURE 1. Change in sputum IL-10 concentration in study participants.

degree of protection against smoke exposure during overhaul, they clearly did not provide complete protection. Poor face-piece fit or incomplete protection against poorly absorbed chemicals, such as carbon monoxide, may have occurred.

No previous studies have evaluated IL-10 concentrations in sputum following acute smoke exposure. The mechanism by which IL-10 concentrations decrease while other cytokines remain unchanged is not clear. IL-10 is produced in the lung by macrophages following activation such as with lipopolysaccharide (LPS), by T cells and B cells, and by bronchial epithelial cells (de Waal Malefyt & Moore, 1998). One possible explanation for our results involves toxicity to or suppression of these IL-10-producing cells. Smoke contains a large number of reactive chemicals, and the IL-10-producing cells may be particularly susceptible to reactive injury. Smoke may also either directly or indirectly suppress IL-10 production through a number of more reversible mechanisms.

The results of other studies are important in interpreting the mechanism of and biological significance of our findings. Sputum samples collected from chronic smokers showed values of IL-10 that were 75% of nonsmoking controls and COPD patients had values that were only 30% of controls (Takanashi et al., 1999). Levels of IL-10 correlated with the number of IL-10 positive cells in induced sputum. IL-10 decreases the levels of RFD1+ stimulatory macrophages while increasing the levels of other macrophage phenotypes (Tormey et al., 1998). IL-10 leads to apop-

tosis of neutrophils, thus limiting the degree of inflammation (Cox, 1996). IL-10 suppresses production of TNF-α and IL-8 (de Waal Malefyt & Moore, 1998). In addition, IL-10 downregulates macrophage expression of matrix metalloproteinases, including interstitial collagenase (MMP-1) and gelatinase B (MMP-9), while upregulating tissue inhibitors of metalloproteinases, specifically TIMP-1 (Busiek et al., 1995; Lacraz et al., 1995; Stearns et al., 1999). Therefore changes in IL-10 concentrations following smoke exposure may result in changes in other inflammatory mediators within the lung that lead to chronic respiratory effects. Although in our study IL-8 and TNF-α did not significantly change following smoke exposure, IL-8 concentrations did increase almost twofold. Our small sample size and choice of a short postexposure interval may have affected our ability to detect increases in IL-8 or TNF-α.

There are a number of limitations to this study. First, only one time point was evaluated, 1 h following cessation of overhaul. Additional studies are necessary to evaluate the time course of this change. IL-8 and TNF-α concentrations could potentially increase at a later time following smoke exposure, either as a direct effect of loss of IL-10 suppression, or directly as a result of smoke exposure. The observed reduction in IL-10 was most likely transient, and the health consequences of this change are not known. Although no products of combustion measured in this study were correlated with change in IL-10, our power to detect such a relationship was limited with our small sample size. Other factors external to this study may also influence IL-10 levels. Finally, IL-10 concentrations in sputum supernatant were highly variable, which may have resulted partially from dilution with saliva. Dilution effects could be controlled in future studies if IL-10 concentrations could be expressed as a ratio with a suitable protein that did not change in concentration with smoke exposure.

In conclusion, low-level occupational smoke exposure may have significant effects on IL-10 concentrations within the lung. In evaluation of smoke exposure, acute health effects need to be clearly separated from chronic effects. While the biological significance of our findings is not clear, acute decline in IL-10 could provide an additional mechanism to explain the proinflammatory effects of occupational smoke exposure.

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