



Inflammatory responses to the occupational inhalation of metal fume

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ABSTRACT: Occupational exposure to metal fume promotes a reversible increase in the risk of pneumonia, but by mechanisms which are unclear. To investigate, the current authors measured various markers of host defence function in welders and nonwelders.

Induced sputum and venous blood samples were collected from 27 welders with regular long-term exposure to ferrous metal fume and 31 unexposed matched controls. In sputum, the present authors measured cell counts, the soluble and cellular iron concentration, and levels of interleukin-8, tumour necrosis factor- α , myeloperoxidase, matrix metalloproteinase-9, immunoglobulin (Ig)A, α_2 -macroglobulin and unsaturated iron-binding capacity. Blood samples were assayed for evidence of neutrophil activation and pneumococcal IgG antibodies.

Welders had significantly higher iron levels and a substantially lower unsaturated iron-binding capacity in their sputum, but, despite a high iron challenge, there was a noteworthy absence of an inflammatory response. Only blood counts of eosinophils and basophils were significantly related to the extent of welding. Weak nonsignificant trends were observed for several other measures, consistent with low-grade priming of neutrophils.

In conclusion, these data suggest that chronic exposure to metal fume blunts responsiveness to inhaled particulate matter. However, the mechanism behind the lack of detectable local inflammatory response requires further investigation.

KEYWORDS: Inflammation, metal fume, occupational, pneumonia, welding

Each year in England and Wales, one person per thousand is admitted to hospital with community-acquired pneumonia (CAP) [1] and four times as many cases are treated outside hospital [2]. Half of cases who are in employment require >2 weeks absence from work [3], and case fatality in patients admitted to hospital ranges from 6–24% [4, 5]. In subjects aged <65 yrs, pneumonia kills as often as all other infectious diseases combined [6].

Some risk factors for CAP are well characterised, including heavy drinking [7], hyposplenism [8], smoking [9] and immunosuppression [10]. An increased risk has also been described in closed communities [11].

Less is known about occupational risk factors, but analyses of mortality in England and Wales have regularly demonstrated a marked excess of deaths from pneumonia in welders [12–14]. A recent analysis [15] showed that the excess is attributable largely to: 1) deaths from lobar pneumonia and pneumonias other than bronchopneumonia; 2) that it is limited to males

below retirement age; and 3) that a similar pattern exists for several other occupations entailing exposure to metal fume, such as moulders and coremakers in foundries. In a case-control study of males of working age [16], CAP was associated with occupational exposure to metal fume in the previous year but not in earlier periods, supporting the hypothesis of a hazard that is reversible following cessation of exposure. Risks were highest for recent exposure to ferrous metal fume with lobar pneumonia as the outcome [16].

The health effects of welding have recently been reviewed [17]. The mechanism by which metal fume promotes pneumonia remains unclear. However, it is suggested that submicron-sized particles may have a direct toxic effect on immunocompetence, or that iron may act as a bacterial growth nutrient or agent of oxidative injury [18].

The host response to airway infection is normally to increase the number and activation state of macrophages and neutrophils. This may be

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Received:

May 04 2005

Accepted after revision:

October 19 2005

SUPPORT STATEMENT

This study was supported by the Worshipful Company of Blacksmiths of the City of London (UK) as a millennium project.

This article has supplementary material accessible from www.erj.ersjournals.com.

enhanced by locally produced immunoglobulin (Ig)A [19] that protects the mucosa and is a stimulus for inflammatory cell activation. Typically, inflammatory mediators, such as tumour necrosis factor (TNF)- α and interleukin (IL)-8, or markers of cellular activation, such as matrix metalloproteinase (MMP)-9 and myeloperoxidase (MPO) are elevated during lung infection. According to experimental evidence, several elements of the response to infection, such as neutrophil recruitment and activation, are also triggered by inhaled particulate matter (PM) [20–22]. However, inhaled iron could promote neutrophil death by apoptosis [23, 24], *via* iron-dependent hydroxyl free radical generation [25], and impair immunocompetence.

To examine the effect of ferrous fume on airways inflammation and host defence, the present authors conducted a study in welders and a matched reference group. The current authors aimed to identify potential biomarkers of the hazard that might subsequently be characterised for their relationship to pneumonia and perhaps be used to assess the relation of risk to levels of occupational exposure. The latter is important for optimal control of the hazard, but, because pneumonia is rare, exposure-response relationships are difficult to establish directly.

Evidence for a generalised inflammatory response was measured as cell counts in blood and sputum, C-reactive protein (CRP) in blood, and TNF- α , IL-8, MMP-9, MPO and α_2 -macroglobulin levels in sputum. Immunocompetence was assessed through the respiratory burst of blood neutrophils, circulating pneumococcal IgG and sputum IgA concentration. With no prior information on the mechanism of predisposition to pneumonia in welders, these measures were chosen *a priori* from a range of candidates as plausible and convenient starting points for investigation. The current authors also measured iron in the cellular residue and supernatant of sputum and unsaturated iron-binding capacity (UIBC) in sputum as biological indices of recent exposure (past 1–2 months).

MATERIAL AND METHODS

Study subjects

Ethical approval was granted by the UK Defence Medical Services Clinical Research Committee. A total of 27 male volunteers with regular long-term exposure to metal fume as welders were recruited from the civilian workforce at HM Naval Base, Portsmouth, England. These were group-matched by age (in 10-yr bands), social class and smoking status to 31 male volunteers from the same workplace who had no exposure to metal fume in the previous 12 months. All subjects were free from diabetes, asthma, chronic obstructive airways disease and clinically overt immunodeficiency.

Study design and methods

Subjects were investigated in seven sessions, each conducted towards the end of a normal working week and including similar numbers from exposed and unexposed groups. A nurse-administered questionnaire provided information on matching variables and history of exposure to metal fumes (including metal type, intensity of welding and use of protective equipment). Smoking history and alcohol intake were noted.

A venous blood sample was tested for full blood count, CRP and pneumococcal IgG antibodies. A dichlorofluorescein

(DCFH) oxidation assay was used to measure the polymorphonuclear cell respiratory burst in whole blood [26]. Following incubation at 37°C with nonfluorescent 2',7'-DCFH diacetate and then with phorbol myristate acetate, the amount of the fluorescent derivative DCFH generated by reactive oxygen species was measured by flow cytometry. Neutrophil oxidative burst was expressed as the percentage of neutrophil-produced reactive oxygen metabolites (hydrogen peroxide (H₂O₂)), as well as their mean fluorescence intensity (relative amount of H₂O₂ produced).

Sputum was induced using a standard protocol [27]. Subjects breathed a nebulised aerosol of 4.5% saline solution for four consecutive 5-min periods and coughed into a petri dish. Expectorated samples were processed in an equal weight of 6.5 mM Sputolysin™ (Merck Biosciences, Nottingham, UK), filtered through a 70 μ m cell strainer, and then separated by centrifugation for 10 min at 4°C. The supernatant was stored at -80°C. The cell pellet was resuspended in 1 mL PBS (Invitrogen Ltd, Paisley, UK), mixed thoroughly, and 10 μ L of the suspension assayed in duplicate for total cell count and percentage viability using Trypan blue stain and a haemocytometer. For differential cell counts, 70 μ L of a cell suspension (1×10^6 cells·mL⁻¹) was loaded into a cytospin funnel and centrifuged at $21 \times g$ for 5 min. Cytospin slides were air dried and stained with the Hema Gurr Rapid staining set (VWR International, Poole, UK). Slides were fixed in methanol, stained with eosin and air dried again before counterstaining with methylene blue and being washed of excess stain. Slides were air dried and mounted with DPX (VWR International). Differential cell counts were based on ≥ 500 cells·slide⁻¹.

Commercially available ELISA or RIA kits were used according to manufacturers' instructions to measure TNF- α (R&D Systems, Abingdon, UK), IL-8 (Pelikine assay kit, Mast Diagnostics, Liverpool, UK), MPO (Pharmacia & Upjohn, Uppsala, Sweden) and UIBC (Sigma Diagnostics, Poole, UK). Concentrations of α_2 -macroglobulin and sputum IgA were measured by an in-house ELISA technique [28]. Total iron in sputum supernatant was measured by electrothermal atomic absorption spectrometry using an Analyst 800 (Perkin Elmer LAS UK Ltd, Beaconsfield, UK) following digestion with concentrated nitric acid. Calibration was carried out using aqueous iron standards in the range 0–250 μ g·L⁻¹. Total iron in the cellular fraction was measured by flame atomic absorption spectrometry (Perkin Elmer 2380 AA; Perkin Elmer LAS UK Ltd) following digestion of the cell pellets in 500 μ L nitric acid overnight. Calibration was carried out using aqueous iron standards in the concentration range 0–5,000 μ g·L⁻¹. MMP gelatinase activity was investigated using quantitative substrate gel zymography [29].

All laboratory analyses were performed blind to the exposure status of the participants.

Statistical analysis

The normality of each variable was assessed using the Shapiro-Wilk test and, where relevant, improved by a Box-Cox power transformation. A small fixed constant was added to measures that occasionally took the value zero (TNF- α , UIBC and epithelial cell count); if frequently zero or undetectable (eosinophils, basophils, lymphocytes and MPO in sputum,

and eosinophils in blood), Poisson regression analysis was used. For IL-8, the equivalent normal scores were used instead.

Following these adjustments it proved possible to compare levels of biomarkers using standard parametric methods. Independent sample t-tests (unpaired) were used to test the overall relationship of age (<40 *versus* \geq 40 yrs), typical alcohol consumption (\leq 28 units \cdot week $^{-1}$ *versus* >28 units \cdot week $^{-1}$) and smoking status (never, ex- and current) to each of the biological measures. Multiple regression modelling was used to assess their association with welding after adjustment for potential confounders. Welders were also subclassified into high and low exposure groups of roughly equal size according to three criteria: 1) the total iron in the cellular residue of the sputum (< or >1.5 $\mu\text{g}\cdot\text{L}^{-1}$); or 2) their typical duration of weekly welding: \geq 2 h \cdot day $^{-1}$ for \geq 3 days \cdot week $^{-1}$ *versus* less than that (use of fume extraction differed little between groups defined in this way); or 3) years spent as a welder (in two bands, <10 yrs or \geq 10 yrs). Two-sided tests for trend were performed across exposure bands.

RESULTS

The characteristics of the study participants are presented in table 1. The exposed group had been employed as professional welders in marine engineering for a median period of 10 yrs (range 6 months to 44 yrs). Their work entailed welding, burning and cutting, plate fabrication and boilermaking. The majority welded for >2 h \cdot day $^{-1}$ on most working days in a typical working week. A variety of gas-shielded welding techniques were employed (metal inert gas welding, tungsten inert gas welding and metal active gas welding), as well as metal core arc and flux core arc welding. All subjects welded mild steel. In addition, some welded galvanised metal (12), aluminium or aluminium alloys (nine), stainless steel (six), chromium plated metals (two), cast iron (one), brass (one), copper alloys (one), zinc alloys (one) and nickel alloys (one). All welders had welded within the past week and most within 48 h of being tested.

The nonexposed group comprised fitters and turners (11), project planners, team leaders and engineers (nine), painters (two), crane drivers (two) and miscellaneous trades (seven). They were similar to the exposed group in terms of age, typical weekly alcohol consumption and proportion of ever-smokers (table 1).

In analyses that included all subjects (welders and controls), there were few significant associations between the biological variables and age, alcohol intake and smoking status (data not presented here; supplementary data of the numerous correlations is available at www.erj.ersjournals.com). However, sputum α_2 -macroglobulin and IgA levels tended to be higher in younger adults (<40 *versus* \geq 40 yrs of age; $p<0.05$), pneumococcal IgG titres in blood, and sputum IgA and MMP-9 tended to be higher in heavy drinkers (\leq 28 *versus* >28 units \cdot week $^{-1}$ of alcohol; $p<0.05$); and blood lymphocyte count and sputum IgA levels were higher in current smokers (*versus* never-smokers; $p<0.05$). Total iron in the cellular residue was higher, although not significantly, in smokers than nonsmokers. When controls and welders were analysed separately, amongst controls, smokers tended to have higher levels than nonsmokers of MMP-9 (median 602 *versus*

TABLE 1 Characteristics of the study groups

	Welders	Nonwelders
Subjects n	27	31
Age yrs		
All	32 (18–60)	40 (20–60)
\geq 40	10 (37)	16 (52)
Weekly alcohol consumption units		
All	12 (0–46)	14 (2–61)
\geq 28 units	7 (26)	7 (23)
Smoking habits		
Current smokers	10 (37)	9 (29)
Ex-smokers	6 (22)	10 (32)
Nonsmokers	11 (41)	12 (39)
Years employed as welder		
All	10 (0.5–44)	
Welding in a typical week		
\geq 3 days \cdot week $^{-1}$	18 (67)	
>2 h \cdot day $^{-1}$	17 (63)	

Data are presented as median (range) or n (%).

191 ng \cdot g $^{-1}$, age- and alcohol-adjusted p -value=0.08), IgA (18.7 *versus* 7.3 $\mu\text{g}\cdot\text{mL}^{-1}$; $p=0.09$), and α_2 -macroglobulin (381 *versus* 126 $\mu\text{g}\cdot\text{mL}^{-1}$; $p=0.07$). In welders, by contrast, differences in these variables by smoking status were minor (MMP-9: 278 *versus* 281 ng \cdot g $^{-1}$, $p=0.92$; IgA: 20.1 *versus* 12.5 $\mu\text{g}\cdot\text{mL}^{-1}$, $p=0.15$; and α_2 -macroglobulin: 184 *versus* 257 $\mu\text{g}\cdot\text{mL}^{-1}$, $p=0.96$).

Table 2 compares biomarkers in welders and nonwelders, with regression analyses adjusted for age, smoking and alcohol consumption. As anticipated, exposure to welding fume was associated with significantly higher levels of iron in the sputum, both in the cellular element ($p<0.001$) and in the supernatant ($p=0.02$), and there was a significant corresponding reduction in UIBC (mean 2.73 $\mu\text{g}\cdot\text{mL}^{-1}$ in welders *versus* 3.32 $\mu\text{g}\cdot\text{mL}^{-1}$ in nonwelders; $p=0.02$). When welders were categorised in two bands, according to the iron levels in the cellular residue of their sputum sample (most and least exposed to iron fume), and compared with nonwelders (unexposed to iron fume), statistically significant trends were observed for total iron in the supernatant (rising values across exposure categories; $p=0.03$) and UIBC (falling values; $p=0.01$).

Only a few other significant differences were detected. A greater proportion of welders than nonwelders had elevated blood counts of basophils and eosinophils ($p\leq 0.02$), with some evidence of a trend by exposure level. Weak nonsignificant trends in relation to exposure level were observed for CRP, neutrophil respiratory burst intensity and sputum IgA (higher in exposed categories); and for eosinophils in sputum (lower in exposed categories). Levels of pneumococcal antibodies in blood and α_2 -macroglobulin and MPO in sputum tended to be higher, and IL-8 in sputum lower, in welders relative to the unexposed comparison group, but not significantly and without apparent trend by exposure level. Most differences were small in absolute terms. The proportion of viable cells in

TABLE 2 Comparison of inflammatory and immunological measures between welders and nonwelders

	Nonwelders	Welders: iron in cellular residue			p-value [#]	
		≤1.5 µg·L ⁻¹	>1.5 µg·L ⁻¹	All	Welders versus nonwelders	Test for trend
Inflammatory markers in blood						
Neutrophils 10 ⁹ cells·L ⁻¹	3.3 (31)	3.6 (13)	3.1 (14)	3.3 (27)	0.99	0.73
Lymphocytes 10 ⁹ cells·L ⁻¹	2.0 (31)	2.0 (13)	1.7 (14)	1.8 (27)	0.10	0.03
Monocytes 10 ⁹ cells·L ⁻¹	0.5 (30)	0.5 (13)	0.5 (14)	0.5 (27)	0.98	0.95
Eosinophils [†] 10 ⁹ cells·L ⁻¹	0.1, 33% ≥0.2 (30)	0.3, 62% ≥0.2 (13)	0.2, 71% ≥0.2 (14)	0.2, 67% ≥0.2 (27)	0.01	0.09
Basophils [†] 10 ⁹ cells·L ⁻¹	0.1, 17% ≥0.2 (30)	0.1, 31% ≥0.2 (13)	0.2, 71% ≥0.2 (14)	0.2, 52% ≥0.2 (27)	0.02	0.04
White blood cells 10 ⁹ cells·L ⁻¹	6.0 (31)	6.5 (13)	5.5 (14)	5.9 (27)	0.77	0.37
C-reactive protein [†] mg·L ⁻¹	<2, 19% >2 (31)	<2, 31% >2 (13)	<2, 36% >2 (14)	<2, 33% >2 (27)	0.65	0.48
Inflammatory markers in sputum						
Macrophages × 10 ⁶ cells·g ⁻¹	0.22 (31)	0.30 (13)	0.16 (14)	0.22 (27)	0.98	0.49
Neutrophils × 10 ⁶ cells·g ⁻¹	0.086 (31)	0.135 (13)	0.060 (14)	0.089 (27)	0.82	0.75
Eosinophils [†] × 10 ⁶ cells·g ⁻¹	0, 48% >0 (31)	0, 38% >0 (13)	0, 29% >0 (14)	0, 33% >0 (27)	0.16	0.34
Epithelial cells × 10 ⁶ cells·g ⁻¹	0.040 (31)	0.063 (13)	0.040 (14)	0.051 (27)	0.34	0.82
Lymphocytes [†] × 10 ⁶ cells·g ⁻¹	0.0009 (31)	0.0027 (13)	0.0001 (14)	0.0006 (27)	0.61	0.17
Total cells × 10 ⁶ cells·g ⁻¹	0.42 (31)	0.64 (13)	0.31 (14)	0.44 (27)	0.77	0.59
Interleukin-8 [†] ng·g ⁻¹	6.5 (31)	5.1 (13)	5.6 (14)	5.6 (27)	0.61	0.75
Myeloperoxidase [†] ng·g ⁻¹	0, 19% >0 (31)	0, 38% >0 (13)	0, 36% >0 (14)	0, 37% >0 (27)	0.15	0.36
TNF-α pg·g ⁻¹	1.74 (28)	1.68 (12)	2.07 (13)	1.87 (25)	0.80	0.87
MMP-9 ng·g ⁻¹	236 (24)	244 (10)	284 (13)	266 (23)	0.84	0.67
α ₂ -Macroglobulin µg·mL ⁻¹	164 (23)	160 (11)	223 (13)	193 (24)	0.78	0.53
Immunological markers						
Pneumococcal IgG antibodies µ·mL ⁻¹	60 (31)	108 (13)	63 (13)	83 (26)	0.24	0.44
Neutrophil fluorescence %	72 (31)	68 (13)	67 (14)	68 (27)	0.17	0.23
Neutrophil intensity	115 (31)	124 (13)	142 (14)	132 (27)	0.18	0.12
IgA in sputum µg·mL ⁻¹	9.7 (25)	11.6 (11)	14.5 (12)	13.0 (23)	0.36	0.21
Markers of exposure and effect						
Total iron in supernatant µg·L ⁻¹	137 (31)	157 (13)	172 (14)	165 (27)	0.02	0.03
Total iron in cells µg·10 ⁶ cells	0.39 (31)			1.32 (27)	<0.001	
Unsaturated iron-binding capacity µg·mL ⁻¹	3.32 (17)	2.89 (6)	2.58 (7)	2.73 (13)	0.02	0.01
Cell viability proportion	0.80 (31)	0.75 (13)	0.83 (14)	0.79 (27)	0.85	0.63

Data are presented as the mean (n), back transformed to original scale where appropriate, unless otherwise indicated. TNF: tumour necrosis factor; MMP: matrix metalloproteinase; Ig: immunoglobulin. #: adjusting for age, average weekly alcohol consumption and smoking status (in three bands); †: data presented as the median (n).

welders' sputum did not differ significantly from that in nonwelders.

When welders were subclassified according to the frequency of welding in a typical week or number of years spent as a welder (table 3), instead of by iron concentration in the sputum, then essentially similar results were obtained. Significantly higher levels of iron were again found in sputum and in cellular residue, and significantly lower levels of UIBC. There were small nonsignificant differences in blood basophil and eosinophil counts and a marginally higher level of pneumococcal antibodies in long-term welders (p=0.09).

Only one welder had a past history of pneumonia since first welding. There were no striking differences in the levels of inflammatory mediators in this individual relative to other welders or controls.

DISCUSSION

Welders in the present study had sufficient exposure to metal fume to result in significantly higher levels of iron and significantly lower levels of UIBC in their sputum, and were in other ways comparable to the metal-fume exposed populations in whom excesses of pneumonia have been previously demonstrated. However, despite this, few meaningful differences were detected in biological indices of host defence or inflammation in their blood and sputum relative to controls.

The present authors employed two surrogates to confirm and subclassify the extent of exposure, *i.e.* biomarkers of iron uptake and exposure history. The current authors consider the former of these two proxies to be a more reliable index, as work patterns may vary and intensity of welding may differ, as may the control measures employed. Moreover, recall and self-reporting of time-varying exposures is challenging and

TABLE 3 Comparison of inflammatory and immunological measures according to frequency and intensity of welding

	Frequency of welding			Duration of welding		
	Infrequent [#]	Frequent [#]	p-value [†]	≤10 yrs	>10 yrs	p-value [†]
Inflammatory markers in blood						
Neutrophils 10 ⁹ cells·L ⁻¹	3.7 (13)	3.1 (14)	0.64	3.2 (14)	3.5 (13)	0.70
Lymphocytes 10 ⁹ cells·L ⁻¹	1.8 (13)	1.9 (14)	0.14	1.8 (14)	1.9 (13)	0.15
Monocytes 10 ⁹ cells·L ⁻¹	0.5 (13)	0.5 (14)	0.58	0.5 (14)	0.5 (13)	0.81
Eosinophils ⁺ 10 ⁹ cells·L ⁻¹	0.2, 69% ≥0.2 (13)	0.2, 64% ≥0.2 (14)	0.02	0.2, 71% ≥0.2 (14)	0.2, 62% ≥0.2 (13)	0.05
Basophils ⁺ 10 ⁹ cells·L ⁻¹	0.2, 62% ≥0.2 (13)	0.1, 49% ≥0.2 (14)	0.14	0.2, 64% ≥0.2 (14)	0.1, 38% ≥0.2 (13)	0.09
White blood cells 10 ⁹ cells·L ⁻¹	6.3 (13)	6.0 (14)	0.43	6.0 (14)	6.2 (13)	0.94
C-reactive protein ⁺ mg·L ⁻¹	3.5, 54% >2 (13)	<2, 14% >2 (14)	0.63	<2, 21% >2 (14)	<2, 46% >2 (13)	0.24
Inflammatory markers in sputum						
Macrophages 10 ⁶ cells·g ⁻¹	0.21 (13)	0.23 (14)	0.98	0.24 (14)	0.20 (13)	0.77
Neutrophils 10 ⁶ cells·g ⁻¹	0.082 (13)	0.096 (14)	0.80	0.084 (14)	0.094 (13)	0.88
Eosinophils ⁺ 10 ⁶ cells·g ⁻¹	0, 38% >0 (13)	0, 29% >0 (14)	0.35	0, 29% >0 (14)	0, 38% >0 (13)	0.40
Epithelial cells 10 ⁶ cells·g ⁻¹	0.049 (13)	0.053 (14)	0.37	0.062 (14)	0.041 (13)	0.57
Lymphocytes 10 ⁶ cells·g ⁻¹	0.0003 (13)	0.0008 (14)	0.69	0.0003 (14)	0.0007 (13)	0.52
Total cells 10 ⁶ cells·g ⁻¹	0.42 (13)	0.46 (14)	0.78	0.48 (14)	0.40 (13)	0.97
Interleukin-8 ⁺ ng·g ⁻¹	12.7 (13)	4.5 (14)	0.96	5.3 (14)	6.0 (13)	0.56
Myeloperoxidase ⁺ ng·g ⁻¹	0, 38% >0 (13)	0, 36% >0 (14)	0.21	0, 43% >0 (14)	0, 31% >0 (13)	0.18
TNF-α pg·g ⁻¹	2.93 (12)	1.23 (13)	0.73	2.19 (13)	1.58 (12)	0.83
MMP-9 ng·g ⁻¹	295 (11)	242 (12)	0.91	303 (12)	231 (11)	0.95
α ₂ -Macroglobulin μg·mL ⁻¹	222 (11)	170 (13)	0.94	233 (13)	151 (11)	0.97
Immunological markers						
Pneumococcal IgG antibodies μ·mL ⁻¹	83 (13)	82 (14)	0.39	51 (14)	133 (13)	0.08
Neutrophil fluorescence %	67 (13)	69 (14)	0.22	69 (14)	66 (13)	0.18
Neutrophil intensity	126 (13)	139 (14)	0.14	136 (14)	129 (13)	0.21
IgA in sputum μg·mL ⁻¹	13.2 (11)	12.9 (12)	0.50	16.5 (12)	9.7 (11)	0.90
Markers of exposure and effect						
Total iron in supernatant μg·L ⁻¹	173 (13)	157 (14)	0.07	165 (14)	165 (13)	0.04
Total iron in cells μg·10 ⁻⁶ cells	1.59 (13)	1.11 (14)	0.001	1.20 (14)	1.46 (13)	<0.001
Unsaturated iron-binding capacity μg·mL ⁻¹	2.72 (8)	2.75 (5)	0.04	2.52 (5)	2.86 (8)	0.06
Cell viability proportion	0.78 (13)	0.80 (14)	0.98	0.79 (14)	0.79 (13)	0.75

Data are presented as mean (n), back transformed to original scale where appropriate, unless otherwise indicated. TNF: tumour necrosis factor; MMP: matrix metalloproteinase; Ig: immunoglobulin. #: frequent defined as welding for ≥3 days·week⁻¹ in an average working week, and for >2 h on welding days; †: adjusting for age, average weekly alcohol consumption and smoking status (in three bands; the test of trend takes, as its baseline, the values in nonwelders presented in table 2); †: data presented as median (n).

sometimes leads to inconsistencies (the present authors found an imperfect concordance between estimated extent of welding and level of iron in the lungs for several comparisons in table 3). Iron levels in the cellular residue are also more likely to represent exposures over the past few months, the time period for which there is epidemiological support for an elevated risk of pneumonia [15, 16]. However, whether welders were subclassified by biomarkers of iron uptake or by intensity or duration of welding in the present study, little evidence was found of a generalised inflammatory response or impaired immunity, even amongst those who were most exposed.

Low levels of serum UIBC have been linked with high fatality from pneumococcal pneumonia [30], but risk by levels of UIBC

in the airways remains essentially unexplored. Dose-related decrements in sputum UIBC were found in the welders. Whether or not free iron was present at a high enough level in the extracellular milieu to act directly as a growth nutrient, as previously theorised [18], is unclear.

Only a few differences were detected in other parameters. The raised level of basophils and eosinophils in blood could have arisen from an allergic response to metal fume, but the welders had no symptoms suggestive of asthma and no corresponding increase in these cellular elements was observed in the airways. Another possibility is that inhaled particulates induced epithelial damage, or activated alveolar macrophages to establish a systemic eosinophilia and basophilia through cytokine signalling [31]. The somewhat higher levels of CRP

and evidence of low grade neutrophil activation in blood would be consistent with a weak effect of this kind.

However, in the airways, there was remarkably little evidence of a corresponding activation of cells or release of cytokines. α_2 -Macroglobulin levels tended to be higher, suggesting that inhaled metal fume induces plasma exudation. However, levels of MPO, a relatively specific marker of neutrophil activation, and of MMP-9 and TNF- α , were only a little higher, and IL-8 levels in sputum only marginally lower, than in nonwelders.

These findings are at odds with the existing experimental data on lung responses to PM arising from environmental air pollution. Metal-rich PM from urban air normally induces a release of pro-inflammatory mediators (including TNF- α and IL-8) when incubated with human bronchial epithelium or alveolar macrophages [20, 21]. Similar responses have been found in the airways of healthy subjects exposed to diesel exhaust [22]. The *in vitro* response to residual oil fly ash, a model for iron-rich PM, can be abolished by incubation with the iron chelator desferrioxamine [21], and it has been hypothesised that air pollution exerts its inflammatory effect *via* oxidative stress, catalysed by metals present in PM. Certain other occupational particulates, such as zinc oxide in metal fume fever [32] and dust from coal mines [33], have also been linked with release of cytokines and influx into the airways of inflammatory cells.

In this context, the absence of an apparent effect on biological indices of host defence in welders with significant lung burdens of iron is noteworthy and, indeed, an unexpected finding. It is unlikely to have arisen from insufficient exposure, or from inadequate technique. The methods used for sputum induction, processing and measurement of fluid phase mediators have been standardised and are well established [27]. Although sputum induction samples are obtained mainly from the bronchi, while the excess risk of lobar pneumonia suggests an alveolar mechanism, there is empirical evidence that bronchoalveolar lavage and sputum induction produce qualitatively and quantitatively similar results in workers exposed to particulates [34].

Susceptible welders with recurrent episodes of pneumonia could have been selected out of the workforce, but the current authors consider this to be unlikely (pneumonia would be considered a rare occurrence in this working population). In addition, the present study sample of volunteers could have been healthier than welders as a whole. However, a "healthy worker" effect cannot readily explain the absence of an expected response to a particulate load (a healthier workforce would, if anything, mimic the general population more closely in reacting to inhaled PM). Nearly all of the welders had recent exposures (within 48 h of testing) and welding fume can cause acute irritation, but again this would tend to make the response in welders different from controls, and therefore does not account for the similarity that was found. Another possibility is that positive findings were missed by chance because of the sample size, or through a failure to measure the parameters that matter most in promoting pneumonia (a selection was made from among the more plausible candidates). However, diesel exhaust has been reported previously

to induce leukocytic infiltration of the airways and raised IL-8 levels in bronchial washings from a smaller sample of subjects than reported here [22, 35], and the current authors' failure to observe any substantial differences in cellular and cytokine response is therefore striking. It is possible that iron-containing metal fume is simply not a potent stimulant of airways, despite some suggestive contrary experimental observations [20–22], but, if so, the reason for this needs to be more fully understood.

The absence of a local response and the existence of a modest systemic reaction may arise if homeostatic mechanisms diminish the local reaction to metal fume. However, it is hard to reconcile this with the response of normal human lung tissue to air pollutants bearing much less iron [35].

A more appealing hypothesis is that excessive iron impairs the normal capacity to respond to inhaled PM and the effect is such as to augment infective risk. In keeping with this idea is some recent experimental evidence that urban air pollution impairs clearance of *Streptococcus pneumoniae* in rats through an iron-dependent mechanism that manifests as a muted capacity to recruit polymorphs and produce ILs and TNF- α [36, 37]. A muted response to inhaled particulate is also suggested by the differential response to cigarette smoke in the presented subjects. Although based upon small numbers and of borderline statistical significance, higher levels of MMP-9, IgA and α_2 -macroglobulin were observed in smokers compared with nonsmokers when analysis was confined to nonwelders, but no such differences were found for welders.

It is conceivable that the unusually high burden of transitional metal in welders' lungs could promote apoptosis *via* iron-catalysed oxidative stress, impairing the activation of neutrophils or other cellular elements in the airways [23, 24]. *Streptococcus pneumoniae* (the organism most clearly identified with infective risk from exposure to metal fume [16]) can promote apoptosis to enhance its own pathogenicity [38], and apoptosis has been induced experimentally by inorganic iron in human endothelium [39]. Such a process could render welders more vulnerable to infection, and a short-term reversible increase in risk of infection (the pattern suggested by epidemiological evidence) could last until exposure ceases, free iron is safely stored and apoptotic cells are replaced with healthy ones. The proportion of viable cells measured by exclusion of trypan blue in welders lungs did not differ significantly from that in nonwelders, which tends not to support this hypothesis. However, during apoptosis the neutrophil surface membrane remains intact and continues to exclude vital dyes. Thus, apoptotic events precede cell death, which can in any case have other causes, and since no staining or biochemical analysis was undertaken to measure apoptosis in the present study (and no *in vitro* tests of cellular stimulation in sputum), the authors cannot confidently reject the possibility.

The increased risk of pneumonia in welders is not yet explained. However, an absence of the expected local inflammatory response to particulate matter provides some encouragement to explore the role of free radical injury and apoptotic cell death in further targeted research. Another sensible step would be to measure directly markers of oxidative activity, such as malondialdehyde and lipid hydroperoxides in

welders' sputum and to test the function *in vitro* of alveolar macrophages and neutrophils from welders' lungs. Finally, if chronic exposure to ferrous fume blunts the response to particulate matter, a difference in inflammatory responsiveness should be evident between long-term welders and naive but acutely exposed subjects, and this would be worth establishing as a first priority.

ACKNOWLEDGEMENTS

The authors would like to thank C. Harris (MRC), J. Ward and the laboratory staff at Southampton General Hospital (Southampton, UK), and L. Marshall, M. Nash and S. Clarke (School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK). The authors would also like to thank J. Henderson, W. Fowler and the occupational health staff of HM Naval Base, Portsmouth, and P. McIntosh, B. Hicks, B. Irvine and K. Parry (Fleet Support Ltd, Portsmouth, UK) and the 58 volunteers from Fleet Support Ltd's staff.

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