Modulation of polymorphonuclear leukocytes function by incubation with human serum from oxidant-challenged individuals

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Polymorphonuclear leukocytes (PMN) from healthy donors were tested for stimulated release of superoxide anions after being incubated with serum of welders and of a group of unexposed individuals. These two groups were further subdivided either according to age or to smoking habits. The experiments showed that stimulated superoxide production from PMN was inhibited ($P < 0{\cdot}05$) by serum from young smokers as compared to that of young nonsmokers, both from the unexposed group. Incubation of PMN with serum from elderly nonsmoking individuals decreased superoxide production as compared to incubation with serum from young nonsmoking individuals, both from the unexposed group. A decrease in superoxide production by incubation with serum of welders as compared to that of unexposed individuals was significant only when the comparison was carried out between the young, non-smoking subgroups. These findings suggest that age, smoking, and exposure to oxidants induce appearance in serum of factors that affect the PMN function.

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1. Introduction

Polymorphonuclear leukocytes (PMN) play a fundamental role in the primary host defense against bacterial pathogens. We have previously reported (Aloufy *et al* 2001) that the stimulated release of superoxide anions by PMN from welders was significantly lower than that of a nonwelding control group. Impaired function of PMN from oxidant-exposed workers or chemicals-exposed workers has already been reported in the literature. Perlingeiro and Queiroz (1994) found reduced phagocytosis and reduced intracellular killing of *Candida albicans* in workers exposed to lead. When chemotaxis was chosen as representing PMN function, Governa *et al* (1988, 1994) found impairment of chemotaxis of PMN from lead-exposed workers and hexane-exposed workers. The age-dependent suppression of superoxide release by PMN was also well documented in the literature (Polignano *et al* 1994; Piazzola *et al* 1998; Di Lorenzo *et al* 1999).

It is possible that soluble mediators of immunity, released into the blood stream due to oxidative stress, might be responsible for altered function and properties of blood PMN. In order to further test this hypothesis, we compared the stimulated superoxide production by PMN from healthy donors after incubation with the serum from welders or from unexposed volunteers, both groups subdivided according to age and smoking habits. The rationale being that smoking, age, and exposure to oxidants have been reported to induce changes in the blood concentration of cytokines, and of other mediators of inflammation, which might affect PMN function.

Keywords. Polymorphonuclear leukocytes; smoking; superoxide release; welders

Abbreviations used: CABG, Coronary artery bypass grafting; FEV, forced expiratory volume; FVC, forced vital capacity; PMN, polymorphonuclear leukocytes.

2. Materials and methods

2.1 *Subjects*

This study has been performed in accordance with the ethical standards laid down by the 1964 Declaration of Helsinki.

Study groups:

(i) 44 full-time welders working (45 h/week) in all types of welding: i.e. electrical arc, metal inert gas (MIG) and tungsten inert gas (TIG) welding. Their ages ranged from 20 to 60 years.
(ii) The unexposed group comprised of 44 teachers, matched to the subjects of the study group, the matching criteria being age and smoking habits.

These two groups were further subdivided into smokers/nonsmokers and young/elderly subgroups.

2.2 *Superoxide anion release by stimulated PMN incubated with serum from subjects*

A sample of 10 ml venous blood was withdrawn upon fasting. The separated serum was stored at – 80°C until incubation experiments were carried out. Isolation of PMN from healthy donors was carried out from 10 ml of blood according to the method of Szücs *et al* (1994). Phorbol myristate acetate (PMA)-stimulated formation of superoxide radical was determined according to the procedure developed by Pick and Mizel (1981) in which ferricytochrome C is reduced to ferrocytochrome C in the presence of superoxide dismutase. All the chemicals used were purchased from Sigma Chemical Co., St. Louis, MO, USA. The assay was carried out with an ELISA reader device (Ceres 900 from Biotek Instruments, Inc, USA) that allows processing of a large number of samples. Each microwell of the 96 well plate contained 10 µl of a PMN suspension ($12{\cdot}5 \times 10^6$ cells/ml), 15 µl superoxide dismutase (300 U/ml) or an equal volume of Hank's balanced salt solution (HBSS), 10 µl cytochrome C (800 µM) and 15 µl PMA (10 mg/ml). In addition, 10 µl serum or an equal volume of HBSS was added and left to incubate at 37°C for 30 min. Usually PMN from donors were incubated in parallel with serum from exposed (welders) and from unexposed subjects. The analysis of data was carried out with normalized results, i.e. superoxide production by PMN incubated with serum divided by that of PMN from the same donor incubated with buffer.

2.3 *Cytokine levels in serum*

We measured TNF-α, IL-2, IL-6 and s-ICAM in serum of subjects. For this purpose, we used ELISA kits purchased from R&D Systems (Minneapolis, MN, USA).

2.4 *Lung function*

Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), their ratio FEV1/FVC, peak expiratory flow (PEF) and forced expiratory function ($FEF_{25–75}$), all as percent from normal values, were evaluated with an Autospiro spirometer (AS-500/AS-300) to which the relevant data on the examined subjects (age, sex, height) was fed.

The level of ozone in the vicinity of the welders was measured with an ultraviolet photometer (model 1008-AH, Dashibi Environmental Corp., Glendale, CA, USA).

2.5 *Statistical analysis*

Superoxide production of donor PMN after incubation with serum of welders and of that of unexposed individuals was compared using the *t*-test. Data were considered statistically significant if $P \leq 0{\cdot}05$. Comparison between subgroups (smoking habits and age) within the welder and the unexposed groups was carried out by unpaired *t*-test and by the nonparametric Wilcoxon test. Comparison between TNF-α, IL-2, IL-6 and s-ICAM in serum of subjects from all subgroups was carried out by the non-parametric Wilcoxon test.

3. Results

Ozone levels at the electric arc site were 0·25 ppm, at the TIG site ozone levels were 0·3 ppm, and at the other sites they were 0·23 ppm. These levels are above the permitted levels of 0·100 ppm.

Stimulated superoxide production of PMN from welders and from unexposed subjects divided in age and smoking habits subgroups is shown in table 1. This table reveals that if we compare the effect of serum from the welders (young and elderly, smokers and nonsmokers) to that of the total unexposed group on PMN production of superoxide, there is no significant difference between them. However, in isolated groups, oxidative stress caused by welding or smoking appears to exhibit a different pattern:

(i) Incubation of PMN with serum from young nonsmoking welders depresses the production of superoxide by PMN as compared to the incubation of cells with serum from young, nonsmoking, unexposed individuals (groups A2 and B2, $P = 0{\cdot}04$).
(ii) Incubation of PMN with serum from elderly, nonsmoking, non-welders depresses their superoxide production as compared to incubation with serum from

young, nonsmoking, nonexposed individuals (groups B4 and B2, $P = 0{\cdot}03$).

(iii) The effect of smoking is evident only in young, nonexposed individuals. Table 1 shows that serum from the smokers suppresses the PMN production of superoxide anion when compared to that of nonsmokers (groups B1 and B2, $P = 0{\cdot}02$).

The subjects from the two main study groups were also subdivided according to lung function (percent decrease from 'normal' FEV1 and FVC). Superoxide production from donor PMN after incubation with serum of subjects from the two study groups subdivided into subgroups according to lung function is shown in table 2. It is apparent that the effect of lung function of subjects on superoxide production is not significant. However, an 'almost significant' effect ($P = 0{\cdot}051$) was found only when normal and nonnormal subjects within the unexposed group were compared (subgroups D1 and D2).

TNF-*a*, IL-2, IL-6 and s-ICAM determined in serum of subjects from the various study groups were not significantly different.

4. Discussion

The present research was focused on the serum-mediated modulation of PMN function. We found a definite effect of incubation with serum from oxidant- challenged individuals on superoxide release from donor PMN, mainly when exposure to oxidants, smoking and age were considered separately. Bugajski *et al* (1999) investigated plasma-mediated modulation of PMN function in experiments with serum from patients during coronary artery bypass grafting (CABG), and reported a stimulatory effect with respect to superoxide production by PMN. Since CABG is considered a state of oxidative stress due to oxygen radicals generated by the ischemia-reperfusion process, it seems that these results are in contradiction with our findings. One should however keep in mind that our case of oxidative stress is quite different from that encountered during CABG, and, therefore, the mechanism of modulation of PMN function might be quite different.

Table 1. Stimulated superoxide (normalized results) production from PMN incubated with serum of subjects of various research groups.

	Welders (oxidant exposed)				Nonexposed			
Age (years)	< 40		> 40		< 40		> 40	
Smoking habits	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers
Superoxide from PMN after incubation with serum divided by that from incubation with buffer	$34{\cdot}5 \pm 3{\cdot}3$ (A1) $n = 8$	$38{\cdot}7 \pm 6^a$ (A2) $n = 8$	$31{\cdot}9 \pm 7{\cdot}8$ (A3) $n = 12$	$31{\cdot}2 \pm 7$ (A4) $n = 16$	36 ± 8^b (B1) $n = 6$	$46{\cdot}9 \pm 5{\cdot}8$ (B2) $n = 6$	$32 \pm 7{\cdot}3$ (B3) $n = 12$	$33{\cdot}8 \pm 7{\cdot}9^c$ (B4) $n = 13$
As above, average of all subgroups	$32{\cdot}6 \pm 7{\cdot}2$				$34{\cdot}9 \pm 8{\cdot}6$			

The results are given as average ± SD of each subgroup.
^{a,b,c}, Significantly less than the respective control group (B2).

Table 2. Stimulated superoxide (normalized results) production from PMN incubated with serum of subjects subdivided according to lung function*.

	Welders (oxidant exposed)		Nonexposed	
	Normal	Nonnormal	Normal	Nonnormal
Superoxide from PMN after incubation with serum divided by that from incubation with buffer	$32{\cdot}8 \pm 7{\cdot}04$ (C1) $n = 36$	$31{\cdot}7 \pm 8{\cdot}29$ (C2) $n = 8$	$36{\cdot}4 \pm 9{\cdot}22$ (D1) $n = 31$	$31{\cdot}3 \pm 5{\cdot}86$ (D2) $n = 10$

The results are given as average ± SD of each subgroup.
*Definition of lung diseases (nonnormal lung functions): Obstructive-FEV1: mild > 70–< 80%; medium > 60–< 70%; severe > 34–< 50%; normal > 80%.
Restrictive-FVC: mild > 70–< 80%; medium > 60–< 70%; severe > 34–< 50%; normal > 80%.

Our results indicate that mediators released into the blood stream might cause the lowering of superoxide production by PMN when incubated with serum from oxidant-challenged individuals. Nonetheless, we could not demonstrate that either TNF or IL-2, IL-6 or s-ICAM are the mediators responsible for this effect. Further, research is needed to establish the factors responsible for this PMN altered function.

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