

A Possible Role for Superoxide Production in the Pathogenesis of Contact Dermatitis

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Superoxide generation by blood monocytes was examined in patients with irritant, nickel, and chromate hand dermatitis. Phorbol myristate acetate stimulated monocytes generated significantly more superoxide in patients with nickel dermatitis, as did patients with hand eczema generally. No significant stimulation of monocyte superoxide generation occurred with either opsonized zymosan or PMA in the presence of excess superoxide dismutase in any of the groups of hand dermatitis. The results indicate a biochemical stimulation of superoxide which may accentuate the immunological damage in the skin that is observed in nickel and perhaps chromate dermatitis.

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Oxygen-derived free radicals (ODFR) are chemical species which owe their reactivity to the possession of an unpaired electron in their outer orbit. Free radicals are known to play an important role in normal cellular metabolism (1), but there is increasing evidence for their involvement in tissue damage (2). Phagocytes such as neutrophils, tissue macrophages and blood monocytes (3–5) possess the capacity to produce large quantities of ODFR and metabolites via the initial production of the superoxide anion. It is suggested that these ODFR may be important

initiators of both acute and chronic inflammatory reactions (6, 7).

Miyachi et al. (8) examined the potential role of ODFR in chronic cement dermatitis. They found that stimulation of polymorphonuclear leukocytes in patients suffering from chronic cement dermatitis generated markedly increased levels of superoxide anion, while there was only a slight increase in cells from cement workers without dermatitis. They concluded that although cement dermatitis is initiated as a contact sensitivity to chromate, it is possible that the inflammatory process is exacerbated by tissue damage from ODFR. In chronic eczema, it is known that there is an increased production of monocytes in the patients' bone marrow, possibly as a result of monocyte recruitment at the site of inflammation (9). Therefore, in the present study we have examined, in patients with both allergic contact and irritant contact dermatitis, oxygen-derived free radical generation by monocytes, which are cells of primary importance in both the induction and mediation of the tissue response in allergic contact dermatitis (10).

PATIENTS AND METHODS

Patients

Seven patients (3 males, 4 females, age range 16–45 years) with irritant contact dermatitis on the hands and who were patch test negative to the ICDRG standard series of contact allergens (11) were studied. All had dermatitis on the backs and sides of fingers. These patients had no history of atopy or other significant medical illness.

Table I. Superoxide production rates by peripheral blood monocytes in patients with contact dermatitis.

	Controls	Irritant contact dermatitis	Nickel dermatitis	Chromate dermatitis	All patient groups
Unstimulated monocytes	10.85 ± 3.11 (n = 12)	10.10 ± 3.13 (n = 7)	10.10 ± 2.52 (n = 6)	11.90 ± 3.45 (n = 9)	10.55 ± 3.13 (n = 22)
STZ stimulated	18.61 ± 7.811 (n = 14)	20.01 ± 5.72 (n = 7)	20.40 ± 5.55 (n = 3)	19.30 ± 3.90 (n = 9)	19.50 ± 3.70 (n = 19)
PMA stimulated	29.42 ± 9.06 (n = 14)	31.20 ± 8.70 (n = 6)	35.80 ± 10.11* (n = 5)	32.35 ± 5.46 (n = 8)	31.80 ± 7.79** (n = 19)
PMA stimulated in presence of SOD	12.85 ± 4.52 (n = 14)	11.90 ± 4.01 (n = 7)	11.10 ± 3.73 (n = 5)	13.25 ± 4.03 (n = 8)	12.97 ± 2.72 (n = 20)

Results expressed as mol/min/monocyte × 10⁻⁹ and given as median ± SD.

n = number of estimations from which result was derived.

*p = 0.035, **p = 0.02.

Nine patients (8 males, 1 female, age range 24–54 years) with active cement dermatitis, patch test positive only to chromate in the ICDRG standard series of allergens, were studied. These patients had no history of atopy and had no other significant medical illness.

Seven patients (all females, age range 15–28 years) with nickel hand eczema and who were patch test positive only to nickel in the ICDRG standard series of allergens were studied. These patients had no history of atopy or other significant medical illness.

Fourteen subjects (6 males, 8 females, age range 25–58 years) with no history of dermatitis or other significant skin or medical illness were included as normal controls. These included healthy volunteer medical and technical staff, as well as patients attending the dermatological clinic with non-inflammatory and non-malignant skin conditions (e.g. benign).

Methods

Twenty ml of blood was drawn from each patient or control subjects at 9 a.m. and placed in EDTA-containing tubes which were quickly taken to the laboratory for processing.

Cell separation

Mononuclear cells were separated on a Ficoll-Hypaque gradient (1.077 g/ml) from freshly-drawn venous blood as previously described (12,13) and resuspended in Hanks' balanced salt solution (HBSS) to give a final monocyte concentrations of 0.3–1.3 × 10⁶/ml. Cells were counted with a Coulter counter (14) and by non-specific esterase staining (15).

Stimulus preparation

Zymosan was opsonized with fresh human serum (STZ) washed in HBSS and stored frozen in aliquots. Phorbol myristate acetate (PMA; 12-O-tetradecanoul-phorbol-13-acetate) was prepared in dimethylsulfoxide (DMSO; 2 mg/ml), stored at -10°C and diluted in HBSS prior to use. Reaction concentrations were 25 µg/ml (STZ) and 1 µg/ml (PMA).

Monocyte superoxide generation

This was assayed by reduction of cytochrome *c* as previously described (12, 13). Briefly, 500 µl reaction mixtures containing separated mononuclear cells, 100 µM cytochrome *c* (Type III, Sigma Chemicals, Poole, England) and stimulus were prepared in 10 mm round bottomed tubes. After incubation at 37°C the reaction was terminated. Following centrifugation at 0°C, cytochrome *c* reduction in the supernatant was measured as absorbance shift at 550 nm. For each stimulus used, a rate constant 'K' for superoxide generation over 15 min was calculated according to the formula:

$$K = 1/t (t \times [\text{mo}]) \times \ln ([\text{cyt } c_0]/[\text{cyt } c_t]) \text{ ml/min/monocyte,}$$

where *t* = time, [mo] = monocyte concentration (Coulter count positive cells), and [cyt *c*₀] and [cyt *c*_t] are the concentrations of oxidized cytochrome initially and after *t* minutes of incubation. Control tubes containing excess bovine erythrocyte superoxide dismutase (SOD) were included in all experiments.

Statistics

Monocyte superoxide generation rates in patients and control subjects were compared using the Mann-Whitney U-test for non-parametric data. *P*-values less than 0.05 were taken as statistically significant.

RESULTS

The results, expressed as medians ± standard deviation (SD), are given in Table I.

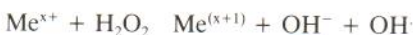
Superoxide generation by monocytes from each form of dermatitis was compared with the controls, and superoxide generation from all dermatitis patients was compared with the controls. Superoxide generation in unstimulated cells (UTZ) and opsonized zymosan stimulated cells (STZ) did not differ significantly from that of controls for each of the

patient groups as well as all patients suffering from dermatitis. Phorbol myristate acetate (PMA) stimulated monocytes generated significantly more oxygen-derived free radicals in patients with nickel dermatitis ($p = 0.035$) and all of the patients with dermatitis ($p = 0.02$), but not in patients with irritant contact dermatitis ($p = 0.137$) or chrome dermatitis ($p = 0.062$) when compared with normal controls. Stimulation of superoxide generation with PMA in the presence of excess superoxide dismutase (SOD) revealed no significant differences between any of the patient groups.

DISCUSSION

In the present study, PMA-stimulated monocytes from patients with nickel dermatitis produced significantly more superoxide than did controls. Superoxide formation in PMA-stimulated monocytes from patients with chromate dermatitis was greater than controls. Although this trend did not reach significance, it may be that a larger study would show a lesser effect than with nickel dermatitis. The present study made no attempt to correlate the severity of dermatitis with monocyte superoxide production but it is possible that the greatest production occurs in patients with the most severe or extensive dermatitis.

These changes following PMA stimulation suggest a biochemical rather than membrane receptor basis for enhanced ODFR production, as no changes were observed following STZ stimulation which is a complement receptor dependent secretagogue. These data suggest the possibility of enhanced superoxide generation in nickel, and perhaps chromate dermatitis. It may be that in nickel, and perhaps chromate, hand dermatitis, initial immunological damage to the skin is greatly accentuated by the generation of ODFR by phagocytes at the site of tissue inflammation. The tissue damaging effects of free radicals are often related to derivatives such as hydroxyl radical rather than the superoxide anion itself (16, 17). Certain transitional metals may be involved in promoting the formation of hydroxyl radical from superoxide anion by acting as electron donors:



where Me^{x+} = metal ion.

It is interesting that in patients with irritant con-

tact dermatitis, where transitional metals are not important, there was no increase in free radical formation, although nickel and chromate are unlikely to act as electron donors.

Superoxide formed *in vitro*, either as a result of normal metabolic processes or during inflammatory tissue injury, is degraded by the specific enzyme, superoxide dismutase (18) by catalysing a reaction that removes superoxide radical, generating hydrogen peroxide and oxygen. In their study, Miyachi et al. (8) found no increase in the superoxide dismutase activity in the skin of patients with severe contact dermatitis, compared with healthy cement workers, although the levels in these workers were significantly above those of normal controls. However, it may be possible to limit some of the damage caused by ODFR formation in the skin of such patients by the use of systemic anti-oxidants such as vitamin E (19) or by agents capable of reducing the capacity to generate ODFR in phagocytic cells, such as dapsone which may produce clinical improvement in cement dermatitis (8). The use of effective anti-oxidants and inhibitors of phagocyte superoxide generation in the treatment of nickel and chromate dermatitis, both of which have an intractable course (20, 21) warrants further investigation.

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