The Molecular Basis of Oxidative Damage by Leukocytes

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CORRELATIONS AND DISSOCIATIONS BETWEEN SUPEROXIDE PRODUCTION AND ADHESION FUNCTION OF HUMAN NEUTROPHILS

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INTRODUCTION

The investigation of the mechanisms that regulate neutrophil functions may take advantage of methods that simultaneously evaluate multiple responses to various cell agonists and antagonists. In this report we describe studies carried out by using a microplate method that enables the measurement of the oxidative metabolism (as superoxide anion production) and of the adhesion of neutrophils in the same assay system.[1] Neutrophils were incubated in foetal calf serum-coated microwells for 10 min in the absence or in the presence of 60.3 monoclonal antibodies (anti-CD18 integrin adhesion protein) and of α-methyl mannopyranoside (α-MM) (competitive inhibitor of binding sites for Concanavalin A), then they were challenged with soluble and particulate stimulants in the presence of cytochrome c. After 30 min incubation, the superoxide produced was detected by reading cytochrome c reduction (absorbance at 550/540 nm), then the plates were washed and the adherent cells were quantitated by measuring the activity of acid phosphatase. By comparing the superoxide production with the adhesion response of the same cells in several different experimental conditions it was possible to get new insights on the activation mechanism(s) of neutrophils.

RESULTS

Human neutrophils, incubated in the absence of stimulants, did neither produce superoxide, nor adhere to the bottom of the wells. Three different stimulants, serum treated zymosan (STZ), concanavalin A (Con A) and n-formyl-leucyl-phenylalanine (fMLP) were tested in this system and they induced both activation of oxidative metabolism and adhesion, but with variable dose-dependence curves: With fMLP and STZ as stimulants there was a similar dose-dependence response for O_2^- and for adhesion, while with Con A the adhesion was activated at doses (2-5 μg/ml) much lower than those required for superoxide production (50-100 μg/ml) (data not shown).

To better understand the relationships between the adhesion and activation of the respiratory burst we pre-treated the cells with agents that are expected to interfere with these responses at different levels of their activation. Figure 1 shows that anti-CD18 monoclonal antibodies inhibited adhesion induced by fMLP and by STZ, while did not inhibit the adhesion stimulated by 5 μg/ml Con A, a dose that did not stimulate the respiratory burst.

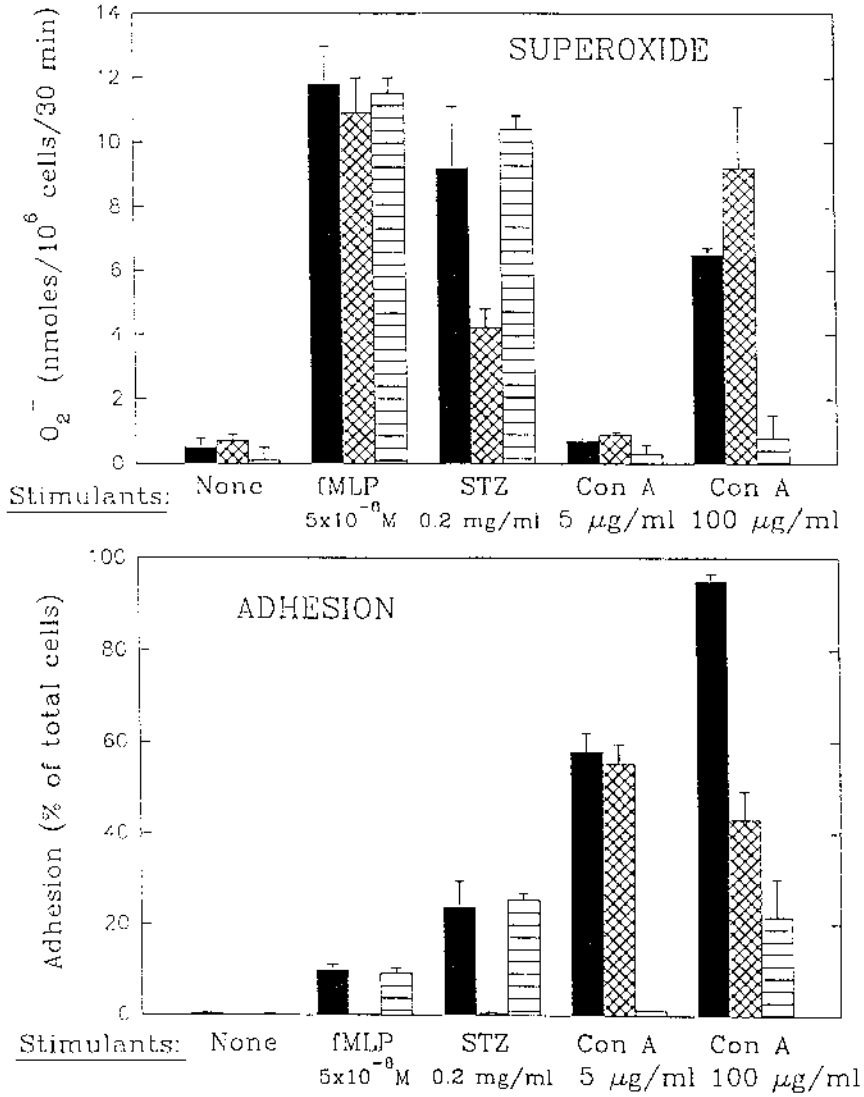

Figure 1. Superoxide production by human neutrophils and concomitant adhesion to serum-coated plastic surfaces in various experimental conditions. In each microplate well, 2 x 10^6 neutrophils were incubated in 100 µl of HBSS containing 5 mM glucose, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.2% human albumin and 0.15 mM cytochrome c, in the absence (solid black bars) or in the presence of 20 µg/ml 60.3 monoclonal antibodies (diagonal crosshatch bars) or of 10 mM αMM (horizontal line bars), at 37 °C for 10 min, then they were activated by addition of the stimulants indicated in the figure. Incubation was carried out for 30 minutes. Values are mean ± S.D. of triplicate assays. The results of a typical (and reproduced) experiment are reported.

With 100 μg/ml Con A, a dose that stimulated superoxide production, adhesion became partially sensitive to inhibition by anti-CD18 antibodies, demonstrating that in these conditions the adhesion was partially integrin mediated. Under the same conditions, the antibodies had no effect on the fMLP- and Con A (low-dose)-induced O_2^- release and affected only partially the O_2^- release induced by STZ. The latter finding is due to the fact that opsonized zymosan interacts with the neutrophil membrane both by integrin-type (IC3b/Mac 1) and non-integrin dependent (glucan, mannan, Fc) mechanisms. The antibodies caused partial stimulation of the Con-A induced O_2^- release, possibly because of a synergistic effect with the lectin, that probably binds also to integrin adhesion molecules.[2] With αMM, the responses to fMLP and STZ were unaffected, while both adhesion and O_2^- release induced by Con A were inhibited, as expected. The residual adhesion observed with 100 μg/ml Con A is probably due to an incomplete competition with the high dose of lectin.

CONCLUSIONS

These results indicate that the superoxide production is not directly linked to adhesion on serum-coated surfaces. In fact, in the presence of low doses of Con A, the neutrophils strongly bind to the surface of the culture wells, but this adhesion is due to (non-integrin type) anchoring systems (possibly the same Con A receptors) that are not associated with activation of the respiratory burst. Higher doses are able of triggering intracellular modifications (possibly through capping of Con A receptors or of CD18 integrins) that lead to activation of NADPH oxidase. On the other hand, the experimental conditions that are able of activating the respiratory burst also concomitantly activate integrin-dependent adhesion, but the two phenomena are not quantitatively proportional.

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REFERENCES

1. Bellavite, P., Chirumbolo, S., Signorini, A., Bianchi, I. and Dri, P., Simultaneous measurement of oxidative metabolism and adhesion of human neutrophils and evaluation of multiple doses of agonists and inhibitors, in Proc. I Int. Congr. on Ultra Low Doses, Doutremepuich, C., Ed., Taylor & Francis Ltd, Basingstoke, U.K., in press.

2. Schmalsteig, F.C., Rudloff, H.E. and Anderson, D.C., Binding of the adhesive protein complex (LFA-1/Mac-1/p150,95) to Concanavalin A, J. Leukoc. Biol., 39,193, 1986.