Positive correlation between superoxide release and intracellular adenosine deaminase activity during macrophage membrane perturbation regardless of nature or magnitude of stimulus

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Letter to the Editor

Summary

Tuftsin stimulates macrophages to release superoxide in direct proportion to intracellular adenosine deaminase activity over a concentration range of 125 to 625 nM tuftsin. This relation is comparable to that previously observed for stimulation by single concentration of several agents. This finding led to the conclusion that the relation between superoxide and adenosine deaminase is independent of the nature or magnitude of the stimulus. In absolute terms, tuftsin increases superoxide secretion up to 375 nM tuftsin; further increases in tuftsin concentration cause a rapid decrease in superoxide secretion to near base line at 500 nM tuftsin. In contrast, the phagocytic response to tuftsin remains maximal up to 10 μM with no indication of inhibition at higher concentrations. Thus, tuftsin stimulation of phagocytosis and superoxide release may be at least partially independent phenomena.

Introduction

We have recently reported that superoxide (O$_2^-$) release during membrane perturbation of macrophages was proportional to intracellular adenosine deaminase (ADA) activity (1, 2). From these studies we concluded that the basis for this correlation was that ADA generates the required metabolic flux through the purine salvage pathway enzyme xanthine oxidase, which produces O$_2^-$ during metabolism of hypoxanthine and xanthine to uric acid. A marked increase in xanthine oxidase activity of polymorphonuclear leucocytes obtained from infected animals as compared with uninfected controls has been reported (3). Because of the broad influence of macrophages on the regulation of immune function (4), we proposed that this accounts for the need of adequate ADA activity for normal immune function, and forms at least a part of the basis of the association of ADA deficiency with immunodeficiency (5).

In this paper we extend these observations with data that indicate that this association of O$_2^-$ with ADA holds over a wide range of concentration of a single membrane perturbant, and that the data fall on a plot very similar to the correlation previously published (2) of these two variables when the macrophages were stimulated with arbitrarily selected concentrations of several agents. The phagocytosis stimulating peptide tuftsin (L-Threonyl-L-Lysyl-L-Prolyl-L-Arginine) was chosen as the stimulant for this study because of its high potency and natural occurrence within the Fc portion of the parent cytophilic gamma globulin leukokinin (6).

Materials and methods

Thioglycollate-elicited peritoneal exudate cells (PEC) were lavaged from ICR Swiss mice of the Roswell Park colony 5 days after i.p. injection of 3 ml brewer's Thioglycollate broth (Becton, Dick-
inson & Co., Cockeysville, MD) as previously described (1, 2). Five tubes were prepared, each of which contained sufficient balanced salt solution (2) to make a final volume of 5.0 ml, 0.125 ml of the lavaged PEC (4 × 10^6 PEC/ml), i.e., 10^5 PEC/ml during the incubation, 0.5 ml of 1.2 mM cytochrome c (Type II from horse heart, Sigma Chemical Co., St. Louis, MO). The reaction was started by the addition of the following amounts of a 2.5 × 10^-6 M tuftsin (Sigma; used without further purification) solution: 0.25, 0.50, 0.75, 1.0, 1.25 ml. The final tuftsin concentrations during the incubations were 125, 250, 375, 500 and 625 nM, respectively, in each of the tubes. Samples of 1.0 ml were removed after 0, 1, 2, 3 and 6 min. at 37 °C, immediately centrifuged at 8 000 g in an Eppendorf ultracentrifuge, and supernatant and residue chilled at 0 °C. The absorbance of the supernatants was determined at 550 nm with the zero time supernatant in the reference beam, and O_2^- produced calculated from the concentration of cytochrome c reduced by use of the equation ΔE = 2.1 × 10^4 M^-1 cm^-1. The sedimented cells were lysed in 0.5% Triton X-100 and used for assay of intracellular ADA activity as described previously (2).

Results and discussion

The results are shown in Fig. 1 in terms of O_2^- production with time, and, in Fig. 2, in terms of O_2^- produced per mole adenosine deaminated. It is evident from Fig. 1 that stimulation by tuftsin is rapid with maximum effects apparent between 1 and 2 min under our experimental conditions. Half-maximum stimulation was observed at about 200 nM tuftsin. The response is sigmoid and proportional to dose up to about 400 nM and then decreases very rapidly to base line upon further increase in tuftsin concentration.

Linear regression analysis of a double reciprocal plot of the top of Fig. 1 (data not shown) was not possible since the double reciprocal data for 150, 250 and 375 nM tuftsin were not linear and clearly suggested positive cooperativity which is consistent with the sigmoid shape of the dose response curve. Thus, no data can be readily calculated for maximum possible stimulation at infinitely high tuftsin concentration or the tuftsin concentration responsible for the half-maximal effect.

The dose–response relation of tuftsin has been reviewed by Najjar (7). Response was expressed in terms of number of cells which contained phagocytized particles. Cells from several species, attached to cover slips or in suspension at cell densities up to 13 × 10^6 per ml, and phagocytizing a variety of particles all showed half-maximal stimulation at about 50 ng per ml, i.e., 100 nM tuftsin, with the maximum response maintained to about 10 μM with no indication of inhibition at higher concentrations. Thus, our observation of decreased O_2^- release at elevated tuftsin concentration is not synonymous with decreased stimulation of phagocytosis.

Of major interest is the similarity of the slope of the best straight line fitted to our present data and the line fitted through data obtained a year ago with different chemotactic and phagocytic stimuli. This similarity is the basis for our suggestions that the relation between O_2^- and ADA activity is independ-