CELLULAR AND EXTRACELLULAR PHOSPHOLIPASE A₂ ACTIVITY

IN ZYMOSAN PLEURISY IN RAT

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SUMMARY

The pleural exudate from rats treated intrapleurally with zymosan contains phospholipase A₂ (PLA₂) activity which is Ca²⁺-independent and optimally active at a neutral pH. This PLA₂ activity was found in approximately equal amounts in both the cellular and extracellular fractions of the exudate. The Ca²⁺-independency of the PLA₂'s in the pleural exudate distinguishes them from plasma PLA₂’s and this suggests that the source of the exudate PLA₂'s is not plasma. The appearance of PLA₂ activity in zymosan-induced pleural exudate correlates temporally with increases in exudate volume and pleural cell number. In all cases, the maximum response was seen 24 hr after zymosan challenge. All parameters of pleurisy and PLA₂ activity are similarly sensitive to the steroid dexamethasone which has been hypothesized to act, in part, through the synthesis of PLA₂ inhibitory peptides. In its entirety, this information suggests that there is a relationship between pleural PLA₂ activity and the appearance of pleural inflammation (exudate volume and cells) and that PLA₂ may play an important role in the initiation and propagation of this inflammatory process in rats. Furthermore, the zymosan-induced pleurisy model may serve as a useful model for the identification of PLA₂ inhibitors with antiinflammatory activity.

INTRODUCTION

Cellular and extracellular phospholipase A₂'s (PLA₂'s) are enzymes associated with inflammatory processes in experimental animals and in man (1). Zymosan-induced pleurisy in rodents is

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characterized by the accumulation of a pleural exudate fluid containing inflammatory cells (2-4) and has been used recently as an in vivo model for the evaluation of potential antiinflammatory agents. We have studied the characteristics of the PLA2 activities in zymosan-induced, pleural exudate fluid in rat to evaluate the relationship of the activities of these enzymes to the development of this experimentally-induced inflammatory reaction.

MATERIALS AND METHODS

Zymosan-Induced Pleurisy

Zymosan (Sigma Chemical, Co., St. Louis, MO) in saline (10 mg/ml) was heated for 1/2 hr in a boiling water bath, washed three times and then resuspended in saline (10 mg/ml). This preparation of boiled zymosan did not contain detectable levels of PLA2 activity. Male Lewis rats (260-300g) were given intrapleural injections of the 10 mg/ml zymosan suspension (0.25-2.0 mg zymosan/rat) to induce the pleurisy response. At the indicated times, the pleural cavity was exposed and heparin (= 20 units/ml) was added to the exudate fluid in the pleural cavity to prevent coagulation. The exudate fluid was collected by aspiration and the volume was measured. An aliquot (10 μl) was removed for quantitating total cell accumulation using a Coulter Counter. The exudate fluid was then centrifuged at 10,000 x g for 30 sec. The supernatant (extracellular fraction) was aspirated and the pellet (cellular fraction) was resuspended in a volume of saline equal to the original exudate fluid volume. All samples were stored frozen and thawed immediately before sonication and assay for PLA2 activity. The cellular fraction, when thawed, was sonicated (Ultrasonic Cell Disrupter, Heat Systems - Ultrasonics, Inc.) 4 times for 5 sec. intervals prior to use.

PLA2 Assay

The activity of PLA2 was quantitated by the method of Vadas et al. (5). The standard reaction mixture (0.5 ml) contained 6.6 mg/ml fatty acid-free bovine serum albumin (Sigma Chemical Co. St. Louis, MO), 5.0 mM CaCl2, 50 mM HEPES buffer (pH 7.4), 125 mM NaCl and [14C]oleate-labeled E. coli (heat-killed, intact) as substrate (2 x 10^8 cells/ml which contained 0.48 μCi radioactivity/μmol phospholipid and 18.2 nmol total phospholipids/ml) (Dr. R. Franson, Medical College of Virginia, Richmond, VA). The reaction was terminated after 30 min. at 37° C by the addition of 0.5 ml 50 mM EDTA. The reaction mixture/EDTA was then filtered through a 0.45 μm Millex-HV filter (Millipore Corp.) to separate the released oleic acid from E. coli. The quantity of [14C]oleic acid in the filtrate was determined by liquid scintillation spectrometry (Beckmann LS 7800 spectrometer). The efficiency of recovery of [14C]oleic acid through the filtration step was 78% ± 4% and 1000 dpm was equivalent to 2.4 nmoles oleate hydrolyzed/hr (corrected for recovery). Heparin at 20