HYPERPHOSPHOLIPASEMIA A₂ IN HUMAN VOLUNTEERS CHALLENGED WITH INTRAVENOUS ENDOTOXIN

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Abstract—Phospholipase A₂ (PLA₂) activity was measured in the serum of 23 individuals infused intravenously with endotoxin (EN) at a dose of 4 ng/kg body weight. A marked increase in PLA₂ was noted 3 h after EN challenge (mean 828 ± 513 units/ml), reached its maximum at 24 h after the challenge (mean 2667 ± 2442 units/ml), and was still evident at 48 h (mean 763 ± 366 units/ml). In contrast, TNF levels were maximal (mean 712 ± 375 pg/ml) 90 min after the EN challenge and subsided to very low values (5 ± 5 pg/ml) 5 h after the challenge. There was a positive correlation between the maximum response of TNF and that of PLA₂ (r = 0.82, P < 0.01). Administration of ibuprofen or pentoxifylline did not alter the PLA₂ response. EN challenge did not affect serum pancreatic PLA₂ concentration or that of the lysosomal cationic enzyme, lysozyme. Neutralizing antibody against human recombinant (synovial type) PLA₂ completely abolished PLA₂ activity in the sera tested. We conclude that EN infusions cause marked intravascular release of non-pancreatic secretory PLA₂ and that the magnitude of this response seems to be related to the prior generation of TNF.
INTRODUCTION

Endotoxemia associated with gram-negative bacteremia or induced by experimental intravenous administration of endotoxin elicits a rapid host response manifesting in the synthesis and release of cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8) (1-10). In rabbits, intravenous infusions of endotoxin also lead to a marked increase in circulating phospholipase A2 (PLA2), (11, 12). Furthermore, infusions of PLA2 in healthy rabbits reproduce hemodynamic features of endotoxemia, whereas inhibition of PLA2 attenuates the above manifestations (11, 12). IL-1 and TNF serve in vitro as signals for the synthesis and extracellular release of PLA2 (13). High levels of circulating PLA2 were found in gram-negative septic shock in man (12, 14). Thus, it was of substantial interest to investigate whether human volunteers infused intravenously with endotoxin develop hyperphospholipasemia A2 and whether a relationship exists between PLA2 activity and TNF response.

MATERIALS AND METHODS

Twenty-three healthy volunteers received an intravenous bolus of endotoxin (EN) at a dose of 4 ng/kg. The specific activity of EN obtained from Escherichia coli, Lot EC-5 (U.S. Reference-Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland) was 5 units/ng. Serum samples were submitted from two institutions and the detailed protocols, including the method for TNF determination, have been published elsewhere (2, 3). Twelve test subjects and 14 controls received intravenous infusion of 0.9% saline at a rate of 0.7 ml/kg/h starting the evening before the study. Eleven test subjects received infusion at the start of experiment. Thirteen persons received EN alone, six received EN and ibuprofen (Motrin, Upjohn, Kalamazoo, Michigan) orally, 800 mg at 90 min before EN, 800 mg at the time of EN infusion, and 800 mg 3 h after endotoxin administration. Four persons received EN and pentoxifylline (Hoechst-Roussel, Somerville, New Jersey) orally, 400 mg every 8 h x 5 doses prior to EN. These three groups were analyzed separately. Since the activity profiles of PLA2 were similar and not statistically different, they were pooled and analyzed together.

Phospholipase A2 activity was estimated as described in detail (15). Briefly, autoclaved E. coli, strain K12 C600, labeled with 14C]oleic acid served as the substrate. Assays were performed in substrate excess using 5.6 nmol of E. coli membrane phospholipid per assay with a specific activity of 4120 cpm/nmol. Reactions were allowed to proceed for 30 min at 37°C. Enzyme activities were corrected for nonenzymatic hydrolysis. The rate of substrate hydrolysis was linear with reaction times of up to 30 min over a fivefold range of enzyme concentration. The effects of calcium, EDTA, and pH on the activity of serum PLA2 were tested as described previously (15). Pancreatic phospholipase A2 was estimated by radioimmunoassay (16). The effect of anti-PLA2 antibody on serum PLA2 was tested (16) using polyclonal neutralizing antibody to human recombinant synovial-type PLA2 diluted 1:500 with 0.1 M Tris HCl, pH 7.5. Antiserum was incubated with the test serum for 60 min at room temperature. Preincubation of endotoxin with PLA2 in vitro did not alter PLA2 enzymatic activity (data not shown). Lysozyme (muramidase) activity was tested as described (17, 18). Normal serum lysozyme concentration varies from 5 to 15 μg/ml (17, 18).