IN VITRO EXPRESSION AND INHIBITION OF PROCOAGULANT ACTIVITY PRODUCED BY BOVINE ALVEOLAR MACROPHAGES AND PERIPHERAL BLOOD CELLS

J. RASHID, D.J. WEISS*, S.K. MAHESWARAN AND M.P. MURTAUGH
Department of Veterinary Pathobiology, University of Minnesota, St Paul, MN 55108, USA
*Correspondence

ABSTRACT


Local and systemic activation of coagulation is frequently associated with bacterial sepsis. The coagulopathy is due, at least in part, to expression of tissue factor (TF) by monocytes and macrophages. The purpose of this study was to evaluate the expression of procoagulant activity by bovine alveolar macrophages, leukocytes and platelets, and to determine the relative potency of three chemical inhibitors of TF expression (pentoxifylline, retinoic acid, and cyclosporin A). Bovine alveolar macrophages were stimulated with lipopolysaccharide (LPS) derived from Pasteurella haemolytica or recombinant bovine tumour nervous factor (TNF) and dose- and time-dependent effects on TF expression were studied. LPS and TNF induced TF expression in alveolar macrophages and LPS treatment of whole blood induced TF expression in mononuclear cells. Neutrophils and platelets also expressed procoagulant activity, but this activity was not inhibited by anti-bovine TF monoclonal antibody. Pentoxifylline (40 µmol/L), retinoic acid (0.01 mmol/L) and cyclosporin A (0.08 µmol/L) inhibited TF expression when added concurrently with LPS or TNF, but not when added 4 h after stimulation. TF mRNA was not detected in unstimulated alveolar macrophages by Northern blot analysis. In contrast, exposure to LPS or TNF for 6 h induced marked expression of TF mRNA, which was inhibited by treatment with pentoxifylline, retinoic acid and cyclosporin A. Expression of TNF by alveolar macrophages stimulated with LPS was also inhibited by these compounds. Our results indicate that procoagulant activity expressed by alveolar macrophages and monocytes is associated with expression of TF, whereas procoagulant activity expressed by neutrophils and platelets is not. The concentrations of pentoxifylline and retinoic acid necessary for inhibition of TF expression in vitro may not be achievable in vivo owing to their toxic effects. However, the in vitro concentration of cyclosporin A that inhibited TF expression did not exceed the plasma concentration observed in humans, and therefore may be useful for inhibition of TF expression in vivo.

Keywords: cattle, cyclosporin A, macrophages, pasteurellosis, pentoxifylline, pneumonia, procoagulant activity, retinoic acid, tissue factor

Abbreviations: BAL, bronchoalveolar lavage; LPS, lipopolysaccharide; cDNA, cloned deoxyribonucleic acid; cAMP, cyclic adenosine monophosphate; GAPDH, glyceraldehyde phosphate dehydrogenase; mRNA, messenger ribonucleic acid; TF, tissue factor; TNF, tumour necrosis factor; DPBS, Dulbecco's phosphate-buffered saline
INTRODUCTION

Tissue factor (TF), also known as tissue thromboplastin, is the primary cell-associated initiator of the coagulation cascade (Nemerson, 1992). TF is a member of the cytokine/haematopoietic growth factor receptor family and is constitutively expressed by extravascular cells, such as fibroblasts and smooth-muscle cells (Gregory et al., 1989). Binding of coagulation factor VII/VIIa to TF initiates both the extrinsic and intrinsic coagulation pathways by activating factors IX and X. Increasing evidence suggests that TF triggers local and systemic coagulation associated with inflammation and sepsis (Warr et al., 1990; Levi et al., 1993).

Tissue factor is expressed in the lungs of calves with experimental pneumonic pasteurellosis and may mediate intra-alveolar fibrin deposition. Expression of TF by alveolar and pulmonary intravascular macrophages was observed by immunohistochemical techniques in the lungs of calves infected with Pasteurella haemolytica (Rashid, 1996). Administration of anti-bovine TF monoclonal antibody to calves before experimental infection with P. haemolytica prevented deposition of fibrin within alveoli and increased TF-dependent procoagulant activity in blood and bronchoalveolar lavage fluid (Rashid, 1996). These findings suggest that deposition of pulmonary fibrin and systemic activation of coagulation in bovine pneumonic pasteurellosis is mediated by TF expression by lung macrophages and monocytes.

Several agents have been reported to attenuate the coagulopathy in animal models of Gram-negative sepsis (Ishii et al., 1992; Faulds et al., 1993; Olivers et al., 1993). Pentoxifylline, a cAMP phosphodiesterase inhibitor, prevented expression of TF on monocytes in vitro and in vivo and improved survival in a rodent septic shock model (Olivers et al., 1993). Retinoic acid upregulates thrombomodulin and downregulates TF expression, and has been used to prevent disseminated intravascular coagulation in leukaemia patients (Ishii et al., 1992). Cyclosporin A, a fungal cyclic oligopeptide, inhibited TF expression by monocytes and macrophages in vitro (Faulds et al., 1993).

In the present study, we evaluated the expression of procoagulant activity by alveolar macrophages, monocytes, neutrophils and platelets, and the relative potency of putative inhibitors of TF expression in vitro.

MATERIALS AND METHODS

Isolation of alveolar macrophages

Bovine alveolar macrophages were collected by bronchoalveolar lavage (BAL) from 3–6-week-old healthy male Holstein calves as described previously (Weiss et al., 1991). Cell culture experiments were performed under endotoxin-free conditions. Alveolar macrophages (2 × 10⁶) were placed in 24-well tissue culture plates (Costar, Cambridge, MA, USA) containing Dulbecco's modified Eagle's medium supplemented with 2% fetal bovine serum, 1 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, 14 mmol/L Hepes buffer, 2.2 g/L sodium bicarbonate, 100 U/ml penicillin and 0.1 mg/ml streptomycin. After a 2 h incubation at 37°C, 5% CO₂, the non-adherent cells were