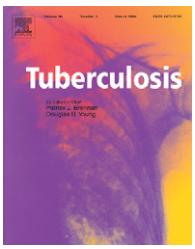
Tuberculosis (2008) **88**, 178–186

Available at www.sciencedirect.com

ScienceDirect

journal homepage: http://intl.elsevierhealth.com/journals/tube



Peripheral cell wall lipids of *Mycobacterium tuberculosis* are inhibitory to surfactant function

Zhengdong Wang[a], Ute Schwab[c], Elizabeth Rhoades[c], Patricia R. Chess[a], David G. Russell[c], Robert H. Notter[a,b,*]

[a]*Department of Pediatrics, University of Rochester School of Medicine, Box 850, 601 Elmwood Avenue, Rochester, NY 14642, USA*
[b]*Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY 14642, USA*
[c]*Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

Received 14 June 2007; received in revised form 7 November 2007; accepted 12 November 2007

KEYWORDS
Mycobacterium tuberculosis;
Trehalose dimycolate;
Lung surfactant;
Surfactant inhibition;
Surfactant dysfunction;
ALI/ARDS

Summary
The transmission of *Mycobacterium tuberculosis* (TB) requires extensive damage to the lungs to facilitate bacterial release into the airways, and it is therefore likely that the microorganism has evolved mechanisms to exacerbate its local pathology. This study examines the inhibitory effects of lipids extracted and purified chromatographically from TB on the surface-active function of lavaged bovine lung surfactant (LS) and a clinically relevant calf lung surfactant extract (CLSE). Total lipids from TB greatly inhibited the surface activity of LS and CLSE on the pulsating bubble surfactometer at physical conditions applicable for respiration *in vivo* (37 °C, 20 cycles/min, 50% area compression). Minimum surface tensions for LS (0.5 mg/ml) and CLSE (1 mg/ml) were raised from <1 mN/m to 15.7 ± 1.2 and 18.7 ± 1.3 mN/m after 5 min of bubble pulsation in the presence of total TB lipids (0.15 mg/ml). TB mixed waxes (0.15 mg/ml) and TB trehalose monomycolates (TMMs, 0.15 mg/ml) also significantly inhibited the surface activity of LS and CLSE (minimum surface tensions of 10–16 mN/m after 5 min of bubble pulsation), as did purified trehalose 6,6′-dimycolate (TDM, cord factor). Phosphatidylinositol mannosides (PIMs, 0.15 mg/ml) from TB had no inhibitory effect on the surface activity of LS or CLSE. Concentration dependence studies showed that LS was also inhibited significantly by total TB lipids at 0.075 mg/ml, with a smaller activity decrease apparent even at 0.00375 mg/ml. These findings document that TB contains multiple lipids that can directly impair the biophysical function of endogenous and exogenous lung surfactants. Direct inhibition by TB lipids could worsen surfactant dysfunction caused by plasma proteins or other endogenous substances induced by inflammatory injury in the infected lungs. TB lipids could also inhibit the effectiveness of exogenous surfactants used to treat severe

*Corresponding author at: Department of Pediatrics, University of Rochester School of Medicine, Box 850, 601 Elmwood Avenue, Rochester, NY 14642, USA. Tel.: +1 585 275 5948.
E-mail address: Robert_notter@urmc.rochester.edu (R.H. Notter).

1472-9792/$ - see front matter
doi:10.1016/j.tube.2007.11.003

acute respiratory failure in TB patients meeting criteria for clinical acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS).

Introduction

Infection with *Mycobacterium tuberculosis* (TB) is a prevalent cause of respiratory disease worldwide,[1–4] and constitutes a severe public health problem in pediatric and adult patients. Although mortality and morbidity from tuberculosis was significantly decreased by the development and use of antibiotic therapy during the last half century, drug-resistant forms of this microorganism are now very common. TB infection also occurs with a high incidence in immuno-compromised patients with the human immunodeficiency virus (HIV) or the acquired immune deficiency syndrome (AIDS) compared to the general population.[3,5,6] It is estimated that between 30% and 40% of individuals with HIV/AIDS will develop tuberculosis in the absence of prophylactic antibiotic or anti-viral therapy.[5,6]

Classical tuberculosis is a reactivation infection that leads to cavitation of the pulmonary granulomas, releasing extracellular bacilli into the lung airways to facilitate transmission. The successful transmission of tuberculosis bacilli depends on the induction of advanced lung pathology, but despite the key importance of this phase of infection, the pathophysiological mechanisms by which TB exacerbates respiratory pathology to enhance transmission are imperfectly understood.[7] Interactions between TB and the pulmonary surfactant system may contribute to this process. Several studies have shown that variants in surfactant genes, most notably those encoding surfactant proteins A and D,[8–11] impact on host susceptibility to tuberculosis. Surfactant proteins A and D bind to TB, modulate bacterial uptake, and influence the activation status of alveolar macrophages.[12–15]

Because of the physiological importance of pulmonary surfactant in respiration, interactions of TB with extracellular alveolar surfactant or with type II pneumocytes have the potential to impact respiratory function in addition to pulmonary biology. The present study addresses the possibility of direct reductions in lung surfactant surface activity induced by lipids from TB organisms. It has recently been shown that mycolic acid and trehalose dimycolate, which are found in TB, can reduce the surface tension lowering ability of solvent-spread interfacial films of synthetic dipalmitoyl phosphatidylcholine (DPPC, a major phospholipid constituent of lung surfactant).[16] However, pulmonary surfactant itself was not studied, and the Langmuir–Wilhelmy balance methodology used did not examine physiological cycling rates and area compressions or include contributions from adsorption that impact the biophysical function of alveolar surfactant.[16]

This study investigates the effects of total lipids extracted from live TB organisms on the surface activity of lavaged bovine lung surfactant (LS) and a clinically relevant calf lung surfactant extract (CLSE, the substance of the exogenous surfactant drug Infasurf®).[17] Also examined are lipid subfractions isolated chromatographically from TB total lipids, i.e., trehalose monomycolates (TMMs), waxes and glycosylated mycoserosates, and phosphatidylinositol mannosides (PIMs). Purified trehalose 6,6′-dimycolate (TDM, cord factor) is also studied. These lipids are associated loosely with the periphery of the cell wall and are released copiously in culture and inside infected macrophages.[18] Surface activity assessments are done on a pulsating bubble surfactometer, which measures a combination of adsorption and dynamic surface tension lowering at physical conditions reflecting those found in the alveoli during respiration (37 °C, 20 cycles/min, 50% area compression).[17,19,20]

Materials and methods

Lung surfactant (LS) and calf lung surfactant extract (CLSE)

Alveolar surfactant was obtained by lavaging intact lungs from freshly killed calves with 2–3 l of cool 0.15 M NaCl given in 2–3 divided aliquots. Active large surfactant aggregates (LS) were isolated by centrifugation of recovered lavage fluid at 12,500 × *g* for 30 min. CLSE, which contains all the lipids and hydrophobic apoproteins in endogenous surfactant, was prepared from LS by chloroform:methanol extraction using the method of Bligh and Dyer.[21] CLSE is the substance of the clinical exogenous lung surfactant Infasurf® (ONY Inc., Amherst, NY).

Isolation of lipids from TB organisms

Methods for the isolation and characterization of TB lipids were reported previously by Rhoades et al.[22] In brief, *M. tuberculosis* CDC1551 (a clinical isolate also referred to as CSU93[23]) was grown to log phase ($OD_{600\,nm}$ 0.55) in Middlebrook 7H9 medium (Difco, Detroit, MI) supplemented with OADC enrichment (Difco), Tween 80, and glycerol. Two-milliliter aliquots (2×10^8 cfu/ml) were spread onto eight Middlebrook 7H11 agar plates (Difco, diameter 150 × 15 mm) and incubated for 20 days at 37 °C. Confluent bacterial growth was then scraped from the agar into chloroform/methanol (2:1, v/v), and extracted twice in 20 ml of the same solvent. Bacterial debris was removed by centrifugation, followed by passage of the extract through a 0.2-μm Teflon filter. One part of the filtered lipid extract was dried and stored at −20 °C for inhibition studies (total TB lipids). The remaining filtered extract was fractionated by liquid chromatography over silica gel-60 (EM Science) in increasing amounts of methanol in chloroform to isolate different subfractions of TB lipids. Waxes and glycosylated mycoserosates eluted in 0–5% methanol, TMMs in 10–25% methanol, and PIMs in 32–100% methanol. Pooled fractions containing these different lipids were further processed by preparative thin layer chromatography (TLC; aluminium-backed, silica gel-60; E. Merck, Darmstadt, Germany). Methanol/