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Induction of inflammatory cytokines and nitric oxide in J774.2 cells and murine macrophages by lipoteichoic acid and related cell wall antigens from *Staphylococcus epidermidis*

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Staphylococcus epidermidis causes infections associated with medical devices including central venous catheters, orthopaedic prosthetic joints and artificial heart valves. This coagulase-negative staphylococcus produces a conventional cellular lipoteichoic acid (LTA) and also releases a short-glycerophosphate-chain-length form of LTA (previously termed lipid S) into the medium during growth. The relative pro-inflammatory activities of cellular and short-chain-length exocellular LTA were investigated in comparison with peptidoglycan and wall teichoic acid from *S. epidermidis* and LPS from *Escherichia coli* O111. The ability of these components to stimulate the production of pro-inflammatory cytokines [interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α] and nitric oxide was investigated in a murine macrophage-like cell line (J774.2), and in peritoneal and splenic macrophages. On a weight-for-weight basis the short-chain-length exocellular LTA was the most active of the *S. epidermidis* products, stimulating significant amounts of each of the inflammatory cytokines and nitric oxide, although it was approximately 100-fold less active than LPS from *E. coli*. By comparison the full-chain-length cellular LTA and peptidoglycan were less active and the wall teichoic acid had no activity. As an exocellular product potentially released from *S. epidermidis* biofilms, the short-chain-length exocellular LTA may act as the prime mediator of the host inflammatory response to device-related infection by this organism and act as the Gram-positive equivalent of LPS in Gram-negative sepsis. The understanding of the role of short-chain-length exocellular LTA in Gram-positive sepsis may lead to improved treatment strategies.

INTRODUCTION

The incidence of infection caused by coagulase-negative staphylococci, particularly *Staphylococcus epidermidis*, has risen significantly over the past two decades. This follows the increased use of implanted medical devices such as central venous catheters, continuous ambulatory peritoneal dialysis catheters, prosthetic hip joints, and cardiac and vascular prostheses (Boyce, 1996). Understanding the mechanisms of pathogenesis of *S. epidermidis* could provide important clues to both prevention and treatment of infection caused by these organisms. Although products such as extracellular slime, lipase, haemolysins and receptors for collagen, laminin, vitronectin and fibronectin enhance pathogenicity of *S. epidermidis* (Farrell *et al.*, 1993; Molnàr *et al.*, 1994; Peters & Schumacher-Perdreau, 1994; Paulsson *et al.*, 1992), no single determinant has proved to be essential for virulence (Lambe *et al.*, 1990).

In previous studies we screened sera from patients with *S. epidermidis* infections for antibodies to secreted antigenic products that are expressed *in vivo* (Lambert *et al.*, 1996). Our approach was based on recognition that *S. epidermidis* infections usually occur in the form of biofilms in which the organisms adhere tightly to the surface of the medical device. Cells are embedded in a polysaccharide matrix and cell-associated antigens are not accessible to contact with cells of the immune system. We reasoned that microbial antigens released from the biofilm were more likely to provoke an antibody response than cellular antigens trapped within the

Abbreviations: IL, interleukin; LTA, lipoteichoic acid; NO, nitric oxide; PGN, peptidoglycan; sce-LTA, short-chain-length exocellular lipoteichoic acid; TNF, tumour necrosis factor; WTA, wall teichoic acid.

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biofilm. We found that patients with device-related infections had elevated levels of IgG to an exocellular form of LTA that is released into the growth medium of *S. epidermidis* cultures without any apparent cell lysis. The antigen was a short-chain-length form of LTA comprising six glycerophosphate units linked to a gentiobiosyldiacylglycerol glycolipid (Fig. 1). This was in contrast to the membrane-bound, cellular form of LTA in which the glycerophosphate chain contains up to 42 units (Lambert *et al.*, 2000).

We referred to the short-chain-length exocellular form of LTA as 'lipid S' (staphylococcal glycolipid) to distinguish it from the full-chain-length cellular LTA, although they are antigenically equivalent as determined by the formation of fused precipitins on double diffusion in agarose. Morath *et al.* (2002) have since suggested the term 'lipid B' for the glycolipid anchor of LTA in *Staphylococcus aureus* to reflect its analogy to lipid A of LPS. Since use of the terms lipid S and lipid B could create confusion in this area we propose to refer to lipid S as short-chain-length exocellular LTA (sce-LTA) in future studies.

The IgG response to the antigenic determinants on sce-LTA and/or LTA can be exploited in the serodiagnosis of Gram-positive infection, including central venous catheter-associated sepsis (Elliott *et al.*, 2000), prosthetic joint infection (Rafiq *et al.*, 2000) and endocarditis (Connaughton *et al.*, 2001). Detection of very high levels of IgG to sce-LTA and/or LTA indicates prominent bacterial expression during infection and stimulation of a strong immune response in the host. Since cellular LTA is an important mediator of the inflammatory response in Gram-positive sepsis (Ginsburg, 2002) we measured the relative activities of sce-LTA and LTA as inflammatory mediators together with other cell surface antigens, peptidoglycan (PGN) and wall teichoic acid (WTA). We measured production of pro-inflammatory cytokines [tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β] and nitric oxide (NO) in the J774.2 murine macrophage-like cell line, chosen because macrophages are the primary source of pro-inflammatory mediators in sepsis. We also measured TNF-α production in macrophages derived from the spleen and peritoneal cavity of mice.

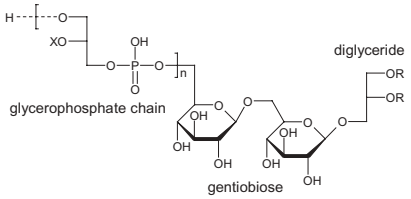

Fig. 1. Structures of sce-LTA ($n = 6$) and LTA ($n = 42$) from *S. epidermidis* (Lambert *et al.*, 2000). R, ester-linked long chain fatty acids; X, ester-linked D-alanine or glycosidically linked *N*-acetylglucosamine.

METHODS

Preparation of bacterial components. *S. epidermidis* NCIMB 40896, originally isolated from a patient with central venous catheter-related sepsis, was grown in the chemically defined liquid medium of Hussain *et al.* (1991a) for 18 h at 37 °C on a rotary shaker. Sce-LTA was recovered from the growth medium by gel permeation chromatography on Superose 12 and its identity confirmed by negative electrospray mass spectrometry, NMR spectroscopy and chemical analysis of glycerol, phosphate, glucose and fatty acid content (Lambert *et al.*, 2000). LTA was prepared from the bacterial cells by extraction with phenol, digestion with nucleases, further phenol extraction and purification by gel permeation chromatography on Superose 12 (Coley *et al.*, 1972). Glycerophosphate chain lengths of cellular LTA and sce-LTE were determined by negative electrospray mass spectrometry and confirmed by elution from an octyl-Sepharose CL-4B hydrophobic interaction chromatography column equilibrated in 0·05 M sodium acetate buffer, pH 4·7, with a linear 15–65 % v/v gradient of n-propanol (Fischer, 1993).

Bacterial cell wall sacculi comprising PGN and covalently linked WTA were prepared by boiling a whole-cell suspension for 30 min in a solution of SDS followed by thorough washing (Ohta *et al.*, 1998). WTA was released from the protein- and lipid-free cell wall sacculi with 0·1 M NaOH and the PGN was solubilized with lysostaphin (Poxton & Hancock, 1988). The identity of WTA as a (1→3)-linked poly(glycerol phosphate) teichoic acid was confirmed by chemical analysis of glycerol and phosphate content, negative electrospray mass spectrometry and NMR spectroscopy (Sadovskaya *et al.*, 2004). Absence of ribitol was confirmed by gas liquid chromatography of alditol acetates following trifluoroacetic acid hydrolysis (Lambert *et al.*, 2000).

All bacterial fractions were freeze-dried and dissolved in pyrogen-free water to 10 mg ml^{-1} (Versol Water for Irrigation; Laboratoire Aguettant). The purity of each isolated component was assessed by chemical, chromatographic and spectroscopic analysis. Levels of any contaminating LPS in the sce-LTA, LTA, PGN and WTA preparations were measured by the quantitative chromogenic limulus amoebocyte lysate assay (QCL-1000; BioWhittaker) according to the manufacturer's instructions. Presence and levels of any contaminating protein or lipoprotein were assessed by SDS-PAGE (11 % w/v acrylamide) and Coomassie blue staining followed by staining of the same gel with the more sensitive Bio-Rad protein silver stain reagent (Lambert *et al.*, 2000). Responses to these Gram-positive products were compared to that of LPS isolated from *Escherichia coli* O111:B4 by phenol extraction (Sigma-Aldrich).

Cell culture and measurement of response to bacterial components. The murine macrophage-like cell line, J774.2 (European Collection of Cell Cultures) was maintained in Dulbecco's modified essential medium (DMEM) containing glucose, Glutamax (Life Technologies), 10 % v/v heat-inactivated fetal bovine serum, penicillin (100 IU ml^{-1}) and streptomycin (25 μg ml^{-1}). Cultures were incubated at 37 °C in an atmosphere of 95 % air and 5 % CO_2. Adherent cells from confluent cultures were detached, centrifuged at 150 ***g*** for 10 min and resuspended in complete culture medium to 1×10^6 cells ml^{-1}. Aliquots (1 ml) were placed in individual wells of 24-well cell-culture plates and allowed to adhere to the surface for 1 h. Stock solutions of the different bacterial fractions prepared in pyrogen-free water (10 mg ml^{-1}) were diluted in tissue culture medium, added to the wells and the plates incubated at 37 °C.

After 24 h, samples of the culture supernatants were collected and stored at −20 °C for subsequent analysis of cytokines by ELISA (R&D Systems). NO was measured as the stable breakdown products nitrite and nitrate. Nitrate was converted to nitrite using nitrate reductase and the total nitrite was then measured using the Griess reagent (Promega). The assays were carried out within 3 days of sampling and the culture

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supernatants were thawed once only. In control experiments it was confirmed that there was no reduction in the amount of cytokines or NO measured following storage at −20 °C and thawing. All experiments were carried out on three separate occasions; standard deviations of the means of triplicate measurements for single experiments were calculated and displayed for each assay.

Murine macrophage isolation and measurement of response to bacterial components. To prepare peritoneal and splenic macrophages, adult male MF1 outbred mice were sacrificed by cervical dislocation under ether anaesthesia. Peritoneal macrophages were extracted by injecting 10 ml of sterile supplemented medium RPMI 1640 (Sigma-Aldrich) into the peritoneal cavity. The area was massaged for 2–3 min and the injected medium then extracted. A further 10 ml of medium was injected and the process repeated. The extracted medium was centrifuged for 10 min at 150 ***g***, and the pellet was suspended in 5 ml of medium, placed in a 10 cm Petri dish containing 5 ml of pre-warmed medium and incubated at 37 °C in an atmosphere of 95 % air and 5 % CO_2 for 16–24 h to allow adherence of macrophages to the plastic surface.

The spleen was removed from each mouse, placed in a Petri dish containing 10 ml pre-warmed medium and mechanically dispersed with forceps to yield a suspension containing resident macrophages, follicular dendritic cells and T- and B-cells. The cell suspension was placed in a 15 ml conical tube and the tissue clumps were allowed to settle. The remaining cell suspension was aspirated and transferred to a new tube, washed twice by centrifugation at 150 ***g*** for 10 min and resuspensed in fresh medium. The suspension was then placed in a Petri dish and incubated at 37 °C in an atmosphere of 95 % air and 5 % CO_2 for 18 h to allow adherence of macrophages to the plastic surface.

The same technique was used to recover adherent splenic and peritoneal macrophages from the Petri plates. Non-adherent cells (lymphocytes and red blood cells) were first removed by aspiration and the Petri dish with remaining adherent cells was washed twice with 3 ml of medium. Washed cells were recovered from the plates with a cell scraper (Sarstedt) and resuspended in 3 ml of medium. Cells were washed twice and resuspended in medium to 5×10^5 cell ml^{-1}. Aliquots (0·25 ml) of these suspensions were placed in individual wells of a 96-well tissue culture plate and were incubated for 24 h at 37 °C in an atmosphere of 95 % air and 5 % CO_2 to adhere. Bacterial products were then added to the wells and the plates incubated for a further 24 h. Supernatants were collected, TNF-α was determined by ELISA and NO by the Griess reagent.

Peritoneal macrophages were also prepared from adult male C3H/HeJ TLR4-deficient, LPS-resistant mice and C3H/HeN TLR4-sufficient, LPS-sensitive mice obtained from Charles River Wiga, Germany (Hoshino *et al.*, 1999). Levels of TNF-α and IL-6 were measured after exposure of 5×10^5 cell ml^{-1} of peritoneal macrophages to LPS, sce-LTA or LTA for 24 h as for the macrophages from MF1 outbred mice. NO production was measured after 48 h of exposure.

RESULTS

Exposure to the *S. epidermidis* products (sce-LTA, LTA and PGN) at concentrations from 1 to 50 μg ml^{-1} induced a progressive increase in IL-6 production in J774.2 cells (Fig. 2a). Sce-LTA was approximately three times more active than LTA and PGN, which had similar activities. The WTA preparation did not elicit IL-6 secretion over the concentration range 1–50 μg ml^{-1} (results not shown). LPS from *E. coli* O111:B4 was approximately 200–600-fold more active than any of the *S. epidermidis* preparations in terms of IL-6 release.

The three Gram-positive products also stimulated release of TNF-α (Fig. 2b) and IL-1β (Fig. 2c), giving the same pattern of relative activities as shown for IL-6 release. Sce-LTA was more effective than LTA or PGN at concentrations between 5 and 50 μg ml^{-1}. Once again LPS was a more powerful secretagogue for TNF-α and IL-1β, and WTA was ineffectual.

All three *S. epidermidis* products induced a dose-dependent increase in total NO production in the J774.2 cell line expressed as nitrite plus nitrate (Fig. 2d). Sce-LTA was the most active in this respect but, as for the cytokine release, was about 200-fold less potent than LPS.

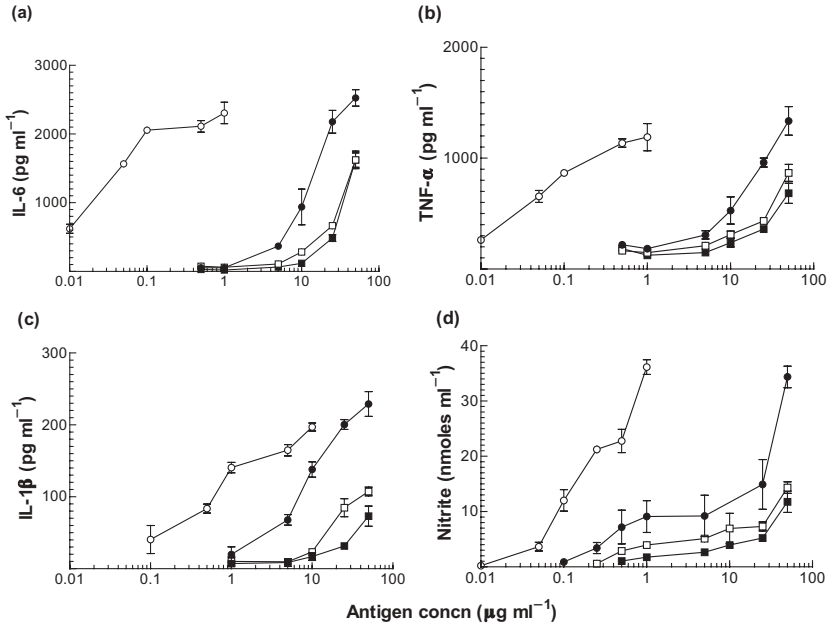

Fig. 2. Induction of pro-inflammatory cytokines and NO in J774.2 cells by *S. epidermidis* products and LPS. Aliquots (1 ml) of 1×10^6 cells ml^{-1} were adhered to wells of the culture plate for 1 h, the bacterial product was added at the concentration shown and the plate was incubated for 24 h at 37 °C. Culture supernatant was then recovered and assayed for IL-6 (a), TNF-α (b) and IL-1β (c) by ELISA and for total NO (d), measured as nitrite and nitrate using the Griess reagent. Error bars show the standard deviations of the means of triplicate assays for single experiments. Results shown are representative of experiments carried out on three separate occasions. ■, PGN; □, LTA; ●, sce-LTA; ○, LPS.

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The comparative activities of sce-LTA and LTA to stimulate TNF-α release in primary murine macrophages obtained from the spleen and peritoneum are shown in Fig. 3(a, b). On a weight-for-weight basis sce-LTA was more active than LTA, but, as with the J774.2 cell line, both materials were less active than LPS. The ability of these components to induce NO production in mouse peritoneal macrophages is shown in Fig. 3(c). There was evidence for reduced TNF-α response for the peritoneal macrophages with sce-LTA concentrations above 10 μg ml^{-1} and LTA concentrations above 50 μg ml^{-1}. However, this effect was not seen for NO production in the same cells (Fig. 3c) or for TNF-α production in the splenic macrophages.

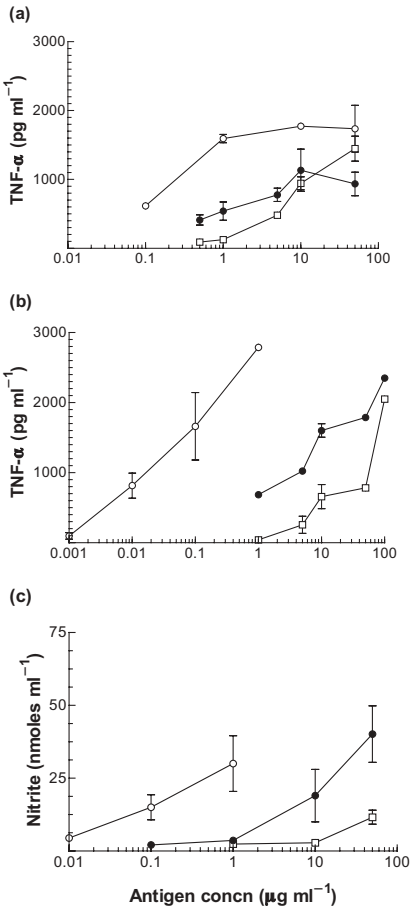

Fig. 3. Induction of TNF-α in peritoneal (a) and splenic (b) murine macrophages and NO in peritoneal murine macrophages (c) by *S. epidermidis* products and LPS. Aliquots (0·25 ml) of 5×10^5 cells ml^{-1} were adhered to wells of the culture plate for 24 h, the bacterial product was added at the concentration shown and the plate was incubated for 24 h at 37 °C. Culture supernatant was then recovered and assayed for TNF-α by ELISA or total NO, measured as nitrite and nitrate using the Griess reagent. Error bars show the standard deviations of the means of triplicate assays for single experiments. Results shown are representative of experiments carried out on three separate occasions. □, LTA; ●, sce-LTA; ○, LPS.

To investigate the nature of the receptors used by the sce-LTA and LTA components, the stimulation of NO, TNF-α and IL-6 production in peritoneal macrophages from C3H/HeJ TLR4-deficient and C3H/HeN TLR4-sufficient mice was measured. The concentrations used were based on those shown to produce comparable responses in peritoneal macrophages from F1 outbred mice (Fig. 3a–c). The NO response of the C3H/HeJ macrophage preparations to LPS at a dose of 0·1 μg ml^{-1} was 78 % lower than that of the C3H/HeN macrophages ($P = 0{\cdot}0002$). By comparison, the NO responses to sce-LTA and LTA at 50 μg ml^{-1} were not significantly different between the two sources of macrophages ($P > 0{\cdot}05$). Similar results were obtained for the TNF-α and IL-6 responses between the two sources of macrophages. LPS at a dose of 0·1 μg ml^{-1} produced 40 % less TNF-α and 47 % less IL-6 in the C3H/HeJ macrophages than in the C3H/HeN macrophages ($P = 0{\cdot}0015$), whereas sce-LTA and LTA tested at 1 and 5 μg ml^{-1}, respectively, produced no significant differences in cytokine response between the two types of macrophage ($P > 0{\cdot}05$). Although the presence of the TLR4-deficiency did not reduce the LPS response completely and did have a small effect on the response to sce-LTA and LTA, these results suggest that the sce-LTA and LTA do not stimulate via TLR4 receptor.

To investigate the possibility that contaminating levels of LPS in the Gram-positive products might be sufficient to provoke the responses observed the LPS content was measured using a limulus amoebocyte assay. At the highest concentration tested for each preparation (50 μg ml^{-1}), the level of LPS detected was 0·0001 μg ml^{-1} for sce-LTA, 0·00005 μg ml^{-1} for LTA and 0·00003 μg ml^{-1} for PGN. These very low levels of LPS contamination would not have initiated production of any of the inflammatory markers, as indicated by the response for the *E. coli* O111:B4 LPS controls in each experiment.

Consideration was given to the presence and potential activity of other Gram-positive products in the sce-LTA, LTA and PGN fractions. Protein was not detected in 0·1 ml samples of the 10 mg ml^{-1} stock solutions of the sce-LTA, LTA, WTA or PGN preparations using either the Folin-Ciocalteau or bicinchoninic acid protein assay reagents. Similarly no bands were detected in lanes of polyacrylamide gels containing 500 μg of each preparation after SDS-PAGE and staining with Coomassie blue and the Bio-Rad protein silver-staining reagent. We therefore conclude that the observed inflammatory activity was not caused by the presence of contaminating protein or lipoprotein.

DISCUSSION

Sce-LTA contains six glycerophosphate units in contrast to the 40–42 in cellular LTA of *S. epidermidis* (Fig. 1, Lambert *et al.*, 2000). It is released into the growth medium, whereas LTA is anchored to the cytoplasmic membrane by its glycolipid, with the glycerophosphate chain protruding through the cell wall (Neuhaus & Baddiley, 2003). Our view on the origin of sce-LTA and its relation to cellular LTA is

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based on consideration of the biosynthetic pathway of LTA in *S. aureus* (Fischer, 1994). Gentiobiosyldiacylglycerol, a glycolipid, is the lipid acceptor to which glycerophosphate units from phosphatidylglycerol are coupled in successive cycles. During the biosynthesis a range of molecules of LTA with increasing chain lengths are produced, culminating in the full-chain-length form. The reason why sce-LTA is released during normal growth of *S. epidermidis* is unclear at present. Whilst cellular LTA could be released by any cell lysis occurring during growth, we found no evidence for full-chain-length LTA in the culture supernatant.

LTA can induce release of pro-inflammatory cytokines and NO from monocytes and macrophages (Bhakdi *et al.*, 1991; Bucher *et al.*, 1997) and, in animal models, it can induce features of sepsis such as delayed circulatory failure with hypotension and multiple organ failure (De Kimpe *et al.*, 1995a, b). There are also suggestions that there may be synergy with PGN in these respects (Kengatharan *et al.*, 1998). The large amounts of sce-LTA produced by rapidly growing *S. epidermidis* in culture (25–30 μg ml^{-1}) and the retention of cellular LTA in cells within the biofilm on medical devices suggest that sce-LTA rather than cellular LTA may be the principle immunogen and inflammatory mediator in Gram-positive sepsis. The relative activities of sce-LTA and LTA as inflammatory mediators therefore need to be examined.

The experiments presented here demonstrate that sce-LTA is a potent activator of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and NO in a murine macrophage cell line. On a weight-for-weight basis the activity of sce-LTA is significantly greater than that of LTA, PGN or WTA, especially for TNF-α and NO production (Fig. 2b, c). Sce-LTA exerted its inflammatory effect over the concentration range 5–50 μg ml^{-1}. Although *S. epidermidis* cells at high density can generate concentrations of this order in culture supernatants (Lambert *et al.*, 2000) such concentrations might not be reached systemically *in vivo*. Nevertheless, if this material were shed from organisms growing as biofilms on catheters or prostheses, local concentrations could be high and might cause pro-inflammatory cytokine and NO release.

By contrast with the sce-LTA, LTA and PGN fractions, we found WTA to be inactive in all of the assays. WTA from *S. epidermidis* comprises a (1→3)-linked poly(glycerol phosphate) chain with no glycolipid anchor (Archibald & Baddiley, 1968; Sadovskaya *et al.*, 2004). It is related to LTA and sce-LTA but is produced by a different biosynthetic pathway (Neuhaus & Baddiley, 2003). Our observations indicate that the presence of the glycolipid is essential for inflammatory activity. It has previously been reported that the major component of the characteristic exocellular slime material produced by *S. epidermidis* in a chemically defined medium is WTA (Hussain *et al.*, 1991b, 1992; Sadovskaya *et al.*, 2004). Our observation that WTA lacks inflammatory activity whereas sce-LTA and LTA (each containing the glycolipid moiety) are potent activators of inflammation suggests that these are significant mediators of shock during sepsis, possibly acting in synergy with PGN (Thiemermann, 2002; Wang *et al.*, 2003).

On a weight-for-weight basis, sce-LTA was a more potent activator than LTA. The molecular masses of sce-LTA and LTA determined by electrospray mass spectrometry are 2414 Da and 18 474 Da, respectively (Lambert *et al.*, 2000), indicating that the two microbial products have approximately similar activities when compared on a molar basis. This is in agreement with recent studies on the activity of LTA produced by chemical synthesis. Morath *et al.* (2002) synthesized LTA from *S. aureus* with a glycerophosphate chain length of six units linked to the glycolipid gentiobiosyl-*sn*-dimyristoylglycerol, i.e. the same structure as sce-LTA produced naturally by *S. epidermidis* (Fig. 1) but with defined fatty acids. The glycerophosphate chain of the synthetic LTA was substituted with four D-alanyl esters and one *N*-acetylglucosamine residue. This synthetic material, which contained no contaminating LPS, was as potent a stimulant of cytokine release in whole human blood as highly purified native LTA from *S. aureus* (Morath *et al.*, 2002; Deininger *et al.*, 2003). These workers found the glycolipid alone to be a much weaker inducer of cytokine release, although its presence in LTA is necessary for activity.

Further information on the structural components of LTA responsible for activity has been derived from the study of chemically modified natural LTA and the development of chemical synthetic routes. The removal of D-alanyl esters from natural *S. aureus* LTA by hydrolysis results in a 10-fold reduction in activity (Morath *et al.*, 2001). Studies by Deininger *et al.* (2003) with various derivatives of the six-glycerophosphate-chain-length synthetic LTA show it to be more active when D-alanyl esters are present than when L-alanyl esters are present. The presence or absence of *N*-acetylglucosaminyl substituents on the glycerophosphate chain or the gentiobiosyl group in the lipid portion had no effect on activity. Taken together, these results show that the important structural features conferring cytokine-inducing activity are a D-alanyl-substituted glycerophosphate chain linked to a lipid anchor, which does not need to contain the gentiobiose unit. Naturally produced LTA molecules contain a variable proportion of D-alanyl substituents, which are highly susceptible to hydrolytic release under slightly alkaline conditions. It seems likely that the alanine residues of sce-LTA and LTA used in the current study were preserved during purification (Lambert *et al.*, 2000) since the synthetic growth medium was buffered at pH 7·0 and all subsequent extraction and purification procedures did not involve incubation in solutions with higher pH values.

The sequence of interactions involved in triggering the cellular response to sce-LTA is unknown. However, LTA probably uses the same molecular pathway as LPS, binding to the CD14 receptor on the macrophage surface but signalling via the TLR2 receptor rather than the TLR4 used by LPS (Cleveland *et al.*, 1996; Hattor *et al.*, 1997; Schroder *et al.*, 2003). The transmembrane Toll-like receptor TLR-2 and

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LPS-binding protein (LBP) are required for maximum binding and the subsequent cellular activation mediated by the transcription factor NF-κB (Schwandner *et al.*, 1999; Schroder *et al.*, 2003). The comparative responses of macrophages from TLR4-deficient and TLR4-sufficient mice to the sce-LTA and LTA presented in this study indicate that they do not utilize the TLR4 receptor. It will be of interest to determine whether sce-LTA interacts with the TLR2 receptor as proposed for LTA (Schroder *et al.*, 2003). The reduced negative charge resulting from the shorter glycerophosphate chain length of sce-LTA might act in the same way as the presence of alanyl esters, aiding its interactions with LPS-binding protein and the cellular receptors above (Morath *et al.*, 2001, 2002; Deininger *et al.*, 2003).

Our results show that sce-LTA is a candidate for initiating the inflammatory response in Gram-positive sepsis and might be equivalent to Gram-negative LPS. Further work needs to be carried out on organisms growing as biofilms to determine the amount of sce-LTA released. Although it has much lower potency as an inflammatory mediator than LPS, sufficient amounts may be released from cells growing as adherent biofilms on medical devices to stimulate the inflammatory response. Release of cell wall fragments including PGN induced by antibiotic therapy would further enhance the inflammatory response (van Langevelde *et al.*, 1999). Thus consideration should to be given to controlling the pro-inflammatory activities of sce-LTA in strategies being developed to moderate the host response to Gram-positive infection (Ginsberg, 2002).

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