Phagocytic Functions and Tumor Necrosis Factor Secretion of Human Monocytes Exposed to Natural Porcine Surfactant (Curosurf)

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ABSTRACT. In this study we have analyzed various phagocytic functions and tumor necrosis factor (TNF) secretion of human monocytes exposed to either a biochemically well-defined porcine surfactant or a purified phospholipid preparation. Adherence, random migration, and chemotactic response to zymosan activated serum and formyl-methionyl-leucyl-phenylalanine were normal in surfactant-treated monocytes; surfactant was not a chemotactic stimulus. In contrast, phagocytosis of *Staphylococcus aureus* by monocytes exposed to surfactant (100 $\mu\text{g/mL}$) or phospholipids (100 $\mu\text{g/mL}$) was slightly impaired [surfactant: at 30 min (t_{30}) $48.5 \pm 11\%$, t_{60} $73.3 \pm 10.1\%$; phospholipids: t_{30} $47.3 \pm 2.5\%$, t_{60} $68.0 \pm 6.6\%$; controls: t_{30} $66.6 \pm 9.9\%$, t_{60} $81.0 \pm 6.6\%$, $p < 0.05$ at t_{30} for both, $p < 0.05$ at t_{60} for phospholipids]. Due to the smaller number of *S. aureus* ingested, bactericidal activity of surfactant- or phospholipid-treated monocytes was slightly reduced when compared with controls. Surfactant or phospholipids had no bactericidal activity. Uptake of *Candida albicans* was identical in surfactant- or phospholipid-treated monocytes and untreated controls; the same was true for the number of *Candida* organisms ingested per cell. Phagocytosis-associated chemiluminescence and production of superoxide anion by monocytes of either source in response to phorbol myristate acetate and opsonized zymosan were also unaffected. Surfactant or phospholipids (500 $\mu\text{g/mL}$), however, effectively suppressed TNF secretion by resting and by lipopolysaccharide (LPS)-stimulated monocytes in a dose-dependent fashion, (LPS-stimulated monocyte controls: 3004 ± 570 pg/mL; LPS + surfactant: 426 ± 162 pg/mL; LPS + phospholipids: 28 ± 9.6 pg/mL; $p < 0.001$ for both). TNF is an important mediator of inflammation, and our data suggest that surfactant or phospholipids, by suppressing monocyte TNF secretion, may have an important role in down-regulating inflammatory reactions in the lung. (*Pediatr Res* 30: 69–74, 1991)

Abbreviations

CL, chemiluminescence
FMLP, formyl-methionyl-leucyl-phenylalanine
HBSS, Hanks' balanced salt solution
LPS, lipopolysaccharide
PMA, phorbol myristate acetate
TNF, tumor necrosis factor

Surfactant replacement therapy in severe neonatal respiratory distress syndrome effectively reduces respiratory distress syndrome-associated pulmonary morbidity and mortality in preterm infants. Immediately after intrabronchial administration of natural surfactant (human, bovine, porcine), there is a dramatic increase in oxygenation with a subsequent reduction in ventilatory requirements (1). However, there is only limited information about the effect of natural surfactant on human pulmonary monocytes and alveolar macrophages. These cells have an essential role in host defense (2, 3) as well as in the regulation of immune functions and inflammation (4–9). Available data on macrophage-surfactant interaction have been mostly derived from *in vitro* experiments using lung lavage materials from various species. However, most investigators have focused on a single aspect of macrophage function.

Our present study was initiated to examine the principal cell functions of monocytes—the macrophage precursors—in response to inflammation: adherence, random migration, chemotaxis, phagocytosis and killing of *Staphylococcus aureus*, uptake of *Candida albicans*, oxidative metabolism, and LPS-stimulated TNF-secretion. Each of these aspects was studied in monocytes either pretreated or directly exposed to a biochemically and physically well-defined porcine surfactant preparation (Curosurf) or a preparation of purified phospholipids; untreated monocytes were used as controls.

MATERIALS AND METHODS

Monocytes from healthy adult donors were isolated by Ficoll-Hypaque density gradient and purified by adherence and a 24-h cultivation in Teflon culture bags at 37°C in 5% CO₂/95% air (10). The resulting cell preparation contained about 90% monocytes identified by nonspecific esterase stain (Technicon, Terry Town, New Jersey). More than 98% of the monocytes were viable as judged by a trypan blue exclusion test. For phagocytic assays, monocytes were resuspended in HBSS at a density of 5×10^6 cells/mL before use.

Preparation of surfactant. Curosurf was isolated from minced porcine lungs by a combination of washing, chloroform-methanol extraction, and liquid-gel chromatography. The isolated polar lipid fraction was dissolved in chloroform (20 mL/g of surfactant) and sterilized by a high-pressure filter system (first filter 0.45 μm , second filter 0.20 μm). Subsequent steps of the procedure, including evaporation of the solvent and suspension of the surfactant in normal saline by gentle sonication (50 W, 48 kHz) at a phospholipid concentration of 80 mg/mL, were performed under sterile conditions with autoclaved glassware. Curosurf contains approximately 99% polar lipids, mainly phospholipids, and 1% hydrophobic, low molecular weight (surfactant protein-B, surfactant protein-C) proteins (11).

The phospholipids were isolated from Curosurf by liquid-gel

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chromatography on Sephadex LH-60 in chloroform/methanol 1:1 vol/vol containing 5% 0.1 M HCl (12). The phospholipids, eluted between 60–120% of the column volume, were neutralized by addition of methanol/water to the fraction (final proportions between chloroform/methanol/water, 8:4:3 by volume) (13). After separation the unpolar phase was evaporated to dryness and analyzed for phospholipids (14) and proteins (12).

The purified phospholipids were suspended in 0.9% NaCl by sonication and repeated freezing and thawing. The final concentration of phospholipids was 80 mg/mL.

Preincubation of monocytes with surfactant. A total of 5×10^6 monocytes/mL HBSS were preincubated with various concentrations of surfactant, phospholipids, or 0.9% saline (controls) in a shaking water bath at 37°C for 30 min. After preincubation the cells were washed twice with PBS and resuspended in HBSS at the optimal concentration; assays of monocyte functions were then immediately performed.

Adherence. Adherence to nylon fiber was determined by a modification of the method of MacGregor *et al.* (15). Forty mg of scrubbed nylon fiber (type 200; Fenwall Laboratories, Morton Grove, IL) were packed in 1-mL plastic syringes. The length of the columns was adjusted to exactly 0.4 mL. A total of 3×10^5 monocytes in 1 mL HBSS were preincubated with either surfactant, phospholipids, or saline and added in 0.5-mL aliquots of prewarmed columns and allowed to filter at 37°C in 5% CO₂/95% air. Monocytes in the effluent samples were counted (esterase stain) and the percentage of monocyte adherence calculated; each assay was performed in duplicate.

Chemotaxis. Random migration and chemotaxis of monocytes were evaluated by the leading front method using a modification of the Boyden Chamber Technique (48-well microchamber; Neuroprobe, Cabin John, MD) (16). Pooled serum activated by zymosan (Sigma, Munich, Germany; 15 mg/mL) and FMLP (5×10^{-5} M) was used as chemoattractant. In some experiments various concentrations of surfactant or phospholipids were added to the lower compartment; the pore size of the nitrocellulose filters was 8 μ m (Sartorius, Göttingen, Germany). Each assay was performed in triplicate.

Phagocytosis and bactericidal activity. A modification of the method of Quie and coworkers was used (10). Phagocytosis by monocytes was determined as the decrease in the number of viable extracellular bacteria (*S. aureus* 502 A) during incubation of bacteria and monocytes in the presence of serum. In addition, various concentrations of surfactant or phospholipids were directly added to the reaction mixture. The number of viable extracellular bacteria was determined by colony counting.

Intracellular killing of *S. aureus* was measured as the decrease in the number of viable intracellular bacteria ingested by monocytes (colony counting); assays were performed in duplicate.

Phagocytosis of *Candida albicans*. A histochemical assay was used to determine phagocytosis of *C. albicans*. Heat-killed *C. albicans* (approximately 2×10^7 /mL) in pooled serum were added to 1×10^7 monocytes/mL and incubated in a shaking water bath at 37°C for 15 min. In representative experiments, surfactant or phospholipids were directly added to the reaction mixture. Cells were stained with trypan blue to discriminate between engulfed and cell-associated nonengulfed yeast particles. The percentage of 200 monocytes that ingested one or more *Candida* organisms (phagocytic index) and the number of *Candida* organisms/200 monocytes were determined.

CL. Luminol-dependent CL was measured using a luminometer (Biolumat LB 9505; Berthold, Wildbad, Germany); each assay was performed in triplicate. The reaction mixture, which was kept at 37°C, consisted of 0.25×10^6 monocytes/mL HBSS, and luminol (Sigma; final concentration 160 μ mol), PMA (Sigma; 2.5 μ g/mL), or zymosan (1 mg/mL) previously opsonized with normal pooled serum was used as a stimulus.

Generation of O₂⁻. Release of O₂⁻ by 1×10^5 monocytes/mL was determined by superoxide dismutase (Sigma) inhibitable

reduction of ferricytochrome c (Sigma) using PMA or opsonized zymosan (17).

TNF release by monocytes. A total of 2×10^4 monocytes in 200 μ L RPMI 1640 (Flow, Meckenheim, Germany) containing 10% FCS penicillin-streptomycin (20 U/mL), and L-glutamine (100 μ g/mL) were plated to each well of a 96-well microtiter plate (Flow). After 90 min incubation at 37°C in 5% CO₂/95% air, nonadherent cells were removed by gently aspirating the supernatant fluid and washing with HBSS three times.

New media (200 μ L) and LPS from *Escherichia coli* 026:B6, (10–500 ng/well) and/or various concentrations of surfactant or phospholipids were added to some wells. After a 24-h cultivation at 37°C in 5% CO₂/95% air, supernatant was removed and immediately frozen at -70°C; TNF-concentrations were measured within 72 h by ELISA technique (T Cell Sciences, Inc., Cambridge, MA).

Statistical methods. Statistical analysis was performed by *t* test for unpaired samples and Wilcoxon-Mann-Whitney test. Values are given as mean $\pm$ 1 SD.

RESULTS

The phospholipid compositions of surfactant (Curosurf) and the purified phospholipid fraction were similar, but the latter only contained trace amounts of proteins (Table 1).

Adherence. Adhesiveness of monocytes exposed to various concentrations of surfactant for 30 min was identical when compared with controls (Table 2). In additional experiments (*n* = 5), monocytes and surfactant (1–100 μ g) were directly added to the column; again, surfactant did not affect monocyte adherence (data not shown).

Random migration and chemotaxis. As demonstrated in Table 2, during a 60-min assay, random migration of monocytes pretreated with surfactant was similar to that of controls. In addition, the chemotactic response to zymosan-activated serum and to the synthetic chemotactic peptide FMLP was identical in monocytes exposed to surfactant and controls.

Surfactant (1–100 μ g) directly added to zymosan-activated serum and FMLP, did not affect the stimulated monocyte migration achieved with either stimulus (Table 2). Surfactant itself, when used as a chemotactic stimulus, had no chemotactic properties [controls, 56.7 ± 12.4 μ m; 100 μ g surfactant, 58.9 ± 10.1 μ m (*n* = 6)].

Phagocytosis and bactericidal capacity. Phagocytosis of *S. aureus* was identical in monocytes preincubated with surfactant (100 μ g/mL) and in controls: $81 \pm 6.6\%$ within 60 min and $83.2 \pm 4.7\%$ in controls (*n* = 4). Similarly, monocytes pretreated with surfactant killed *S. aureus* as efficiently as control monocytes. However, when surfactant (100 μ g) was directly added to the assay mixture, bacterial uptake was significantly reduced when compared with controls (*p* < 0.05 at 30 min (*t*₃₀)) (Fig. 1a). In addition, purified phospholipids (100 μ g) present in the assay mixture similarly reduced the phagocytic capacity of monocytes (*p* < 0.05 at *t*₃₀, *t*₆₀). The presence of surfactant or phospholipids

Table 1. Composition of Curosurf (mean $\pm$ SD for 22 batches used in clinical trials) and purified phospholipids

Component	Curosurf	Purified phospholipids
Phospholipids (mol %)		
Phosphatidylcholine*	75.1 ± 3.4	75.3
Phosphatidylethanolamine	8.5 ± 2.0	5.4
Phosphatidylserine	1.0 ± 0.7	0.3
Phosphatidylinositol	6.4 ± 1.5	8.4
Phosphatidylglycerol	3.4 ± 1.0	2.4
Lysophosphatidylcholine	0.7 ± 0.4	1.8
Sphingomyelin	4.9 ± 1.7	6.5
Proteins (% total phospholipids)	1.0 ± 0.3	≤ 0.1

*Dipalmitoylphosphatidylcholine (% total phosphatidylcholine) in Curosurf 46.4 ± 1.7 , in purified phospholipids 42.4.

Table 2. Adherence, random migration, and chemotaxis of monocytes preincubated for 30 min with various concentrations of surfactant (1–100 $\mu\text{g}/\text{mL}$; controls: monocytes preincubated in HBSS)*

Parameters	No. of experiments (n)	Controls	Surfactant ($\mu\text{g}/\text{mL}$)		
			1	10	100
Adherence (%)	5	99.2 $\pm$ 0.7	98.8 $\pm$ 0.6	98.3 $\pm$ 1.7	96.2 $\pm$ 3.1
Random migration ($\mu\text{m}/\text{h}$)	6	46.7 $\pm$ 7.1	44.0 $\pm$ 11.4	56.1 $\pm$ 4.4	55.7 $\pm$ 5.3
Chemotaxis					
ZAS ($\mu\text{m}/\text{h}$)	6	140 $\pm$ 14.4	132 $\pm$ 23.4	142 $\pm$ 14.5	145 $\pm$ 12.1
FMLP ($\mu\text{m}/\text{h}$)	6	88 $\pm$ 13.4	86.1 $\pm$ 10.7	88.3 $\pm$ 9.2	87.3 $\pm$ 8.1

* Values are mean $\pm$ 1 SD. ZAS, zymosan-activated serum.

Fig. 1. Phagocytosis (a) and killing (b) of *S. aureus* by untreated monocytes (controls) and monocytes exposed to surfactant (100 $\mu\text{g}/\text{mL}$) or to phospholipids (100 $\mu\text{g}/\text{mL}$). Values represent mean $\pm$ 1 SD for six experiments performed in duplicate. *, $p < 0.05$; **, $p < 0.01$.

Fig. 2. Effect of surfactant on the growth curve of *S. aureus* during a 4-h assay (37°C). Aliquots from the experimental and control tubes were removed at the given time interval and serially diluted in PBS to make agar pour plates for colony counting. Data represent mean $\pm$ 1 SD of four experiments performed in duplicate.

in the killing assay effectively reduced the bactericidal activity of monocytes ($p < 0.01$ at t_{60}) (Fig. 1b). Interestingly, lower doses of surfactant or phospholipids (1 and 10 μg) did not affect bacterial uptake and killing.

Growth of *S. aureus* was not inhibited by surfactant (100 μg) present during a 4-h assay (Fig. 2).

Phagocytosis of *C. albicans*. Preincubation of monocytes with surfactant (100 μg for h) did not affect the capacity of the phagocytes to ingest *C. albicans* (data not shown). In addition,

surfactant (100 μg) directly added to the assay influenced neither phagocytic index nor *C. albicans* uptake per monocyte when compared with controls (Table 3).

Production of O_2^- . Generation of O_2^- by monocytes preincubated with surfactant (100 μg for 30 min) was identical when compared with untreated controls (5×10^6 cells pretreated with surfactant (nmol/h): resting cells, 0.9 ± 0.4 ; PMA, 9.2 ± 0.7 ; opsonized zymosan, 6.5 ± 1.7 ; controls (nmol/h): resting cells, 1.2 ± 0.4 , PMA, 8.9 ± 0.7 , opsonized zymosan, 6.3 ± 1.8 ($n = 5$). Kinetic registration of PMA-stimulated O_2^- production was 1.67 ± 0.18 nmol/min in surfactant-pretreated monocytes and 1.53 ± 0.13 nmol/min in controls. As summarized in Table 3, resting and stimulated production of O_2^- by monocytes was not affected by surfactant present in the assay system.

Generation of CL. After stimulation with PMA, monocytes preincubated with surfactant (100 μg for 30 min) as well as untreated monocytes generated identical amounts of CL. Additionally, CL by monocytes stimulated with opsonized zymosan was also similar in cells of either source (Table 4).

TNF secretion of monocytes exposed to surfactant. As shown in Figure 3, unstimulated monocytes secreted only small amounts of TNF during a 24-h assay. However, monocytes exposed to surfactant (500 $\mu\text{g}/\text{mL}$) or phospholipids (500 $\mu\text{g}/\text{mL}$) released even less TNF when compared with controls (surfactant, $p < 0.05$; phospholipids, $p < 0.01$).

Monocytes stimulated by LPS (50 ng–2.5 $\mu\text{g}/\text{mL}$) secreted significant levels of TNF as compared to unstimulated monocytes (data not shown); the maximal release of TNF was induced by 2.5 $\mu\text{g}/\text{mL}$ LPS. LPS-stimulated monocytes exposed to surfactant (final concentration 500 $\mu\text{g}/\text{mL}$), however, secreted significantly less TNF activity ($p < 0.001$). LPS-stimulated monocytes cocultivated with phospholipids (final concentration 500 $\mu\text{g}/\text{mL}$) released even less TNF when compared with monocytes exposed to identical concentrations of surfactant ($p < 0.001$). As shown in Figure 4, surfactant or phospholipids inhibited TNF release by LPS-stimulated monocytes in a dose-dependent manner.

Viability of unstimulated and LPS-stimulated monocytes as well as monocytes exposed to surfactant or phospholipids was $>90\%$ in all experiments as assessed by exclusion of trypan blue. Surfactant or phospholipids (1–100 $\mu\text{g}/\text{mL}$) that were added to the standards of the TNF-assay did not interfere with the assay system.

DISCUSSION

Monocytes—the precursors of pulmonary macrophages—play an essential role in cellular host defense as circulating phagocytes as well as immunoregulatory cells. In this study we evaluated the effect of a natural porcine surfactant preparation (Curosurf) on various functions of human monocytes. Curosurf contains about 99% phospholipids and about 1% low molecular weight apoproteins (SP-B, SP-C); its biochemical and physical properties are well defined (11). Structure and biophysical activity of the two hydrophobic apoproteins have been characterized (13).

Adherence is an important premigratory event in the initiation of the inflammatory response. Adhesiveness of monocytes that

Table 3. Phagocytosis of *C. albicans* and generation of superoxide anion by monocytes directly exposed to surfactant (100 µg/mL, n = 5) and controls*

	Phagocytosis of <i>C. albicans</i>		Generation of O ₂ ⁻ (nmol/10 ⁵ monocytes/h)		
	Phagocytic index	No. of ingested <i>C. albicans</i> per cell	Resting value	PMA	Opsonized zymosan
Without surfactant	78 ± 6.3	2.6 ± 0.2	1.3 ± 0.8	9.7 ± 0.8	5.5 ± 1.3
With surfactant	78 ± 5.6	2.6 ± 0.2	1.4 ± 0.6	9.7 ± 1.1	5.8 ± 1.2

* Values are mean ± 1 SD.

Table 4. Generation of CL by monocytes preincubated with surfactant for 30 min [100 µg/mL (n = 4)] and controls*

	Stimulus			
	PMA		Opsonized zymosan	
	Peak (cpm)	t _{max} (min)	Peak (cpm)	t _{max} (min)
With surfactant	6.67 ± 2.6 × 10 ⁷	1.92 ± 0.4	1.26 × 10 ⁷ ± 0.1 × 10 ⁶	35.9 ± 2.2
Without surfactant	5.75 ± 3.5 × 10 ⁷	1.72 ± 0.3	1.17 × 10 ⁷ ± 2.8 × 10 ⁶	35.8 ± 2.7

* Values are mean ± 1 SD. t_{max}, time interval between stimulation of cells and maximal generation of CL.

were preincubated with surfactant or directly exposed to Curosurf was normal when compared with untreated cells. In addition, random migration and chemotaxis in response to zymosan-activated serum and FMLP was identical to monocytes exposed to surfactant and controls. Surfactant, in the concentrations used, was not a chemotactic stimulus. Similar observations have been recently reported with rat alveolar macrophages; whole pulmonary surfactant and surfactant phospholipids did not affect macrophage chemotaxis. In contrast, a delipidated preparation of rat surfactant, consisting mainly of protein, augmented macrophage migration (18). The decreased migration of alveolar macrophages collected from monkeys exposed to ozone was nearly restored by addition of surfactant lining material (19), whereas pretreatment of rabbit alveolar macrophages with dipalmitoyl phosphatidylcholine caused decreased stimulated migration (20).

As demonstrated in our study, phagocytosis and killing of *S. aureus* 502 A were slightly impaired with monocytes that were directly exposed to Curosurf or to a purified phospholipid fraction. Previous studies on the effect of lung lavage surfactant on phagocytic functions of human alveolar macrophages have yielded conflicting results; in one study uptake of *S. aureus* by macrophages was increased in the presence of surfactant isolated from lung lavage fluid (4). Enhanced phagocytosis and killing of staphylococci were also observed with rat alveolar macrophages exposed to rat surfactant or human alveolar lining material (21, 22). The same phenomenon was observed in a rabbit model (23). Another study demonstrated that phagocytosis and killing of *Streptococcus pneumoniae*, *Hemophilus influenzae*, and *S. aureus* by macrophages were not affected by alveolar lining material (5). In contrast, data from *in vivo* animal experiments on newborn rabbits indicate that administration of homologous surfactant and some artificial phospholipid vesicle preparations reduces the phagocytic killing of group B streptococci by alveolar macrophages (24). These contradictory results can be explained, at least in part, by differences in the composition of surfactants used and by interspecies variations in macrophage activity. In fact, high concentrations of proteins in the surfactant material, especially Ig, may enhance bacterial uptake by improving opsonization of various pathogens.

Our own data suggest that lipid components of Curosurf rather than the small amounts of low molecular weight apoproteins could be responsible for impaired phagocytosis of staphylococci by interacting with membrane receptors of monocytes. A recent report has shown that the incubation of rat peritoneal and alveolar macrophages with alveolar lining material or purified lysophospholipids decreased the number of detectable Fc and complement receptors (25). Possible mechanisms could be un-

specific binding of phospholipids to the macrophage membrane, *i.e.* covering of receptors that are involved in bacterial incorporation, or a modulation of surface receptor density by phospholipids. There is evidence that exogenous lipids, phospholipids, lipoproteins, and fatty acids can be incorporated in the macrophage membrane (26, 27) and that these substances can either suppress (28) or enhance cellular functions of phagocytes.

As shown in our experiments, the uptake of *C. albicans* by macrophages exposed to Curosurf was identical in surfactant-treated and untreated cells; the same was true for the number of *Candida* organisms ingested per cell. With high doses of Curosurf (1–2.5 mg/mL) used by other investigators, the ingestion of *Candida* particles by monocytes was enhanced (6, 7). Because uptake of *Candida* is mainly mediated by mannose receptors on the monocyte membrane, surfactant could exert a different effect on these structures; this may explain the disparity between bacterial and fungal uptake by phagocytic cells in the presence of surfactant.

During phagocytosis of opsonized zymosan or perturbation of the cell membrane with PMA, monocytes exposed to Curosurf generated identical amounts of CL as untreated monocytes. It has been shown that the amount of CL produced depends on the extent of ingested particles (29). We did not observe an increased generation of CL in monocytes preincubated with Curosurf, although this has previously been described in rat alveolar macrophages pretreated with several unsaturated lipids (30). In a recent study, a suppressive effect of rabbit lung surfactant on CL generated by rabbit alveolar macrophages was demonstrated; again, lipid components were thought to be responsible for this alteration of cell function (31).

As shown in our experiment, production of O₂⁻ in response to PMA or opsonized zymosan was identical in surfactant-treated monocytes and untreated cells. Additionally, with high doses of surfactant (1–2.5 mg/mL), an increase in the nitroblue tetrazolium reduction of monocytes challenged by *E. coli* has been observed (6, 7). These data clearly demonstrate that, even in the presence of surfactant, monocytes have an intact oxidative metabolism. Conflicting results may be explained by differences in the assay procedures and the assay design and functional differences of alveolar macrophages from different species (32). Most importantly, there may be quantitative and qualitative differences in the phospholipids present in different species and surfactant materials. The role of surfactant phospholipids in the regulation of immune reactions in the lung has not been extensively studied. A previous investigation demonstrated that phosphatidylcholine and phosphatidylglycerol suppressed the stimulation of peripheral blood lymphocytes by concanavalin A,

Fig. 3. TNF secretion by resting (a) and LPS-stimulated monocytes (b) (2.5 $\mu\text{g}/\text{mL}$). Monocytes were exposed to surfactant (500 $\mu\text{g}/\text{mL}$) or phospholipids (500 $\mu\text{g}/\text{mL}$); untreated monocytes were used as controls. *, $p < 0.05$, statistical significance of difference between untreated and surfactant- or phospholipid-treated monocytes. **, $p < 0.001$, statistical significance of difference between LPS-stimulated cells and cells that were exposed to either LPS and surfactant or LPS and phospholipids. Values represent mean $\pm$ 1 SD for five experiments performed in triplicate.

Fig. 4. Dose-dependent suppression of TNF release by LPS-stimulated monocytes exposed to various concentrations of surfactant or phospholipids (final concentration 500–50 $\mu\text{g}/\text{mL}$).

pokeweed mitogen, or cocultivation with mitomycin-treated allogeneic lymphocytes (33). In addition, whole surfactant and purified surfactant lipids from human, pig, and rabbit lungs were shown to suppress *in vitro* proliferative responses to T cell and B cell mitogens as well as allogeneic mixed lymphocyte cultures; in all species the purified lipid fraction appeared to cause more suppression than did the whole surfactant (9). In contrast, phospholipids in surface active material were found to enhance cytotoxicity of human monocytes and alveolar macrophages; this enhancement of monocyte and macrophage cytotoxicity was only caused by some phospholipids, especially sphingomyelin, phosphatidylcholine, and phosphatidylglycerol, whereas phosphatidylinositol inhibited monocyte cytotoxicity (8). With our data, we provide evidence that porcine natural surfactant and purified phospholipids effectively suppress TNF secretion by resting and by LPS-stimulated monocytes in a dose-dependent fashion. TNF, a potent inflammatory mediator, appears to alter the hemostatic properties of the vascular endothelium by inducing the production of a procoagulant activity (34, 35). It also alters the pattern of antigenic expression (36–38) and cellular rearrangement of endothelial cells (39). TNF seems to be directly

toxic to vascular endothelial cells (40), and pulmonary edema may be attributed to the resulting endothelial lesions. It is quite likely that TNF, among other factors, triggers the synthesis of leukotrienes, prostaglandin E₂, and platelet activating factor and that the infiltration of polymorphonuclear leukocytes in the lungs may result in part from elaboration of these secondary mediators (41). Additionally, TNF stimulates adhesion of polymorphonuclear cells to endothelial cell surfaces (42) and mediates production of reactive oxygen radicals by granulocytes. It is likely that TNF has an important pathogenic role in the inflammatory process of chronic lung disease after severe neonatal respiratory distress syndrome, *i.e.* bronchopulmonary dysplasia. Decreased TNF secretion by monocytes and alveolar macrophages exposed to Curosurf may well contribute to a suppression of pulmonary inflammatory reactions. There is evidence, however, that TNF has an essential role in the host defense against *Listeria monocytogenes*: antilisterial resistance may be decreased after the exposure of macrophages to surfactant (43).

In conclusion, we have shown that human monocytes exposed to natural porcine surfactant and purified phospholipids exhibit basically normal phagocyte functions; only phagocytosis and killing of *S. aureus* were slightly impaired.

Secretion of TNF, however, was markedly depressed in monocytes exposed to surfactant or phospholipids. These data suggest that surfactant may have an important and beneficial role in down-regulating inflammatory reactions in the lung.

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