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Effects of alveolar surfactant aggregates on T-lymphocyte proliferation

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Abstract

The effects of alveolar large aggregate (LA) and small aggregate (SA) surfactant subfractions isolated from healthy adult rats on mitogen-stimulated proliferative responses of human peripheral blood mononuclear cells (PBMC) was examined. Various concentrations of total surfactant suppressed proliferation of stimulated lymphocytes by up to 95% of mitogen-stimulated cells alone. LA subfractions of total surfactant had no effect on proliferation, whereas SA significantly enhanced the lymphocyte proliferation at lower concentrations (7.8 μg/ml) compared to mitogen-stimulated cells alone. Higher concentrations of SA (62.5 μg/ml) inhibited lymphocyte proliferation. This concentration-dependent effect of SA on proliferation of PBMC was also present when cells were stimulated with various lectins including anti-CD3, concanavalin A and phytohemagglutinin. Analysis of the supernatant of mitogen-stimulated cell cultures treated with inhibitory concentrations of SA showed decreased amounts of interleukin (IL)-2, compared to cells alone, which could be reversed by adding exogenous IL-2 to the cell cultures with the SA. These results suggest that alveolar surfactant subfractions have distinct functions within the alveoli, both biophysically and with respect to their effects on the host's immunomodulatory responses.

Keywords: Pulmonary surfactant aggregate; Lymphocyte proliferation; Interleukin-2

1. Introduction

Pulmonary surfactant isolated from broncho alveolar lavage can be separated into two distinct subtypes [1,2], designated as large surfactant aggregates (LA) and small aggregates (SA) [3]. LA represent freshly secreted surfactant in large lamella or tubular myelin-like forms and are responsible for maintaining alveolar stability by reducing the surface tension at the air–liquid interface. SA are small vesicular forms, which are not surface active and have been shown to be the metabolic products of LA formed through a conversion process within the alveolar space [4,5].

In addition to its biophysical role of lowering surface tension, current evidence suggests that surfactant may affect the pulmonary inflammatory re-

Abbreviations: ARDS, acute respiratory distress syndrome; bLES, bovine lipid extract surfactant; LA, large aggregate; SA, small aggregate; SP-A, surfactant-associated protein A; SP-D, surfactant-associated protein D; anti-CD-3, monoclonal antibody UCHT1 (T-cell receptor); PHA, phytohemagglutinin; PBMC, peripheral blood mononuclear cells; Con A, concanavalin A

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sponse and participate in immune regulation within the lung. Previous studies have shown for example, that whole surfactant, as well as individual components of surfactant can influence the function of lymphocytes. Alveolar lymphocytes obtained from lungs via broncho alveolar lavage are relatively hyporesponsive to various mitogens in comparison to lymphocytes isolated from peripheral blood [6]. These differences presumably serve to protect the host from chronic alveolar immune-cell activation, which potentially would lead to progressive lung damage. Since pulmonary surfactant lines the alveolar space and is in close proximity to both alveolar and vascular cellular populations, it is reasonable to assume that the presence of an intact endogenous surfactant system is important for lung homeostasis, and may therefore influence lymphocyte activation and proliferation.

Recent in vitro studies have shown that both the phospholipid and surfactant-associated proteins (SP-) SP-A and SP-D altered lymphocyte function [7,8]. We have previously shown that mitogen-induced lymphocyte proliferation was inhibited when these cells were pre-incubated with purified bovine SP-A [7]. Subsequent studies demonstrated that SP-D also inhibited lectin and anti-CD3-stimulated proliferation of human peripheral blood mononuclear cells (PBMC) [8]. Although these findings support the hypothesis that surfactant plays an important role in downregulating mitogen-induced lymphocyte proliferation, the experimental approach utilized in these studies was limited. For example, surfactant does not exist as isolated lipid or protein components within the airspace, but as a complex lipoprotein mixture, which can be separated into large and small aggregates. Indeed, it is possible that these complex aggregate structures may alter the availability of the individual surfactant components, specifically the surfactant-associated proteins, to various cellular populations within the lung. The objective of the current study therefore, was to characterize the effects of these different alveolar surfactant subfractions on mitogen-induced lymphocyte proliferation to more adequately reflect the interactions of alveolar surfactant with these cells in vivo.

2. Materials and methods

2.1. Isolation of the surfactant aggregates

Total alveolar surfactant and alveolar surfactant aggregate subfractions from four different groups of normal adult (Wistar) rats were utilized for these experiments. Whole lung lavage was performed in healthy animals immediately after sacrifice using sterile Tris-buffered saline at room temperature. Ten ml of this fluid was infused through the endotracheal tube into the lungs until they were fully distended. The fluid was then withdrawn and reinfused two more times and this procedure was repeated a total of five times for each animal. All lung lavage samples were pooled and centrifuged at $150\times g$ for 10 min to yield a pellet containing cellular debris. The $150\times g$ supernatant was referred to as 'total surfactant'. Large aggregate (LA) and small aggregate (SA) forms were isolated from the $150\times g$ supernatant, via centrifugation of this material at $40\,000\times g$ for 15 min at 4°C. The LA pellet was resuspended in a small volume of sterile, serum-free RPMI-1640 media and aliquots were removed for determination of phosphorus content while the remainder of the sample was stored in separate aliquots at −70°C until further use. The $40\,000\times g$ supernatant containing the SA fraction was dialyzed against distilled deionized water using a dialysis membrane with a molecular mass cutoff of 6000–8000 Da, and then transferred into 50-ml sterile plastic centrifuge tubes and concentrated by speed-vac. The SA powder was then resuspended in a small volume of sterile, serum-free media similar to the LA fractions, and aliquots were obtained for phosphorus determination. The remaining SA sample was frozen at −70°C until further use. Phospholipid content was measured by a modified method of Duck-Chong [9] and composition was determined by separating the various lipids via thin-layer chromatography followed by phosphorus assay [10]. The phospholipid composition was generally similar between the two aggregate types, with the sole exception of lysophosphatidylcholine which comprised only $0.4\pm0.3\%$ of the large aggregates whereas it was $2.3\pm0.5\%$ of the small aggregates ($P<0.05$).

Aliquots of the LA and SA fractions were resuspended in 0.15 M NaCl/1.5 mM $CaCl_2$ to obtain a

final concentration of 2.5 mg of phospholipid/ml. The surface tension-reducing activity of these samples was then assessed using a pulsating bubble surfactometer (Electronetics Corp., NY) as described by Enhorning [11]. With this technique the surface tension-reducing ability of the LA was high, reducing tension from an initial value 24.9 ± 1.2 to 4.8 ± 2.6 mN/m after 20 pulsations and this was further reduced to 0.7 ± 0.2 mN/m after 40 pulsations. With the SA forms both basal surface tension was greater at a value of 46.2 ± 1.7 mN/m and this was only reduced to 27.3 ± 2.4 mN/m after some 40 pulsations. At all points tested there was a significant ($P < 0.05$) difference between the values obtained from the two differing aggregate types, with the large aggregates being superior throughout.

2.2. Surfactant protein (SP-A and SP-D) analyses

Similar quantities (based on phospholipid-phosphorus) of LA and SA fractions isolated from the four different groups of rats utilized for these experiments were subjected to a 12% polyacrylamide protein gel electrophoresis containing 0.1% sodium dodecyl sulfate using the method of Laemmli [12]. After electrophoresis, proteins were transferred to nitrocellulose using a Bio-Rad transfer apparatus (Bio-Rad Laboratories, Mississauga, ON, Canada). Transfer was carried out at 100 V for 1 h. Western blot analysis was then performed using the nitrocellulose paper that was blocked overnight with 3% milk in 50 mM Tris buffer at 4°C and incubated with a primary antibody in phosphate-buffered saline (PBS)/0.2% normal rat serum for 1 h. The primary antibodies utilized for these experiments included a polyclonal rabbit-anti-rat SP-D antibody (a generous gift of Dr Jo Rae Wright, UNC, Chapel Hill, NC) and a polyclonal rabbit-anti-rat SP-A antibody (a generous gift of Dr Fred Possmayer, UWO, London, ON, Canada). Blots were then washed three times in PBS with 0.1% Tween-20 and incubated with the second antibody, horseradish peroxidase (HRP)-labeled donkey anti-rabbit sera. After ECL (enhanced chemiluminescent) reagent development, the blots were exposed to X-ray film (Eastman Kodak, Rochester, NY). The ECL kit employed was purchased from Amersham Life Science (Little Chalfont, Bucks, UK) and used according to the instructions provided.

2.3. Isolation of lymphocytes

Human peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers via venipuncture and collected in 15-ml heparin-containing vacuum tubes (Becton Dickinson, Rutherford, NJ, USA). The blood was diluted 1:1 in sterile tissue culture media (RPMI-1640) containing penicillin (100 μg/ml), streptomycin (100 μg/ml), amphotericin-B (2.5 μg/ml), β-mercaptoethanol (5.5×10^{-5} M), and gentamycin (0.1 μg/ml) (Gibco BRL, Burlington, ON, Canada). Ten ml of the diluted blood sample was layered over 4.5 ml of Histopaque-1077 (Sigma, Oakville, ON, Canada) in 15-ml conical tubes. Mononuclear cells were harvested from the interface after centrifugation of these tubes at $400 \times g$ for 40 min. PBMC were then washed two times in 4°C tissue culture media and resuspended in sterile tissue culture media containing 10% (v/v) newborn calf serum (Gibco BRL). Cells were cultured at a concentration of 2×10^5 cells per well in flat-bottomed, 96-well sterile plates (Corning, NY).

2.4. Lymphocyte proliferation assays

Three different T-lymphocyte cell mitogens, phytohemagglutinin (PHA; 1 μg/ml), concanavalin A (Con A; 2 μg/ml) (Sigma, St. Louis, MO), and anti-CD3 (UCHTI; 50 ng/ml; ID Labs, London, ON, Canada) were used for these experiments. In separate experiments, varying amounts of either total surfactant, LA or SA (7.8, 15.6, 31.3 or 62.5 μg phospholipid/ml) were added to the lymphocytes together with the mitogen in 96-well plates. The cultures were then incubated at 37°C with 5% CO_2 in a humidified atmosphere for 64 h. At the 48-h time point, 1 μCi of [^{3}H]thymidine (Amersham International, Oakville, ON, Canada) suspended in 50 μl medium was added to each well (specific activity: 6.7 Ci/nmol). Cells were then harvested at the 64-h time point with a semiautomatic Skatron cell harvester (Sterling, VA) and the filter papers were allowed to dry. The amount of [^{3}H]thymidine incorporated into the DNA was measured via liquid scintillation spectrophotometry. Of note, cell viability was confirmed using both the Trypan blue exclusion technique, as well as measuring the release of lactate dehydrogenase from the cells (Sigma Diagnostics, St. Louis, MO).

In some experiments, exogenous interleukin-2 (IL-2) (R&D Systems, Minneapolis, MN) was added at doses of either 10 or 100 IU/ml at the initiation of the cell culture.

2.5. Interleukin-2 (IL-2) assay

In separate experiments, PBMC were isolated as described above and cultured in 0.5 ml of RPMI-1640 medium containing 10% (v/v) newborn calf serum in 24-well flat-bottomed plates (Corning). Cells, 2×10^6 per well, were plated in the presence or absence of 50 ng/ml of anti-CD3 (UCHTI; ID Labs, London, ON, Canada) and varying amounts of either LA or SA (7.8, 15.6, 31.3, or 62.5 μg phospholipid/ml). After 24 h of culture, cells were then transferred into sterile Eppendorf tubes and centrifuged in a microcentrifuge at 14 000 rpm for 1 min to precipitate cells and debris. The supernatants were frozen at −70°C until IL-2 assays were performed. Human IL-2 enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Systems and used according to the manufacturer's specifications. Each culture condition was performed in triplicate and the resultant supernatants were assayed in duplicate.

2.6. Statistics

Statistical analyses were performed by comparing the results between positive control (cells with mitogen alone) and treated cell cultures (cells with mitogen and surfactant preparation) using a two-way analysis of variance (ANOVA) with the Tukey post hoc test. Comparisons between two experimental groups were made using the Student's *t*-test. A probability value of less than 0.05 was considered significant.

3. Results

Surfactant protein analyses are shown in Fig. 1. When equal amounts of LA and SA phospholipid were loaded onto gels, LA contained relatively more detectable SP-A in all four batches of surfactant utilized for these experiments than the SA fractions (Fig. 1A). Moreover, among the four batches, similar amounts of SP-A were detected with both LA and SA samples respectively. In addition, Western blot analysis showed that LA contained minimal amounts of SP-D, whereas variable amounts of SP-D were detected among the SA samples (Fig. 1B).

3.1. Proliferation assays

Different quantities of 'total surfactant' (i.e., before aggregate isolation) were tested for their effects on lymphocyte proliferation. These results are shown in Fig. 2. Total surfactant had no effect on lymphocyte proliferation at a concentration of 15.6 μg

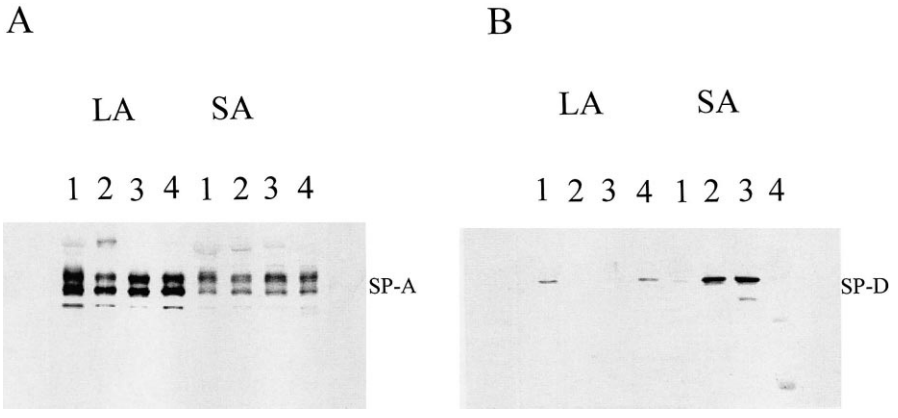

Fig. 1. Western blot analysis of the surfactant protein A and D in LA and SA. Different batches (1, 2, 3, 4) of normal rat LA and SA with equivalent amounts of phospholipid (2.5 and 5.0 μg, respectively) were electrophoresed on 12% SDS–PAGE gel, transferred to the nitrocellulose membrane, and blotted with polyclonal anti-rat SP-A (A) and anti-rat SP-D (B) sera followed by HRP-labeled donkey anti-rabbit antibody. The blots were exposed to X-ray film for 90 s.

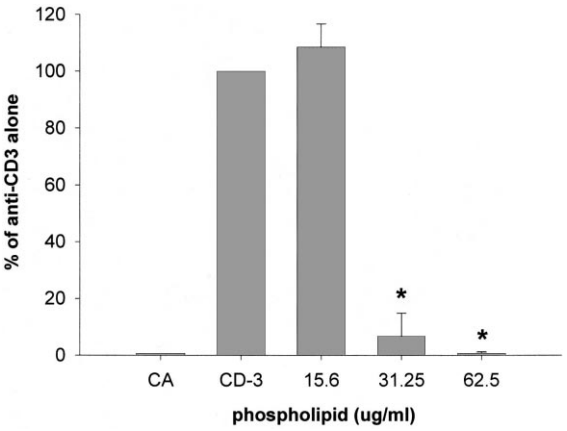

Fig. 2. Incorporation of [^{3}H]thymidine into cultures of human PBMC (2×10^5/well) stimulated with 50 ng/ml of anti-CD3 (UCHT1) in the absence or presence of increasing doses of total surfactant obtained from normal rats. Each bar represents the mean ± S.E.M. of six determinations. CA (cells alone) represents the negative control. *$P \leq 0.05$, significantly different from positive control cells plus CD-3.

PL/ml, but significant inhibitory effects were observed at higher concentrations when compared to the mitogen-stimulated control group ($P \leq 0.05$ vs. 31.3 and 62.5 μg PL/ml).

The effects of the individual surfactant subfractions on lymphocyte proliferation are shown in Fig. 3. Although LA had no significant effects on anti-CD3-stimulated lymphocyte proliferation at all concentrations tested (Fig. 3A), SA had significant concentration-dependent effects (Fig. 3B). While lower concentrations (7.8 μg/ml) resulted in approximately a 2-fold increase in lymphocyte proliferation, higher concentrations (≥ 62.5 μg/ml) inhibited lymphocyte proliferation. In a separate experiment, samples of both LA and SA were heated to 95°C for 10 min prior to adding these aggregates to cell cultures. Heating had no effect on the activity of these surfactant subfractions with respect to lymphocyte proliferation (results not shown). Furthermore, cell viability testing performed at the final time point of all cultures confirmed over 90% viability.

In order to examine if the observed effects of SA on lymphocyte proliferation were mitogen-specific, additional experiments were performed using different T-cell mitogens (Fig. 4). Lymphocytes stimulated with either Con A or PHA were also affected by SA in a similar concentration-specific manner as those observed with the anti-CD3 mitogen, although there was some variability in the enhanced proliferative effect of SA at lower concentrations.

3.2. IL-2 assays and addition of exogenous IL-2

Fig. 5A shows IL-2 concentrations measured in the supernatant obtained from cultured PBMCs, 24 h after anti-CD3 stimulation with or without various amounts of SA added. Unstimulated culture cells alone are indicated as CA. The pattern of the proliferative response (Fig. 5B) in these experiments was similar to those shown in Fig. 3B, and the amount of IL-2 detected in the supernatant of the centrifuged cells was significantly lower than the anti-CD3-stimulated cells. Moreover, IL-2 levels were low even at

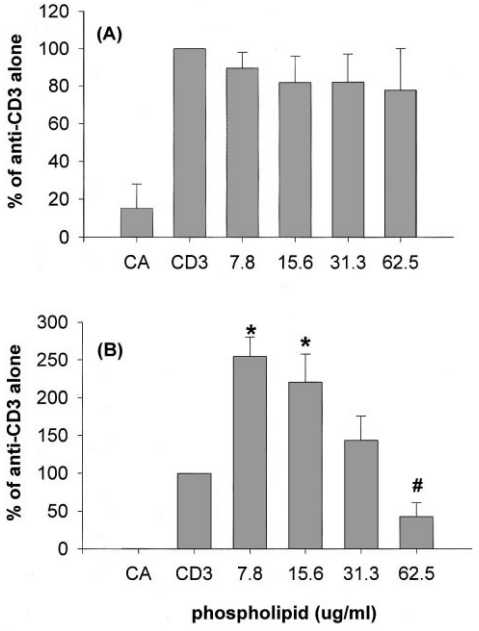

Fig. 3. (A) Incorporation of [^{3}H]thymidine into cultures of human PBMC (2×10^5/well) stimulated with 50 ng/ml of anti-CD3 (UCHT1) in the absence or presence of increasing concentrations of large aggregates (LA; $40\,000\times g$ pellet) obtained from normal rat lung lavage. Each bar represents the mean ± S.E.M. of six determinations. CA (cells alone) represents the negative control. (B) Incorporation of [^{3}H]thymidine into cultures of human PBMC (2×10^5/well) stimulated with 50 ng/ml of anti-CD3 (UCHT1) in the absence or presence of increasing concentrations of small aggregate (SA; $40\,000\times g$ supernatant) obtained from normal rat lung lavage. Each bar represents the mean ± S.E.M. of six determinations. CA (cells alone) represents the negative control. *$P \leq 0.05$, significantly different from positive control cells plus CD-3. #Significantly different ($P \leq 0.05$) from * values.

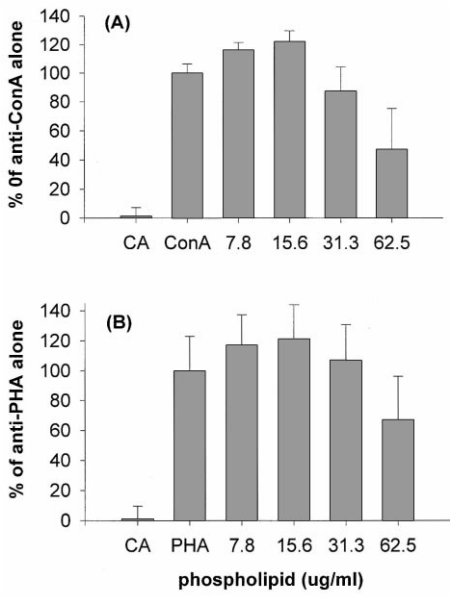

Fig. 4. Incorporation of [^{3}H]thymidine into cultures of human PBMC (2×10^5/well) stimulated with either 1.0 μg/ml of concanavalin A (A), or 1.0 μg/ml of phytohemagglutinin (B) in the presence or absence in increasing doses of small aggregates (SA; $40\,000\times g$ supernatant) obtained from normal rat lung lavage. Each bar represents the mean ± S.E.M. of six determinations. CA (cells alone) represents the negative control.

concentrations of SA which were shown to augment lymphocyte proliferation.

Fig. 6 shows the results of experiments in which exogenous IL-2 was added to the cell cultures. When concentrations of either 10 IU/ml (Fig. 6A) or 100 IU/ml (Fig. 6B) of IL-2 were added to PBMC cultures, the effects of adding SA were abolished at all concentrations up to 62.5 μg/ml. Moreover, both concentrations of IL-2 tested actually augmented the anti-CD3 driven proliferative response, which was approximately 3-fold at 10 IU/ml of IL-2, and 9-fold at 100 IU/ml compared to both anti-CD3 responses. These data suggest that exogenous IL-2 either overcame or reversed the inhibitory effects of higher concentrations of SA on proliferation.

4. Discussion

Although the primary biophysical function of pulmonary surfactant is to reduce surface tension at the air–liquid interface, surfactant is also the first substance that inhaled pathogens encounter when deposited within the alveoli. This fact, together with the extensive in vitro data demonstrating that specific surfactant components may affect cellular inflammatory responses, suggests that an intact surfactant system may protect the host from chronic immune responses to foreign particles. Initial studies evaluating the effects of surfactant components on host immune-cell responses showed that the lipid component of surfactant exhibited anti-inflammatory properties [13,14]. Specifically, phosphatidylglycerol and phosphatidylcholine significantly inhibited cellular immune function [15]. More recently, the two hydrophilic surfactant-associated proteins, SP-A and SP-D, have also been implicated in downregulating immune responses [7,8]. The present study is the first to evaluate the effects of isolated surfactant aggregate forms on these types of responses. Our results showed that SA subfractions had a significant effect

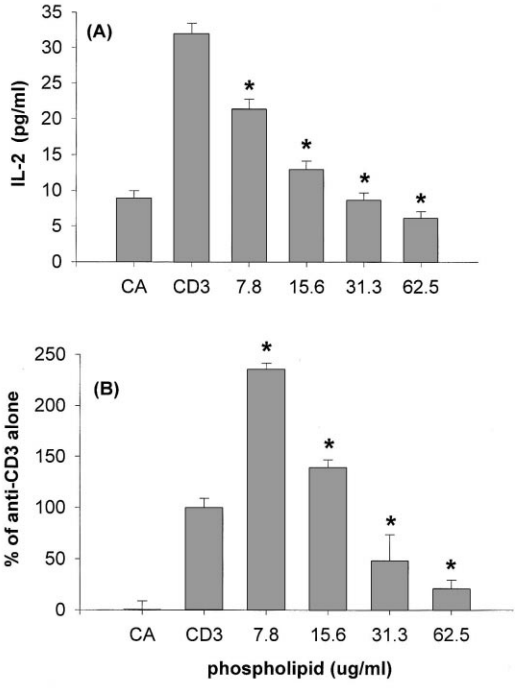

Fig. 5. Human PBMC (1×10^6/well) either untreated (cell alone; CA) or stimulated with 50 ng/ml of anti-CD3 and cultured in the presence or absence of increasing doses of small aggregates for 24 h. The supernatants were assayed for soluble IL-2 using a commercial enzyme-linked immunoassay (EIA) (A). A parallel assay for proliferation was completed and the results are shown in B. CA represents the untreated negative control. $^*P\leq0.05$, significantly different from positive control cells stimulated with CD-3.

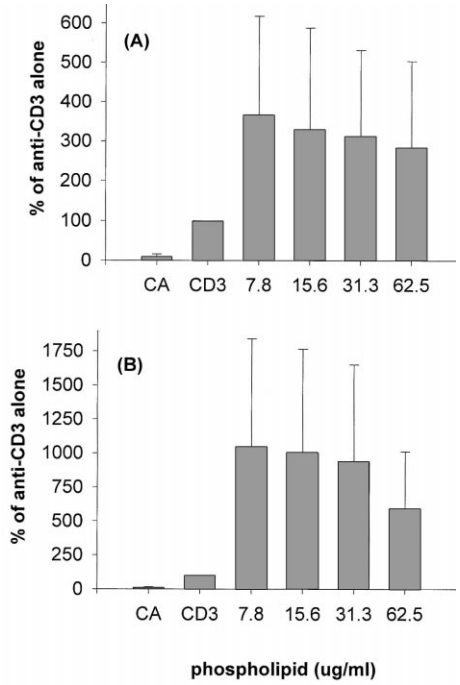

Fig. 6. Effect of the addition of exogenous IL-2 on the antiproliferative effects of SA. The incorporation of [^{3}H]thymidine (expressed as percentage of CD3 alone) in cultures treated with increasing concentrations of SA from 7.8 to 62.5 μg/ml equivalents of phospholipids including 10 IU/ml (A) or 100 IU/ml (B) of recombinant human IL-2. Group mean of pooled data from experiment conducted on PBMC from three donors.

on lymphocyte proliferation depending on the concentrations used, whereas the LA subfractions had no effect on cellular proliferation in this assay system.

The rationale for utilizing lymphocytes for these experiments stems from data that suggest T lymphocytes play an important role in the host's innate immune response within the lung. For example, functional T cells and the various mediators secreted from these cells, including IL-2, regulate the influx of other inflammatory cells such as monocytes, neutrophils, and eosinophils into the lung in response to a local insult. These latter cells are central to a host's inflammatory response aimed at controlling or minimizing the deleterious effects of the insult [16]. In some situations, however, this alveolar inflammatory response may be overwhelming, resulting in acute lung injury and respiratory failure. A clinical example of such a condition is the acute respiratory distress syndrome (ARDS), which is characterized by marked pulmonary inflammation in response to a variety of potential insults. Once established, ARDS has a mortality of 40–60%. A number of other lung diseases also involve a significant alveolar inflammatory response, including interstitial pulmonary fibrosis, tuberculosis and sarcoidosis. These conditions are all mediated via an upregulated delayed, hypersensitivity immune response that involves T lymphocytes, and hence may be influenced by alterations in the pulmonary surfactant system [17]. Indeed, pulmonary surfactant alterations have been demonstrated in patients with these various diseases.

The amount of surface-inactive SA subtypes was shown to be increased relative to LA in broncho alveolar lavage samples obtained from patients with severe ARDS [18] compared to normal subjects. This observation was consistent with the numerous animal studies utilizing models of acute lung injury [19,20]. Moreover, these studies showed that the increased SA forms within the injured lung contributed to the lung dysfunction associated with the lung injury. The results of the present study suggest that in addition to the obvious biophysical consequences of altered surfactant aggregate forms, the host's immune response to various insults may also be compromised. These changes may therefore contribute to the pathophysiology of the inflammatory response occurring within the injured lung.

In our experiments, the whole lung lavage fraction, or 'total surfactant' obtained from normal adult rats inhibited mitogen-stimulated proliferation of human PBMCs. Interestingly, the LA and SA isolated from this total surfactant sample had markedly different effects on cellular proliferation when tested separately. While LA had minimal effects on proliferation, SA fractions exhibited a biphasic response. While exposure to SA at doses of less than 31.25 μg/ml tended to exert a modest stimulatory effect, at concentrations of 62.5 μg/ml and above (of phospholipid equivalents), SA resulted in less T-cell proliferation compared to mitogen-stimulated cells alone. At the present time it is not possible to suggest a potential mechanism whereby low doses of SA tend to enhance proliferation. One can only look to other immune modulators where, for example, transforming growth factor-β can be a mitogen at low concentrations ($<$nM) and an anti-mitogen at high doses

(>nM). The results presented here suggest that the inhibitory effects on proliferation exhibited by the total surfactant sample may have been due to the overriding influence of the SA component of total alveolar surfactant. The specific mechanisms responsible for these effects are unknown, although it was evident that this inhibitory effect was not mitogen-specific (Fig. 4). That the anti-proliferative activity was not removed by heating the samples to 95°C might lead to the suggestion that the factor(s) responsible are unlikely to be proteins. However, it may also be that the interaction with the lipid components of SA leads to protection from denaturation for the factors. One of the definite effects seen in these experiments was the inhibition of the release of IL-2 into the supernatant of cultured lymphocytes which the inhibition of proliferation as the dose of SA increased. Furthermore, as addition of exogenous IL-2 not only reversed the inhibitory effects of SA, but enhanced proliferation with high concentrations of IL-2 added, it is likely that SA inhibited lymphocyte proliferation via inhibiting IL-2 release from the stimulated cells.

In our previous studies evaluating the effects of surfactant components on lymphocyte proliferation, we utilized purified SP-A and/or SP-D. These studies showed similar inhibitory effects of these proteins on human PBMC proliferation as those observed with the SA in the present study [7,8]. Other studies have shown that surfactant phospholipids alone inhibited T-cell proliferation [14,15,21]. Based on current data, it appears that the effects of total surfactant on lymphocyte proliferation may differ compared to those effects observed with the individual subfractions isolated from alveolar surfactant. The reason for these differences are likely related to the nature of the material being tested in the various experiments. For example, the majority of SP-A is contained in the LA fraction of alveolar surfactant (Fig. 2), although LA had no significant effect on T-lymphocyte proliferation (Fig. 3A). Alveolar SP-A is thought to be closely associated with multilamellar and tubular myelin-like structures. This lipid association may serve to inactivate the immune-modulating properties of SP-A by preventing its accessibility to the cells in culture. On the other hand, SP-A was present in significantly lower amounts within the SA fractions, although this protein may not be as tightly associated with the lipid components of the small vesicular structures making up SA. SP-A may be more accessible to the responder cells in culture and therefore exert effects somewhat similar to purified SP-A. In addition, since the Western blots of SP-D showed variable amounts of this protein in the different batches of LA and SA utilized in this study, it is unlikely that SP-D was a major factor responsible for the results of our experiments. The phospholipid profiles of both LA and SA were also quite similar, suggesting that a specific phospholipid component of the aggregate forms did not account for the differences observed between the aggregate forms. Although lysophosphatidylcholine (LPC) was observed to be slightly higher in SA compared to LA, separate experiments in which the specific effects of LPC on T-cell proliferation indicated that there was no significant effect of LPC on anti-CD3-stimulated T-cell proliferation (data not shown). Therefore, based on the present observations it is not possible to propose with any certainty what the nature of the anti-proliferative factor(s) are responsible for the SA effect. It may be that the surfactant-associated proteins are important and that their composition, as well as their conformation within the different aggregate subtypes within the alveolar space, are critical. Further studies evaluating the specific mechanisms involving in these protein–cellular interactions are required.

In view of the abundant evidence of altered surfactant aggregate forms in various disease states, it is tempting to speculate on the relevance of these in vitro findings in relation to the in vivo situation. In normal lungs, the relative proportion of surfactant aggregate forms within the airspace is quite consistent among various species, and likely contributes to the maintenance of the hyporesponsive state of lymphocytes within the lung. In acute lung injury, including patients with ARDS, a significant increase in the SA subfraction within the airspace occurs as injury progresses. These aggregate changes may affect the host's inflammatory response either in a positive or negative manner. In the latter situation, for example, these changes may represent a major factor contributing to a host's maladaptive inflammatory response and the subsequent acute lung injury. A more extensive evaluation of the surfactant system at different stages of lung injury, and correlation of

these changes with cellular immune responses, are required in order to determine the specific role of the surfactant system in this complex immune/inflammatory response. Results of the present study represent an important link between previous in vitro studies utilizing specific surfactant components, and the alveolar surfactant forms as they exist within the alveolar space in vivo.

In summary, we have evaluated the effects of large and small surfactant aggregates on mitogen-stimulated T-lymphocyte proliferation. LA fractions were able to reduce surface tension but had no effect on lymphocyte proliferation. The surface-inactive SA fraction had significant, albeit biphasic effects on lymphocyte proliferation. We speculate that SA may play an important role in the regulation of lymphocyte activation and proliferation within the lung, and that these changes may contribute to the pathophysiology of various inflammatory lung diseases.

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