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Surfactant protein-A levels increase during *Pneumocystis carinii* pneumonia in the rat

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Surfactant protein-A levels increase during Pneumocystis carinii *pneumonia in the rat. D.S. Phelps, T.M. Umstead, R.M. Rose, J.A. Fishman. ©ERS Journals Ltd 1996.*

ABSTRACT: In bronchoalveolar lavage (BAL) of human immunodeficiency virus (HIV)-infected patients with *Pneumocystis carinii* pneumonia and in lungs of glucocorticoid-immunosuppressed rats infected with *P. carinii*, surfactant phospholipid levels are reduced. However, levels of the surfactant-associated protein-A (SP-A) in BAL are 4–5 times higher than normal in patients with *P. carinii* pneumonia. In this study, we examined the effects of glucocorticoid immunosuppression and *P. carinii* infection on SP-A messenger ribonucleic acid (mRNA) and protein levels in rat lungs.

Rats were immunosuppressed by adding dexamethasone to their drinking water and were infected with *P. carinii* by intratracheal instillation of the organism. SP-A was measured by enzyme-linked immunosorbent assay (ELISA) and SP-A mRNA by hybridization of Northern blots with an SP-A complementary deoxyribonucleic acid (cDNA) probe.

There was a severalfold increase in SP-A protein and mRNA levels in uninfected glucocorticoid-treated rats. However, contrary to what has been reported with the surfactant-associated lipids, SP-A mRNA and protein levels in *P. carinii*-infected animals were significantly higher than those found in the uninfected, immunosuppressed animals.

Our results demonstrate that SP-A increases, probably as a result of elevated mRNA levels, in immunosuppressed rats with *P. carinii* infection and are consistent with our findings in HIV-positive patients with *P. carinii* pneumonia.
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Pneumonia due to *Pneumocystis carinii* is a major cause of morbidity and mortality in immunocompromised patients, especially in those infected with the human immunodeficiency virus (HIV), and in those receiving chemotherapy or immunosuppression for organ transplantation or other conditions [1, 2]. We have reported that surfactant-associated protein-A (SP-A) is markedly elevated in bronchoalveolar lavage (BAL) fluid from patients with acquired immune deficiency syndrome (AIDS)-related pneumonias caused by *P. carinii* [3]. Significant decreases [4, 5], or abnormal lipid profiles [6], in surfactant phospholipid levels have recently been reported in BAL from HIV-positive patients. The finding of reduced phospholipids is consistent with experimental observations made in the glucocorticoid- immunosuppressed rat, a model commonly used to study *P. carinii* pneumonia. In animals receiving corticosteroids and infected with *P. carinii*, the phospholipid components of surfactant were found to be markedly reduced, whilst a large increase in the lipids was present in uninfected, immunosuppressed animals [7].

In recent years, the structure of SP-A has been elucidated. A number of striking similarities have been noted between SP-A and proteins involved in host defence function [9]. Ultrastructurally, the native conformation of SP-A resembles that of the complement component, C1q [8]. There are also similarities in the structure, sequence, and carbohydrate-binding domains of SP-A and the mannose-binding protein and conglutinin. These proteins and others belong to a group referred to as "collectins" [9]. Many of the collectins have been shown to affect phagocytic function. The possibility that SP-A might modulate phagocyte function has been supported by recent *in vitro* experiments [10–16], although little is known about its role *in vivo*. In addition, recent studies from our laboratory have demonstrated that SP-A can also influence immune cell function by stimulating mitogen-induced proliferation and cytokine production [17, 18].

SP-A expression is increased within 24 h of dexamethasone injection [19], but no information is available about the effects of long-term oral administration of low doses of glucocorticoids, like those used in the present study, on SP-A metabolism. We hypothesized that SP-A expression would be altered by the infection of glucocorticoid-immunosuppressed rats with *P. carinii*. The inability of glucocorticoid-immunosuppressed rats to adequately remove *P. carinii* from the alveolus by phagocytosis may be related to these changes in SP-A levels

in the alveolar lining layer. Interaction between SP-A and *P. carinii* has been demonstrated [20], and studies by our group suggest that this interaction interferes with the phagocytosis of *P. carinii* by alveolar macrophages ([10] - manuscript submitted). In the present study, we examined the production of SP-A messenger ribonucleic acid (mRNA) and protein in rats over time, in the presence and absence both of glucocorticoid-induced immunosuppression and infection with *P. carinii*.

Materials and methods

Animal model

Viral antibody-free (VAF) barrier-raised Sprague-Dawley rats (150 g body weight (BW)) were obtained from Harlan Laboratories (Indianapolis, IN, USA) and were housed in microisolator cages or in cage racks kept in isolation cubicles. All *P. carinii*-infected rats and uninfected rats were housed in separate isolation cubicles. There was no spontaneously occurring *P. carinii* pneumonia in the immunosuppressed control rats, confirming the absence of latent *P. carinii*. The animals were allowed to feed *ad libitum* on normal rat chow or a pelleted normocaloric low protein (8%) diet (ICN Biomedicals, Irvine, CA, USA). For immunosuppressed animals, drinking water was freely available and contained dexamethasone (Sigma Chemical, St. Louis, MO, USA) at a concentration of 1 $mg \cdot L^{-1}$. All animals were weighed weekly. Rats consumed a daily average of 1–2 µg of dexamethasone·kg^{-1} BW during the course of the experiment.

After at least 1 week of immunosuppression or comparable control treatment, some animals were anaesthetized, the trachea was surgically exposed and 0.1 mL of a saline suspension of *P. carinii* was injected [21]. Approximately 10^7 organisms, containing about 5% cysts, were instilled into each animal. Control animals were injected with an equal volume of sterile normal saline. Each inoculum was either inspected microscopically or was cultured for bacteria on blood agar or for fungi using Sabouraud's medium. Infected animals were sacrificed at intervals ranging 1–7 weeks. At sacrifice, lungs were removed, impression smears made, cultures taken, and the lung tissue was frozen in liquid nitrogen and stored at -70°C for later ribonucleic acid (RNA) and protein analysis. Methenamine silver or modified Giemsa (Diff-Quik, Sigma Chemical, St. Louis, MO, USA) staining of histological sections and impression smears were used to assess *P. carinii* infection. For every time-point and condition, a minimum of three animals was analysed.

Control conditions

Several types of control animals were used for these studies. The first of these were animals that were immunosuppressed with glucocorticoid and fed the low protein diet, but were not infected with *P. carinii*. A second group of control animals was fed low protein diet, but drank normal water. These "diet only" animals were compared to similarly treated animals that were infected with *P. carinii*.

Measurement of SP-A levels

In order to measure SP-A protein levels in the lung tissue, lungs were pulverized after freezing in liquid nitrogen. The pulverized, frozen lung tissue was weighed, added to an extraction solution (3 M urea, 1% 2-mercaptoethanol) at a concentration of 100 mg tissue·mL^{-1} solution, mixed with a vortex mixer, sonicated, and centrifuged at 1,000×g for 10 min. An aliquot of the extract was then removed, centrifuged at 10,000×g and diluted 1:10 with distilled water. For SP-A enzyme-linked immunosorbent assay (ELISA) this aliquot was further diluted 1:100 with 0.5 M carbonate-bicarbonate coating buffer (pH 9.6), and duplicate samples were applied to Immulon 2 ELISA plates (Dynatech Corp. Arlington, VA, USA).

SP-A levels were measured using an indirect ELISA protocol with a rabbit anti-human SP-A polyclonal antiserum as the primary antibody [22]. Rat SP-A purified from surfactant by preparative isoelectric focusing was used as a standard. The total protein content of the samples was determined using the BCA protein assay (Pierce, Rockford, IL, USA) with ribonuclease A (Boehringer Mannhein, Indianapolis, IN, USA) used as a standard protein.

RNA isolation and hybridization

Total lung RNA was isolated by homogenization with RNAzol B (Cinna/Biotecx, Houston, TX, USA). Electrophoresis, hybridization, and analysis were performed as described previously [19]. Multiple lung RNA samples from animals of each condition were subjected to formaldehyde gel electrophoresis, loading 20 µg of lung RNA for each lane. The RNA was transferred to a nylon membrane (Genescreen, DuPont Research Products, Boston, MA, USA). A photograph of each blot was taken under ultraviolet illumination to record the presence of 18S and 28S ribosomal RNA markers and to assess the quality and evenness of loading of the RNA aliquots (fig. 1A). The membranes were prehybridized, hybridized and washed in the manner recommended by the vendor (DuPont Research Products, Boston, MA, USA). The complementary deoxyribonucleic acid (cDNA) for rat SP-A was labelled with ^{32}P-deoxycytidine triphosphate (dCTP) (3,000 Ci·$mMol^{-1}$) to specific activities of 6–8×10^7 counts per minute (cpm)·μg^{-1} DNA by nick translation (Oncor, Gaithersburg, MD, USA). The hybridization was routinely performed with 1–2×10^7 cpm of probe per mL of hybridization solution. The hybridized membrane was exposed to Kodak XAR5 film (Eastman Kodak Co., Rochester, NY, USA) with Cronex Lightning Plus intensifying screens (DuPont Research Products, Boston, MA, USA) at -70°C. The film was developed after an exposure time sufficient to produce bands that could be adequately quantified by laser densitometry (fig. 1B). Densitometry was performed on samples of control rats, those treated with dexamethasone or on low protein diets, and those infected with *P. carinii*.

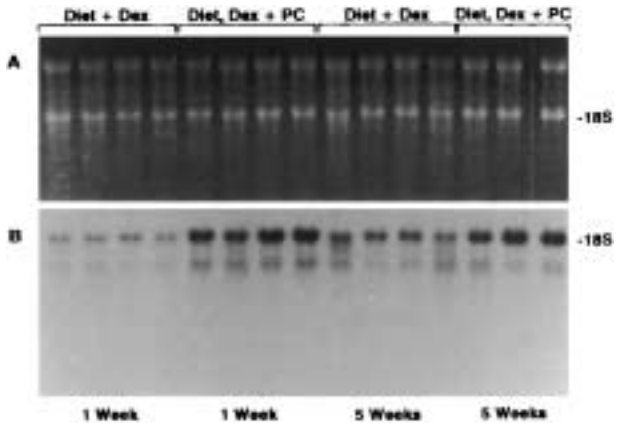

Fig. 1. – Northern blot for SP-A mRNA in lung tissue. Samples containing 20 µg of RNA were subjected to electrophoresis, blotting, hybridization and autoradiography. Samples are from immunosuppressed animals with and without *Pneumocystis carinii* infection for 1 and 5 weeks. Panel A has been stained to demonstrate ribosomal RNA bands. Panel B depicts an autoradiograph of the same gel after hybridization with radiolabelled SP-A probe. Densitometry was performed on both labelled bands and the sum used for statistical analysis. SP-A: surfactant-associated protein-A; mRNA: messenger ribonucleic acid; RNA: ribonucleic acid; Dex: dexamethasone; PC: *P. carinii*.

Statistical methods

In each experiment, the mean of the control protein and mRNA values for that experiment were set equal to 100% and other experimental points were expressed as the percentage of the indicated control group. Groups were then compared by t-test using the Sigma Stat program (Jandel Scientific, San Rafael, CA, USA).

Results

Pneumocystosis model

By examination of Giemsa-stained impression smears, all of the rats inoculated with *P. carinii* and receiving dexamethasone had diffuse pneumocystosis at the time of sacrifice. None of the control or experimental animals had evidence of bacterial or fungal infection. None of the *P. carinii* inocula had bacteria or fungi isolated in culture. Impression smears made of lung tissue and Giemsa-stained from these immunocompetent ("diet only") animals sacrificed at 5 weeks showed no evidence of *P. carinii*. Also, *P. carinii* mRNA was not detected in these animals when Northern blots were hybridized with two different rat *P. carinii* cDNA probes [23]. Both of these methods detected *P. carinii* in lung tissues from histologically infected glucocorticoid-immunosuppressed rats.

SP-A mRNA and protein determinations during immunosuppression

SP-A mRNA levels from uninfected glucocorticoid-immunosuppressed rats were compared with a control group receiving the low protein diet, but no glucocorticoid ("diet only" control). SP-A mRNA levels in the glucocorticoid-treated animals were three to five times

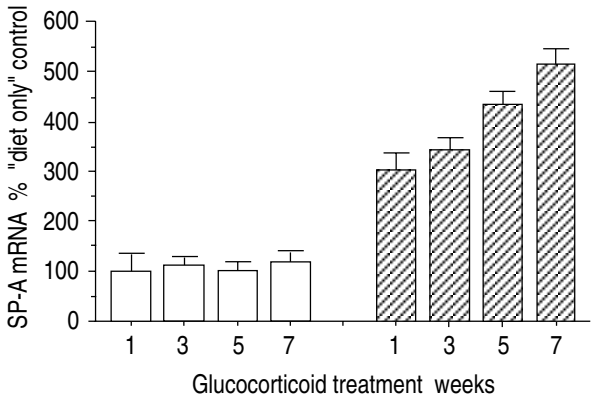

Fig. 2. – Effect of dexamethasone treatment on SP-A mRNA levels. Northern blots containing total RNA were hybridized with a ^{32}P-labelled SP-A cDNA probe and subjected to autoradiography and densitometry to quantitate levels of SP-A mRNA during the course of immunosuppression. Values are presented as mean±SEM. Low protein "diet only" control values n≥3 for each data point. All dexamethasone-treated (Dex) values are significantly different from controls ($p<0.003$) n≥4 for each data point. cDNA: complementary deoxyribonucleic acid. For further abbreviations see legend to figure 1. ▭ : diet only; ▨ : diet and dexamethasone.

greater than those of the "diet only" controls over 1–7 weeks (fig. 2). In figure 2, control values at 1 week are set equal to 100% and all other data are expressed as percentage of control. Lungs were taken for analysis after 1, 3, 5 and 7 weeks of treatment and SP-A mRNA levels were determined by hybridization. As seen in figure 2, lungs from rats treated with glucocorticoid (Dex) contained SP-A mRNA in amounts well above control levels ($p<0.05$) within 1 week. SP-A mRNA levels gradually increased over time to about five times control values.

Total lung SP-A protein content was measured by ELISA in the same animals (fig. 3). These measurements were made in a homogenate of lung tissue and, therefore, include both intracellular and secreted SP-A. Levels of SP-A in rats receiving dexamethasone in addition to the low protein diet were increased over control values at 1 week, and continued to rise for the duration of the experiment. At 7 weeks, there was a greater than fivefold elevation over control levels.

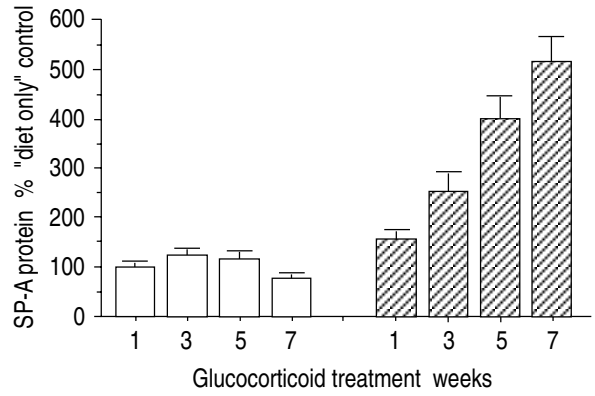

Fig. 3. – Effect of dexamethasone (Dex) treatment on SP-A protein levels in a lung homogenate. Values are presented as mean±SEM. n≥4 for each data point. Dexamethasone-treated samples are significantly different from control samples ($p<0.02$). ▭ : diet only; ▨ : diet and dexamethasone. SP-A: surfactant associated protein A.

SP-A mRNA and protein during P. carinii *infection*

Further increases in SP-A mRNA levels were observed when immunosuppressed animals were infected with *P. carinii*, as compared to immunosuppressed but uninfected control rats after 1 and 5 weeks of infection. Figure 1 shows an autoradiograph of a representative Northern blot hybridized with SP-A cDNA. SP-A mRNA is comprised of both bands [19]. Animals received the low protein diet and dexamethasone for either 1 or 5 weeks. Infection with *P. carinii* increased SP-A mRNA levels at both time-points. The densitometric quantitation of these changes is graphically depicted in figure 4a. Similar increases after *P. carinii* infection were not seen in rats that were fed the low protein diet without dexamethasone (fig. 4b). When SP-A protein levels were measured (fig. 5) in glucocorticoid-immunosuppressed rats a 2.5 fold increase over control values (~800 μg SP-A·100 mg^{-1} lung tissue) in SP-A was detected within 1 week of instillation of *P. carinii* (fig. 5a). SP-A levels continued to increase to more than five times the initial control values by 5 weeks. SP-A levels in the infected rats at 5 weeks were twice those of the uninfected control animals treated for the same time-period despite rising levels of SP-A observed in uninfected rats.

SP-A levels in rats receiving the low protein diet but no dexamethasone treatment ("diet only" control) changed little over 5 weeks (fig. 5b). In this group of animals, inoculation with *P. carinii* caused no significant change

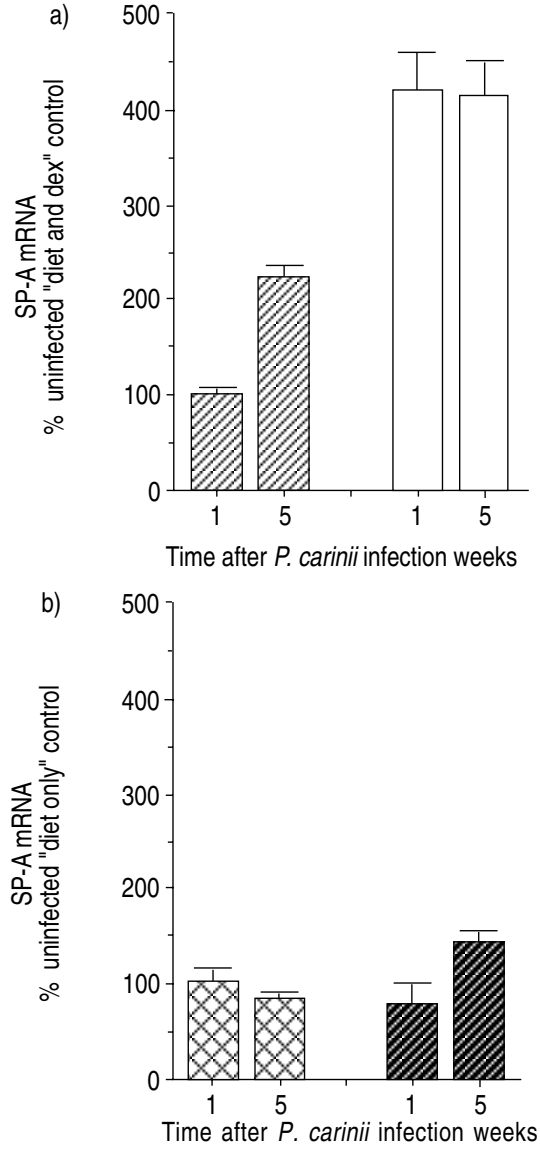

Fig. 4. – Effect of *P. carinii* infection on SP-A mRNA levels. a) Values for dexamethasone-treated rats with and without *P. carinii* infection. b) Values from animals receiving the low protein diet with and without *P. carinii* infection. All data are expressed as the mean±SEM of the SP-A mRNA levels at 1 and 5 weeks after *P. carinii* infection. In each panel the appropriate controls ("diet and dex" or "diet only") have been set equal to 100%. n≥4 at each point. ▨: diet and dex; □ : diet, dex and PC; ▩ : diet only; ▩ : diet and PC. For abbreviations see legend to figure 1.

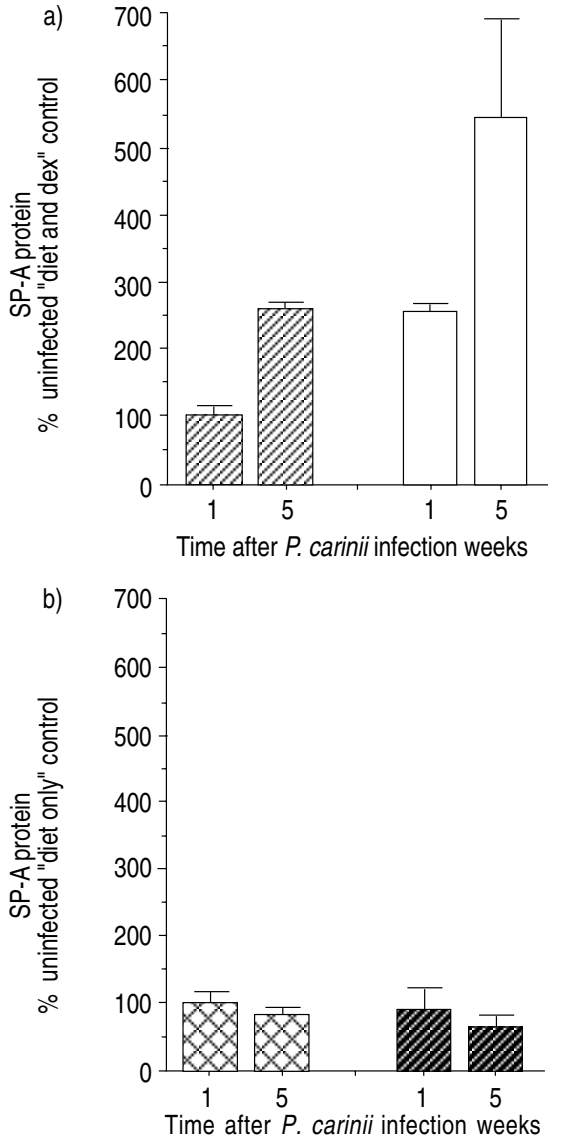

Fig. 5. – Effect of *P. carinii* infection on SP-A levels. Levels of SP-A in lung homogenate from rats treated with a) glucocorticoid and diet; or b) with diet only, and infected with *P. carinii* along with controls. In each panel the appropriate controls ("diet and dex" or "diet only") have been set equal to 100%. n≥4 at each point. ▨ : diet and dex; □ : diet, dex and PC; ▩ : diet only; ▩ : diet and PC. For abbreviations see legend to figure 1.

in SP-A levels.

Discussion

Pneumonia due to *P. carinii* is a major cause of morbidity and mortality in immunocompromised patients. In an earlier study, we found elevated SP-A levels in bronchoalveolar lavage fluids from HIV-infected patients with pneumonia resulting from *P. carinii* and from other pathogens [3]. In the present study, we demonstrated that both SP-A mRNA and protein levels were increased in the lung tissue of rats that were immunosuppressed by daily glucocorticoid treatment. We reported similar increases in SP-A levels within 24 h following a single injection of dexamethasone at much higher doses [19]. In that study, the injection of low doses of dexamethasone (2 $\mu g \cdot kg^{-1}$ BW), like those received orally by the rats in the present study, did not increase SP-A levels [19]. However, continued daily consumption of low levels of dexamethasone in the drinking water increased both SP-A mRNA and protein levels within 1 week, and these increases persisted for the duration of the experiment. These elevated SP-A values continued to increase significantly after *P. carinii* infection. The augmentation both of mRNA and protein suggest increases at the transcriptional or pretranslational level, although mechanisms such as decreased degradation of SP-A cannot be ruled out. In the absence of glucocorticoid-induced immunosuppression, intratracheal injection of *P. carinii* did not result in infection and neither SP-A mRNA nor protein levels were increased.

A number of recent *in vitro* studies have suggested that SP-A might be involved in pulmonary host-defence, and in phagocytosis in particular [11–18], and an interaction between SP-A and *P. carinii* has been demonstrated [20]. Preliminary studies suggest that although SP-A may stimulate the *in vitro* phagocytosis of some organisms [10, 11, 15], such as *Candida albicans*, *Staphylococcus aureus* and Herpes simplex, it may interfere with the phagocytosis of *P. carinii* by alveolar macrophages [10]. Similar organism-specific regulation in the inflammatory response has also been shown for the secretion of cytokines by macrophages [24]. Elucidating the role of SP-A and the other surfactant components in the pathogenesis of infection has been difficult due to the redundancy that characterizes many aspects of host defence function. Glucocorticoids inhibit many aspects of host defence function involving immune cells. However, the enhancement of SP-A expression when other immune cells are suppressed provides a situation where the effect of SP-A on host defence function can be examined independently.

In earlier analyses of BAL samples from HIV-infected patients with *P. carinii* pneumonia, SP-A levels that had been elevated in response to the *P. carinii* infection could not be differentiated from levels that had been elevated due to pre-existing HIV infection. It is possible that the type II cell hyperplasia in *P. carinii* pneumonia contributes to elevations in SP-A levels, although the glucocorticoid-induced increases are probably due to increased transcription. A significant aspect of the present study was the finding that SP-A was elevated by glucocorticoid prior to *P. carinii* infection. This suggests that although SP-A enhances some aspects of alveolar macrophage function *in vitro*, its presence in the lung in increased amounts may be involved in the development of pneumonia due to *P. carinii* in a glucocorticoid-immuno- suppressed animal. These findings raise questions about whether SP-A has a stimulatory effect in host defence against *P. carinii in vivo*. This lack of a protective effect may be related to the fact that the effects of SP-A on phagocytosis depend on the specific organism involved, its growth phase, and the phagocytic cell type under investigation [11–13].

Recent reports analysing the surfactant lipids in BAL from HIV-infected pneumonia patients report either reductions [4, 5] or abnormalities in lipid composition in these individuals [6]. A study using the rat model found that although the glucocorticoid treatment markedly elevated surfactant lipid levels, these levels were reduced to less than half of untreated control values when the animals became infected with *P. carinii* [7]. These reports and the results of the present study suggest the independent regulation of surfactant lipids and SP-A in *P. carinii* pneumonia. Other situations where individual surfactant components have been independently regulated have been described for the differential regulation of SP-A, other surfactant proteins, and the surfactant lipids by some cytokines and growth factors [25–27].

These alterations in the composition of surfactant could influence other aspects of immune cell function in addition to phagocytosis. Recently, the ability of surfactant components to influence proinflammatory cytokine production has been reported [18, 28, 29]. In these studies, isolated surfactant lipids have an inhibitory influence, isolated SP-A stimulates cytokine production, and the combination of both can exert either stimulatory or inhibitory actions depending on the relative amounts of SP-A and surfactant lipids in surfactant. Therefore, in pneumonia due to *P. carinii*, the reduction in levels of surfactant lipids and the increases in SP-A may contribute to the development of the inflammation that often accompanies this condition.

SP-A has been reported to stimulate the phagocytosis of some pathogens *in vitro*. However, the immunosuppressive effects of steroids on alveolar macrophage function may predispose to infection both by altering phagocytosis and by organism-specific mechanisms related to altered metabolism of surfactant components. Whether the elevated levels of SP-A influence the macrophage or affect the phagocytosis of *P. carinii in vivo* remains to be determined, as does the question of whether altered metabolism of surfactant proteins and lipids may participate in susceptibility of the host to *P. carinii* pneumonia.

References

1. Fishman JA. *Pneumocystis carinii* pneumonia. *In*: Fishman AP, ed. Update: Pulmonary Diseases and Disorders. New York, McGraw-Hill, 1992; pp. 263–286.
2. Walzer PD. *Pneumocystis carinii*: recent advances in basic biology and their clinical application. *AIDS* 1993; 7: 1293–1305.

3. Phelps DS, Rose RM. Increased recovery of surfactant protein-A in AIDS-related pneumonia. *Am Rev Respir Dis* 1991; 143: 1072–1075.
4. Escamilla R, Prevost MC, Hermant C, Caratero A, Cariven C, Krempf M. Surfactant analysis during *Pneumocystis carinii* pneumonia in HIV-infected patients. *Chest* 1992; 101: 1558–1562.
5. Hoffman AGD, Lawrence MG, Ognibene FP, *et al*. Reduction of pulmonary surfactant in patients with human immunodeficiency virus infection and *Pneumocystis carinii* pneumonia. *Chest* 1992; 102: 1730–1736.
6. Rose RM, Catalano PJ, Koziel H, Furlong ST. Abnormal lipid composition of bronchoalveolar lavage fluid obtained from individuals with AIDS-related lung disease. *Am J Respir Crit Care Med* 1994; 149: 332–338.
7. Sheehan PM, Stokes DC, Yeh Y, Hughes WT. Surfactant phospholipids and lavage phospholipase A_2 in experimental *Pneumocystis carinii* pneumonia. *Am Rev Respir Dis* 1986; 134: 526–531.
8. Voss T, Melchers K, Scheirle G, Schafer KP. Structural comparison of recombinant pulmonary surfactant protein SP-A derived from two human coding sequences: implications for the chain composition of natural human SP-A. *Am J Respir Cell Mol Biol* 1991; 4: 88–94.
9. Thiel S, Reid KBM. Structures and functions associated with the group of mammalian lectins containing collagen-like sequences. *FEBS Lett* 1989; 250: 78–84.
10. Koziel H, O'Riordan D, Phelps D, *et al*. Surfactant protein-A inhibits binding and internalization of *Pneumocystis carinii* by alveolar macrophages. *Am Rev Respir Dis* 1992; 145: A247 (Abstract).
11. Manz-Keinke H, Plattner H, Schlepper-Shafer J. Lung surfactant protein-A (SP-A) enhances serum-independent phagocytosis of bacteria by alveolar macrophages. *Eur J Cell Biol* 1992; 57: 95–100.
12. McNeely TB, Coonrod JD. Comparison of the opsonic activity of human surfactant protein-A for *Staphylococcus aureus* and *Streptococcus pneumoniae* with rabbit and human macrophages. *J Infect Dis* 1993; 167: 91–97.
13. McNeely TB, Coonrod JD. Aggregation and opsonization of Type A but not Type B *Hemophilus influenzae* by surfactant protein-A. *Am J Respir Cell Mol Biol* 1994; 11: 114–122.
14. Van Iwaarden F, Welmers B, Verhoef J, Haagsman HP, Van Golde LMG. Pulmonary surfactant protein-A enhances the host-defense mechanism of rat alveolar macrophages. *Am J Respir Cell Mol Biol* 1990; 2: 91–98.
15. Van Iwaarden JF, Van Strijp JAG, Ebskamp MJM, Welmers AC, Verhoef J, Van Golde LMG. Surfactant protein-A is opsonin in phagocytosis of Herpes simplex virus type-1 by rat alveolar macrophages. *Am J Physiol* (*Lung Cell Mol Physiol* 5) 1991; 261: L204–L209.
16. Wright JR, Youmans DC. Pulmonary surfactant protein-A stimulates chemotaxis of alveolar macrophage. *Am J Physiol* (*Lung Cell Mol Physiol* 8) 1993; 264: L338–L344.
17. Kremlev SG, Umstead TM, Phelps DS. Effects of surfactant protein-A and surfactant lipids on lymphocyte proliferation *in vitro*. *Am J Physiol* (*Lung Cell Mol Physiol* 11) 1994; 267: L357–L364.
18. Kremlev SG, Phelps DS. Surfactant protein-A stimulation of inflammatory cytokine and immunoglobulin production. *Am J Physiol* (*Lung Cell Mol Physiol* 11) 1994; 267: L712–L719.
19. Floros J, Phelps DS, Harding HP, Church S, Ware J. Postnatal stimulation of rat surfactant protein-A synthesis by dexamethasone. *Am J Physiol* (*Lung Cell Mol Physiol* 1) 1989; 257: L137–L143.
20. Zimmerman PE, Voelker DR, McCormack FX, Paulsrud JR, Martin WJ. 120 kDa surface glycoprotein of *Pneumocystis carinii* is a ligand for surfactant protein-A. *J Clin Invest* 1992; 89: 143–149.
21. Bartlett MS, Fishman JA, Queener SF, Durkin MM, Jay MA, Smith JW. New rat model of *Pneumocystis carinii* infection. *J Clin Microbiol* 1988; 26: 1100–1102.
22. Rooney SA, Gobran LI, Umstead TM, Phelps DS. Secretion of surfactant protein-A from rat type II pneumocytes. *Am J Physiol* (*Lung Cell Mol Physiol 9*) 1993; 265: L586–L590.
23. Fishman JA. A gene family related to the major antigen gene of Pneumocystis. Bristol-Myers Squibb Symposium on Cell and Molecular Biology of Bacterial-Host Cell Interactions. Monterey, CA, February 1993.
24. Kondil O, Fishman JA, Koziel H, Pinkston P, Rose RM, Remold HG. Human immunodeficiency virus type I infection of human macrophages modulates the cytokine response to *Pneumocystis carinii*. *Infect Immun* 1994; 62: 644–650.
25. Ballard PL, Liley HG, Gonzales LW, *et al*. Interferon-gamma and synthesis of surfactant components by cultured human fetal lung. *Am J Respir Cell Mol Biol* 1990; 2: 137–143.
26. Dranoff G, Crawford AD, Sadelain M, *et al*. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science* 1994; 264: 713–716.
27. Whitsett JA, Clark JC, Wispe JR, Pryhuber GS. Effects of TNF-alpha and phorbol ester on human surfactant protein and MnSOD gene transcription *in vitro*. *Am J Physiol* (*Lung Cell Mol Physiol* 6) 1992; 262: L688–L693.
28. Thomassen MJ, Meeker DP, Antal JM, Connors MJ, Wiedemann HP. Synthetic surfactant (Exosurf) inhibits endotoxin-stimulated cytokine secretion by human alveolar macrophages. *Am J Respir Cell Mol Biol* 1992; 7: 257–260.
29. Thomassen MJ, Antal JM, Connors MJ, Meeker DP, Wiedemann HP. Characterization of Exosurf (surfactant)-mediated suppression of stimulated human alveolar macrophage cytokine responses. *Am J Respir Cell Mol Biol* 1994; 10: 399–404.