

Surfactant strengthens the inhibitory effect of C-reactive protein on human lung macrophage cytokine release

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Casals, Cristina, Javier Arias-Díaz, Fernando Valiño, Alejandra Sáenz, Cruz García, José L. Balibrea, and Elena Vara. Surfactant strengthens the inhibitory effect of C-reactive protein on human lung macrophage cytokine release. *Am J Physiol Lung Cell Mol Physiol* 284: L466–L472, 2003. First published November 15, 2002; 10.1152/ajplung.00325.2002.—In this study we investigated the effect of acute-phase levels of C-reactive protein (CRP) on cytokine production by pulmonary macrophages in the presence or absence of pulmonary surfactant. Both human alveolar and interstitial macrophages as well as human surfactant were obtained from multiple organ donor lungs. Precultured macrophages were stimulated with LPS alone or together with IFN- γ in the presence or absence of CRP, surfactant, and combinations. Releases of TNF- α and of IL-1 β to the medium were determined. We found that CRP could modulate lung inflammation in humans by decreasing the production of proinflammatory cytokines by both alveolar and interstitial macrophages stimulated with LPS alone or together with IFN- γ . The potential interaction between CRP and surfactant phospholipids did not overcome the effect of either CRP or surfactant on TNF- α and IL-1 β release by lung macrophages. On the contrary, CRP and pulmonary surfactant together had a greater inhibitory effect than either alone on the release of proinflammatory cytokines by lung macrophages.

human lung surfactant; interstitial macrophages; alveolar macrophages; lipopolysaccharide

C-REACTIVE PROTEIN (CRP) is a member of the pentraxin family of plasma proteins. These proteins are highly conserved in evolution and precede the development of the adaptive immune response (12). They are made up of five identical globular subunits arranged in a cyclic pentamer shape appearing as a doughnut in electron imaging (31). All pentraxins bind two Ca²⁺ per subunit, which are necessary for expression of phosphocholine binding activity (34). CRP binds to the pneumococcal cell wall C-polysaccharide, other phosphocholine-containing polysaccharides present in the cell surface of various gram-negative bacteria, and *Mycoplasma*, as well as phosphatidylcholine in lipid bilayers (34). CRP also binds to C1q (31) and phagocytic cells in mice and in humans

(39, 32) through FC receptors. Fc γ RIa, the high-affinity IgG receptor, binds CRP with low affinity, whereas Fc γ RIIa, the low-affinity IgG receptor, binds CRP with high affinity (30). It is proposed that the binding site of CRP to C1q or cells is located on the face of each subunit opposite the phosphocholine-binding site (31).

CRP is considered the prototypical acute-phase reactant in humans. The synthesis and secretion of CRP notably increases in serum following tissue injury, infection, or inflammation (12). Normal human serum concentrations of CRP are <1 μ g/ml; however, after inflammation or sepsis, serum levels can increase as much as 1,000-fold (12, 17). The magnitude of this increase correlates with the extent of tissue injury or the severity of the inflammatory state. CRP is produced predominantly by hepatocytes in response to proinflammatory cytokines (12). Alveolar macrophages can also produce and secrete CRP to the alveolar space (11). The CRP mRNA levels in isolated macrophages are upregulated by in vitro lipopolysaccharide (LPS) stimulation (11). Patients with sepsis-induced acute respiratory distress syndrome (ARDS) have elevated levels of CRP in both plasma and the bronchoalveolar lavage (BAL) (17, 20). In addition, elevated levels of CRP are found in pulmonary surfactant from transplanted lungs (10).

The increased concentration of CRP in both serum and alveolar fluid after inflammation or sepsis suggests that it fulfills an important biological role. Many of the known properties of CRP are manifested by its interactions with immunologic effector systems. CRP activates the complement system via the classic pathway, promotes phagocytosis and activation of platelets, and enhances LPS-induced production of interleukin (IL)-1 β by human blood monocytes (12). These data are consistent with proinflammatory action of CRP in serum. However, the precise function of CRP in the alveolar fluid remains unclear. There is controversy over whether CRP enhances or suppresses the host inflammatory response to LPS in the alveolar space (13, 25). In these studies, the interaction of CRP with alveolar macrophages was studied in the absence of

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pulmonary surfactant, a membranous material that lines the alveolar epithelium and the alveolar macrophages.

We recently found that CRP present in the cell-free BAL of transplanted lungs is recovered in the floating pulmonary surfactant fraction (10), indicating that CRP binds to surfactant membranes. Because CRP binds avidly to these membranes, it is reasonable to think that surfactant membranes could affect the mode of CRP presentation to its receptors on macrophages. The first objective of the present study was to explore whether CRP stimulates or decreases the inflammatory response of human lung macrophages activated with LPS or the combination LPS and interferon (IFN)- γ . The second objective was to investigate whether pulmonary surfactant affects the CRP-mediated cytokine production by pulmonary macrophages. These studies were done in both human interstitial and alveolar macrophages. Although alveolar macrophages are continuously in contact with pulmonary surfactant in vivo, interstitial macrophages contact surfactant particularly during lung inflammation because the alveolar epithelium is disrupted.

METHODS

Human lung tissue procurement. As a source of lung tissue we used male multiple organ donors. The review board and the ethics committee of the San Carlos Hospital approved this study. Ages ranged from 19 to 50 yr, and cranial trauma or spontaneous intracranial hemorrhage was the cause of death in all of them. Donors with recent history of tobacco smoking, >72 h of mechanical ventilation, or any radiological pulmonary infiltrate were excluded from this study. Immediately after obtaining the left lung, we performed a BAL at 4°C, and the lung was then placed in cold saline solution. The cold ischemia period was <3 h in all cases.

Isolation and analysis of human surfactant. Pulmonary surfactant from human lungs was obtained as previously described (9, 10). Briefly, cell-free BAL was centrifuged at 100,000 *g* for 2 h at 4°C to obtain the large surfactant aggregates in the resulting pellet. The separation of pulmonary surfactant from blood components was performed by NaBr density-gradient centrifugation at 116,000 *g* for 2 h at 4°C. Surfactant has a density of ~1.085 at 4°C, which is lower than that of most of the contaminating components of serum.

Total phospholipid was determined from aliquots of both surfactant and lipid extracts of surfactant by phosphorus analysis as described by Rouser et al. (26). Surfactant concentration was expressed in terms of phospholipid concentration. Total surfactant cholesterol was determined enzymatically with the Sigma diagnostic cholesterol kit. Total proteins in human surfactant was measured with the method of Lowry and associates (21).

Human surfactant apolipoproteins (SP)-A, SP-B, and SP-C were detected in large surfactant aggregates by Western blotting analysis. Electrophoretic analysis of surfactant was performed under reducing conditions (5% β -mercaptoethanol) by one-dimensional SDS-PAGE, using running gels of 12, 16, and 18% acrylamide for SP-A, SP-B, and SP-C, respectively. Isolated human SP-A (2) and isolated pig SP-B and SP-C (24) were used as standards. After electrophoresis, samples were transferred to nitrocellulose using a Bio-Rad Trans-Blot Cell. Transfer was carried out at 100 V of con-

stant voltage and 100 mA of total increment of intensity, using 25 mM Tris, pH 8.3, 192 mM glycine, and 20% (vol/vol) methanol as a transfer buffer. Blotting of SP-A and SP-B was done as previously described (10). For SP-A, an anti-(human-SP-A) polyclonal antibody was used. For SP-B, an anti-(porcine SP-B) polyclonal antibody was utilized.

Western blot analysis of SP-C was performed on polyvinylidene difluoride membranes as described in Towbin et al. (35). Transfer was carried out as reported for SP-A using 25 mM Tris, pH 8.3, 192 mM glycine, 20% (vol/vol) methanol, and 0.01% SDS as blotting buffer. Afterward, the membrane was blocked with 5% dried skimmed milk in 136 mM NaCl, 2 mM KCl, 25 mM Tris·HCl buffer, pH 7.4, and 0.05% (vol/vol) Tween 20. After washing, the antirecombinant human SP-C antiserum (generously donated by Altana Pharma, Konstanz, Germany) was added and incubated overnight. Proteins were visualized using chemiluminescence detection (Hyperfilm ECL; Amersham Pharmacia).

The ability of surfactant to adsorb onto and spread at the air-water interface was tested at 25°C on a Wilhelmy-like, highly sensitive surface microbalance coupled to a Teflon dish of small size (2.9×1.4 cm), as previously reported (10). We studied the influence of human CRP on the adsorption rate of surfactant by adding different amounts of CRP into the hypophase chamber of the Teflon dish, which contained 6 ml of 5 mM HEPES buffer, pH 7.0, 150 mM NaCl, and 5 mM CaCl₂. After surfactant injection into the hypophase, surfactant interfacial adsorption was measured following the change in surface tension as a function of time.

Cell isolation and culture. Bronchoalveolar cells were separated from lavage fluid by centrifugation. The sedimented cells were washed twice with HBSS. Cell suspension was centrifuged (250 *g*, 10 min), and the cell pellet was resuspended in RPMI 1640 medium (10% heat-inactivated FCS, 100 IU/ml penicillin G, and 50 μ g/ml gentamicin). Alveolar macrophages were purified by adherence for 90 min at 37°C under a 95% air-5% CO₂ atmosphere in RPMI 1640 medium in 75-cm² culture flasks. Macrophages from lung interstitium were isolated as described elsewhere (4, 7). Briefly, tissue fragments underwent an enzymatic digestion with elastase (27 U/ml) in a 37°C shaking bath for 60 min. Digestion was stopped by addition of 4°C FCS. The tissue was filtered through nylon mesh (200 and 20 μ m), and the cell suspension was washed twice with HBSS and centrifuged (250 *g*, 10 min). The cell pellets were resuspended in RPMI 1640 medium, and poured into 75-cm² culture flasks. Interstitial macrophages were also purified by adherence. After 90 min of incubation (37°C, under O₂-CO₂ atmosphere), the supernatants were removed and the cells were washed four times with phosphate-buffered saline to remove contaminating nonadherent cells. Adherent cells were found to be $99.5 \pm 0.3\%$ viable (trypan blue exclusion test) and composed of $92.5 \pm 3.1\%$ macrophages, as judged by Wright-Giemsa-stained cytocentrifuge preparations. Flow cytometry analysis of macrophages immunostained with anti-HLA-DR and antibodies to CD14 confirmed the purity of macrophage preparations. The cells were gently scraped, plated onto collagen-coated 96-well plastic dishes (5×10^5 cells per well), and precultured for 24 h. Under these conditions, ~95% of the cells were attached; cell viability was >97%, and macrophage purity was always >98%. Cells were cultured for another 24 h in the presence or absence of smooth LPS (*Escherichia coli* 0111:B4, 10 μ g/ml), LPS + IFN- γ (100 U/ml), human CRP (Calbiochem), human surfactant, and combinations. Human CRP preparations were tested for bacterial endotoxin using a *Limulus* amoebocyte lysate assay (Bio-Whittaker, Walkersville, MD). Human CRP contained 0.53 ± 0.11 pg

endotoxin/ μg protein. The amount of contaminating LPS from the CRP source was not significant compared with the LPS concentration used in this study.

The concentrations of smooth LPS and IFN- γ used as stimulus were selected according to previous dose-response studies in human pulmonary macrophages (7) and monocytes (14). In this study we used a surfactant phospholipid concentration of 0.5 mM. Gregory et al. (15) analyzed total phospholipid recovered from BAL fluid of normal subjects (median value of 7.54 $\mu\text{mol/ml}$) and ARDS patients (<1 $\mu\text{mol/ml}$). On the basis of such studies, the concentration of surfactant phospholipids used in this study was reasonably low and might simulate that found in patients with ARDS. Because acute-phase response may lead to high CRP concentrations in blood (17) and alveolar fluid (20), we have used acute-phase levels of CRP (125 and 250 $\mu\text{g/ml}$) (25) in these experiments. Concentrations of either surfactant or CRP were not cytotoxic for the cells.

Cytokine assays. Cell-free culture supernatants were collected and assayed for TNF- α and IL-1 β with enzyme-linked immunoassay kits (Biosource International). An aliquot of the cell suspension was used for protein quantification, performed by the Coomassie brilliant blue dye method.

Statistical analyses. The number of separate macrophage preparations ($n \geq 6$) employed (each from a different donor lung) is represented by n . The assays from each macrophage preparation were performed at least twice, the replicate values were averaged, and their mean was treated as a single point. The results are presented as the means \pm SE, obtained by combining the results from each cell preparation. Mean comparison was done by Friedman's analysis of variance of ranks, followed by a two-tailed Wilcoxon's rank sum test for paired data to identify the source of the found differences; a confidence level of $\geq 95\%$ ($P < 0.05$) was considered significant.

RESULTS

Effect of CRP on TNF- α and IL-1 β production by human lung macrophages. CRP levels highly increase in both BAL fluid (20) and serum (17) from patients with ARDS. Furthermore, patients who survived the acute lung injury tended to have more CRP levels in their blood and BAL fluid than those patients who died (18). Therefore, we investigated the dose-response effect of human CRP on human lung macrophages at acute-phase levels of CRP (125 and 250 $\mu\text{g/ml}$). Figure 1 shows that unstimulated alveolar and interstitial macrophages isolated from nine different donors produced low concentrations of TNF- α and IL-1 β after 24 h of culture in the absence or presence of acute-phase levels of CRP (125 and 250 $\mu\text{g/ml}$). The production of TNF- α and IL-1 β by either interstitial or alveolar macrophages greatly increased after 24 h of culture in the presence of LPS; that increase was even greater when macrophages were incubated with IFN- γ together with LPS. The addition of 125 $\mu\text{g/ml}$ CRP to alveolar macrophages significantly inhibited LPS-induced production of TNF- α (by 67%) and IL-1 β (by 60%). CRP caused TNF- α release inhibition of 85% and IL-1 β release inhibition of 70% when these cells were stimulated with LPS + IFN- γ . The effect of 125 $\mu\text{g/ml}$ CRP on TNF- α and IL-1 β production by stimulated interstitial macrophages was less pronounced; CRP (125 $\mu\text{g/ml}$) caused an inhibition of TNF- α and IL-1 β production of $\sim 50\%$. However, at higher levels of CRP (250 $\mu\text{g/ml}$), TNF- α and IL-1 β production by both interstitial and alveolar macrophages, stimulated with

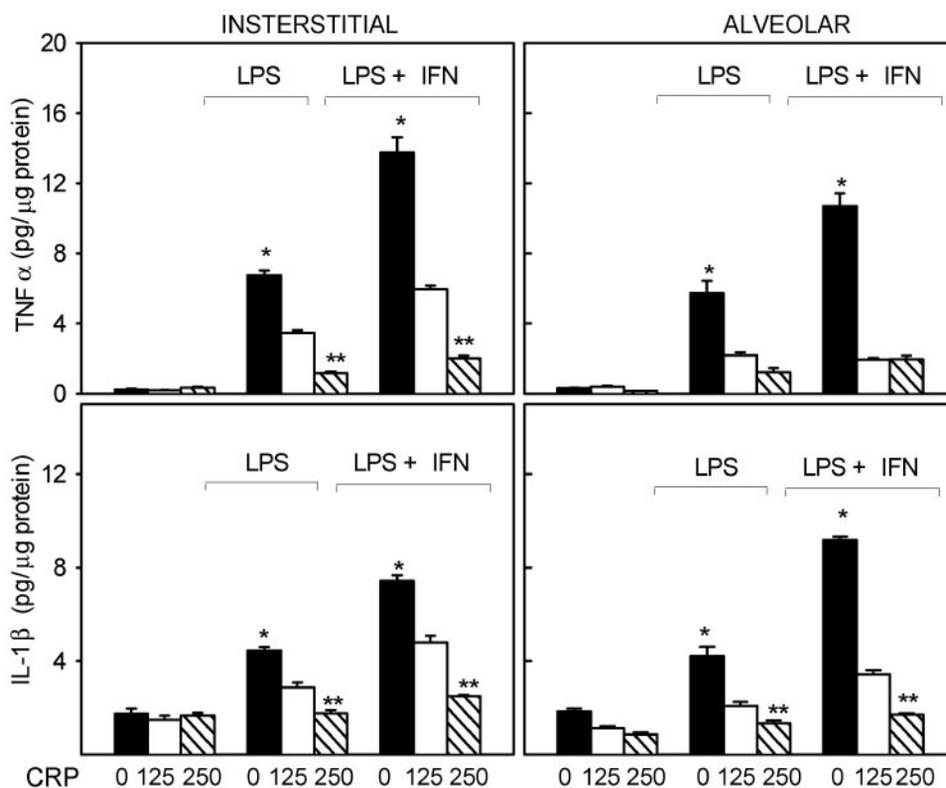


Fig. 1. Effect of C-reactive protein (CRP, 125 or 250 $\mu\text{g/ml}$) on TNF- α (top) and IL-1 β (bottom) release by human interstitial and alveolar macrophages stimulated with LPS alone (10 $\mu\text{g/ml}$) or LPS + IFN- γ (100 U/ml). Nine different interstitial and alveolar macrophage cultures from 9 different donors were used. * $P < 0.01$ vs. all others; ** $P < 0.01$ vs. CRP 125 $\mu\text{g/ml}$.

Table 1. *Human surfactant characteristics*

Human Pulmonary Surfactant	Age, yr	Sex	% Molar Ch/PL	$\mu\text{mol PL/ mg protein}$	$\mu\text{mol Ch/ mg protein}$
Sample 1	20	M	17.52	6.69	1.17
Sample 2	40	F	19.77	8.55	1.69
Sample 3	46	M	27.66	7.66	2.11

Human pulmonary surfactants were obtained from bronchoalveolar lavages from the left lung of multiple organ donors. Donors were previously healthy and without recent history of tobacco smoking. Ch, cholesterol; PL, phospholipid; M, male; F, female.

IFN- γ and/or LPS, was inhibited by >80%. These data indicate that CRP could modulate lung inflammation in humans by decreasing the LPS-induced production of proinflammatory cytokines by both interstitial and alveolar macrophages.

Human surfactant analysis. Pulmonary surfactant is a complex material that contains several specific apolipoproteins (SP-A, SP-B, and SP-C) and a variety of lipids, in particular a high proportion (~80%) of phosphatidylcholine. Different components of surfactant have very different effects on the modulation of immune cell function (38). Because of the complexity of these membranes, we analyzed some lipid characteristics of human surfactants used in this study (Table 1). Human surfactant appears to contain a higher cholesterol-to-phospholipid molar ratio compared with surfactant membranes from other mammalian species (36). The presence of surfactant apolipoproteins in samples of human surfactant was determined by immunoblot analysis of SP-A, SP-B, and SP-C (Fig. 2). SP-A immunoreactive bands of ~35 and ~70 kDa were detected in all surfactant samples analyzed. The non-reducible dimer (~70 kDa), described for SP-A isolated from alveolar proteinosis patients, is also characteristic of human SP-A from healthy persons. Monomeric SP-C, but not dimeric/depalmitoylated SP-C [found in surfactants from alveolar proteinosis patients (16)], was detected. SP-B consisted of dimers of ~18 kDa and

higher oligomeric forms under nonreducing conditions. Only monomeric SP-B (~9 kDa) was recognized under reducing conditions.

The inhibitory effect of human CRP on the adsorption rate of surfactant was determined. CRP caused almost total inhibition of surfactant adsorption at surfactant phospholipid-CRP weight ratio of ~3 (data not shown), suggesting maximum binding of CRP to the phosphocholine head group of surfactant phospholipids.

Effect of pulmonary surfactant on CRP modulation of proinflammatory cytokines production by interstitial and alveolar macrophages. Because CRP binds avidly to surfactant membranes, we next investigated the effect of these membranes on CRP-modulation of both TNF- α and IL-1 β production by lung macrophages. Figure 3 shows that unstimulated interstitial and alveolar macrophages isolated from six different donors produced low concentrations of TNF- α after 24 h of culture in the absence or presence of acute-phase levels of CRP (125 $\mu\text{g/ml}$), human pulmonary surfactant (0.5 mM), or the combination of pulmonary surfactant and CRP (3:1 weight ratio). Figure 3 also indicates that the addition of surfactant membranes to interstitial or alveolar macrophages significantly inhibited the production of TNF- α by these cells stimulated by LPS alone or LPS together with IFN- γ . The incubation of these cells with pulmonary surfactant together with CRP led to a greater decrease in the production of TNF- α by both interstitial and alveolar macrophages stimulated with LPS alone or together with IFN- γ .

Figure 4 shows the effect of CRP, pulmonary surfactant, and the combination of human pulmonary surfactant and CRP (3:1 weight ratio) on the release of IL-1 β by interstitial and alveolar macrophages stimulated with LPS alone or together with IFN- γ . Pulmonary surfactant inhibited the production of IL-1 β by stimulated interstitial or alveolar macrophages, and the combination of pulmonary surfactant and CRP re-

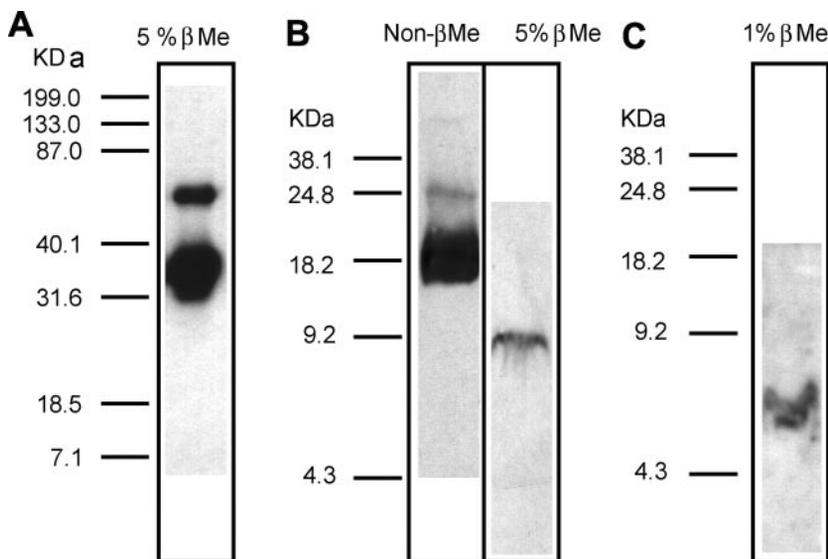
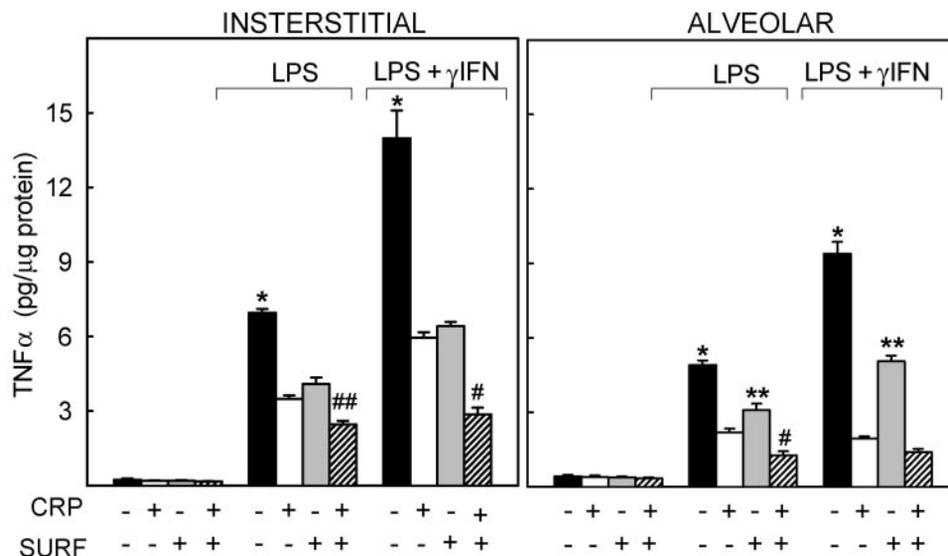


Fig. 2. Immunoblot analysis of surfactant protein (SP)-A (A), SP-B (B), and SP-C (C) in human pulmonary surfactant. Total surfactant protein (5 μg) was separated by PAGE-SDS under reducing [5 or 1% β -mercaptoethanol (Me)] or nonreducing conditions, transferred to a membrane, and probed using polyclonal antibodies anti-SP-A, -B, and -C. Proteins were visualized using chemiluminescence detection. Numbers at left denote molecular mass.

Fig. 3. Effect of CRP (125 $\mu\text{g/ml}$), human pulmonary surfactant (Surf, 360 $\mu\text{g/ml}$), or the combination CRP-pulmonary surfactant (0.35:1, CRP-to-surfactant phospholipid weight ratio) on TNF- α release by human interstitial and alveolar macrophages stimulated with LPS alone (10 $\mu\text{g/ml}$) or LPS + IFN- γ (100 U/ml). Six different interstitial and alveolar macrophage cultures from 6 different donors were used. * $P < 0.01$ vs. all others; ** $P < 0.01$ vs. CRP; # $P < 0.01$ vs. CRP and surfactant groups; ## $P < 0.05$ vs. CRP and surfactant groups.



sulted in a greater inhibition of the release of this proinflammatory cytokine by lung macrophages.

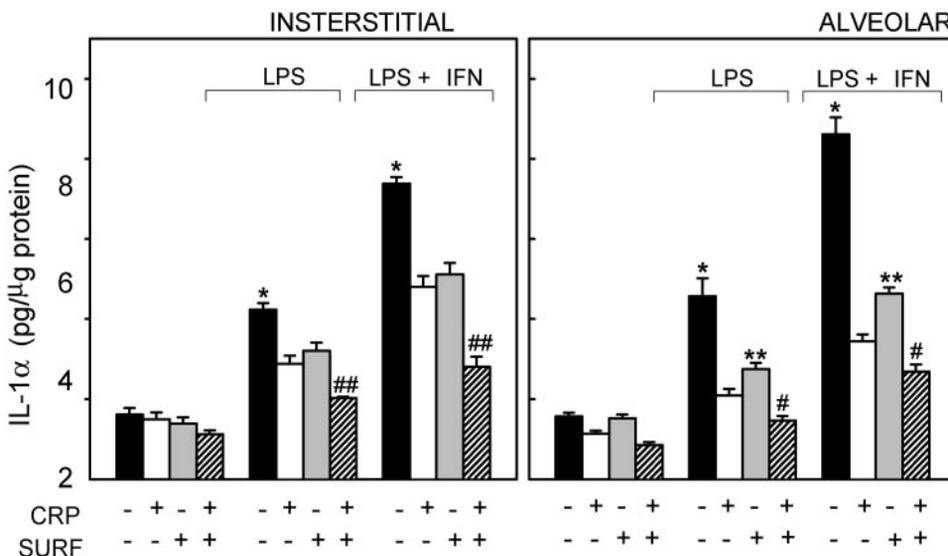
DISCUSSION

The present study indicates that 1) CRP could modulate lung inflammation in humans by decreasing the production of proinflammatory cytokines by both interstitial and alveolar macrophages stimulated with LPS alone or together with IFN- γ and 2) the potential interaction between CRP and surfactant phospholipids did not overcome the effect of either CRP or pulmonary surfactant on TNF- α and IL-1 β release by lung macrophages. On the contrary, both together had a greater inhibitory effect than either alone on the release of proinflammatory cytokines by lung macrophages.

Our observation that CRP decreased TNF- α secretion from LPS-stimulated alveolar macrophages is in agreement with the data from Pue and colleagues (25), who indicated that acute-phase levels of CRP down-regulated human alveolar macrophage production of

IL-1 β in response to endotoxins. However, our results appears to contradict the data from Galve-de Roche-monteix et al. (13), who showed an enhancement of the human alveolar macrophage production of cytokines after stimulation by CRP. Although our study focused on the effects of CRP on LPS-activated interstitial and alveolar macrophages, we did test the ability of CRP to induce production of TNF- α and IL-1 β by unstimulated cells. We could not detect any significant increase in the baseline liberation of these cytokines. We currently have no unequivocal explanation for these contrasting results, although there were some differences in experimental design. A major difference in the two studies is the dissimilar origin of human alveolar macrophages. In the study by Galve-de Roche-monteix et al., alveolar macrophages were obtained from patients with pulmonary cell carcinoma. Alveolar macrophages from patients with lung cancer have shown in vitro greater TNF- α and IL-1 secretion than healthy controls (3, 28). In the present study, these

Fig. 4. Effect of CRP (125 $\mu\text{g/ml}$), human pulmonary surfactant (360 $\mu\text{g/ml}$), or the combination CRP-pulmonary surfactant (0.35:1, CRP-to-surfactant phospholipid weight ratio) on IL-1 β release by human interstitial and alveolar macrophages stimulated with LPS alone (10 $\mu\text{g/ml}$) or LPS + IFN- γ (100 U/ml). Six different interstitial and alveolar macrophage cultures from 6 different donors were used. * $P < 0.01$ vs. all others; ** $P < 0.01$ vs. CRP; # $P < 0.01$ vs. CRP and surfactant groups; ## $P < 0.05$ vs. CRP and surfactant groups.



cells were obtained from previously healthy organ donors. In addition, we performed parallel experiments with interstitial macrophages, which reside within the interstitial space and are thought to be precursors of alveolar macrophages. These cells are not solely an intermediate maturation stage of alveolar macrophages but contribute actively to the process of inflammation in the lung, with potential beneficial or destructive effects on the surrounding tissue (8). To our knowledge, all previous studies involving interactions of CRP with lung macrophages were carried out with the alveolar variety. The present study demonstrates that CRP has an inhibitory effect on the release of proinflammatory cytokines by both alveolar and interstitial macrophages.

Neutrophil accumulation and vascular permeability are common events in ARDS, and CRP appears to offer a protective effect in this disease (18). In animal models of inflammation-mediated lung injury, CRP acts as an anti-inflammatory agent mediating inhibition of neutrophil alveolitis and vascular permeability (17). However, the mechanism by which CRP elicits this inhibitory effect is undefined. The downregulation of proinflammatory cytokine production by macrophages could be involved in the inhibition of neutrophil alveolitis.

The alveolar fluid from normal lungs contains low levels of CRP and a high concentration of pulmonary surfactant, which is involved in reducing the surface tension of the fluid lining the alveoli and in host defense (37). The large excess of SP-A and surfactant membranes (containing SP-B and SP-C) in normal air spaces probably minimizes the biological effects of the low concentration of endotoxins that enter the alveolus. Given the lipophilic nature of LPS, it might incorporate in surfactant membranes, making LPS unavailable for signaling. The hydrophobic surfactant protein SP-C interacts with LPS (5, 6), indicating that this surfactant component could participate in the recognition and neutralization of inhaled LPS. On the other hand, SP-A, the most abundant surfactant apolipoprotein, might mediate inhibition of LPS-induced activation by either direct interaction of SP-A with cellular binding sites of immune cells (in the case of smooth LPS) (27) or direct interaction with LPS (in the case of rough LPS), which blocks the binding of rough LPS to LPS-binding protein (LBP); this prevents the initiation of the LBP/CD14 pathway for inflammatory responses to rough LPS (29).

In patients with acute lung injury in which proinflammatory cytokines and neutrophils accumulate in the air spaces, the concentration of SP-A and surfactant lipids decreases, whereas the concentration of CRP and LBP rises 100-fold or more (17, 22). Although LBP would likely amplify the biological effect of LPS in the lungs, CRP would limit excessive proinflammatory cytokine release by LPS-stimulated alveolar macrophages. We found that the inhibitory effect of CRP on the release of proinflammatory cytokines by stimulated human lung macrophages was not reduced but strengthened by interaction with surfactant membranes. The

interaction of CRP with these membranes could favor the mode of presentation and binding of CRP to its receptors on macrophages. CRP molecules are known from electron microscopy to form ordered two-dimensional arrays at surfaces, and this effect is likely to occur on the lipid bilayer. Furthermore, CRP binds to cells and/or effector molecules via the face of its subunits opposite to the face where the specific site for phosphocholine head groups of phospholipids on membranes is located (31, 34). On the other hand, the fact that CRP and pulmonary surfactant together had a greater inhibitory effect than either alone on the release of proinflammatory cytokines could also be explained by an additive effect. In support of this possibility, we recently found that human SP-A from normal subjects inhibits the smooth LPS-induced TNF- α response of both human interstitial and alveolar macrophages when assayed alone (2) or in the presence of dipalmitoyl-L- α -phosphatidylcholine vesicles (C. Casals, J. Arias-Díaz, and E. Vara, unpublished results). However, the smooth LPS-elicited release of IL-1 β in human alveolar macrophages is not suppressed by human SP-A (2) but by a synthetic surfactant (Exosurf) (33) or bovine-derived surfactant lipid extracts (Survanta) containing the hydrophobic surfactant proteins SP-B and SP-C (1).

ARDS represents an enormous clinical problem that is therapeutically unresolved. Because alterations in the surfactant system significantly contribute to the pathophysiology of the lung injury of patients with ARDS, surfactant replacement therapy has been used in many animal models of lung injury with some encouraging results (19, 37). Alternatively, the potential therapeutic use of CRP in the treatment of inflammation-mediated lung diseases in humans has been proposed (17). However, a high CRP-to-surfactant phospholipid weight ratio might be harmful for the lung function because the binding of CRP to surfactant membranes inhibits the tension-reducing properties of surfactant (10, 23). Future studies will investigate the mechanism by which CRP inhibits LPS-induced activation of lung macrophages. In addition, experiments are in progress to determine whether specific interactions of CRP with surfactant components influence CRP-mediated inhibitory effects on cytokine production.

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