Pneumocystis carinii induces ICAM-1 expression in lung epithelial cells through a TNF-α-mediated mechanism

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Yu, Mariette L., and Andrew H. Limper. Pneumocystis carinii induces ICAM-1 expression in lung epithelial cells through a TNF-α-mediated mechanism. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L1103–L1111, 1997.—Inflammatory cell recruitment contributes to respiratory impairment during Pneumocystis carinii pneumonia. We evaluated expression of intercellular adhesion molecule-1 (ICAM-1), a key participant in leukocyte accumulation, in rats with P. carinii pneumonia. Immunostaining for ICAM-1 was most marked on bronchial epithelium but was also evident on type II pneumocytes, endothelium, and macrophages. Lung from normal and dexamethasone-treated uninfected animals exhibited markedly less ICAM-1. We hypothesized that P. carinii promoted ICAM-1 expression in epithelium through tumor necrosis factor-α (TNF-α) release from macrophages or that P. carinii directly stimulated ICAM-1 expression. Alveolar macrophages were incubated with P. carinii, and the medium was added to A549 epithelial cells. Treatment of macrophages with P. carinii enhanced A549 ICAM-1, which was inhibited with antibody to TNF-α. To determine whether P. carinii alone also stimulated ICAM-1, A549 cells were cultured with P. carinii, also augmenting ICAM-1. Of note, A549 ICAM-1 expression from P. carinii alone was less than with P. carinii-exposed macrophages. Thus ICAM-1 is enhanced in lung epithelium during P. carinii infection, in part, through TNF-α-mediated mechanisms.

Pneumocystis carinii pneumonia is an opportunistic fungus causing severe pneumonia in immunocompromised hosts. Despite effective prophylaxis and treatment regimens, this infection remains a major complication of patients with human immunodeficiency virus infection or hematologic or solid malignancies or after organ transplantation (13, 22, 41). Among patients with acquired immunodeficiency syndrome (AIDS), severe P. carinii pneumonia is the most frequent cause of acute respiratory failure requiring admission to the intensive care unit (7, 39). Furthermore, the mortality of P. carinii pneumonia in all patients ranges between 15 and 40% (7, 22, 39). Prior investigations indicate that lung inflammation contributes significantly to respiratory impairment and clinical outcome during P. carinii pneumonia (22, 35). In particular, increased numbers of neutrophils in bronchoalveolar lavage predict poorer oxygenation and survival during P. carinii pneumonia (22). The contention that pulmonary inflammation potentiates lung injury during P. carinii pneumonia is further supported by clinical observations that corticosteroids prevent deterioration and improve outcome in moderate to severe P. carinii pneumonia (28).

The mechanisms regulating lung inflammation during P. carinii pneumonia remain poorly understood. Recent investigations have focused on a family of cell surface adhesion molecules that enable circulating leukocytes to accumulate in areas of lung inflammation (1, 33). Of primary importance is intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin (Ig) supergene family, that, together with its ligands lymphocyte function-associated antigen-1 and macrophage-1 antigen, promotes the firm adherence of leukocytes onto vascular endothelium, thereby permitting transmigration into injured tissues. In addition to vascular endothelium, ICAM-1 is also expressed by epithelial cells, lymphocytes, platelets, monocytes, and macrophages (6, 12, 33). The expression of ICAM-1 is upregulated by proinflammatory cytokines, most notably tumor necrosis factor-α (TNF-α) and γ-interferon (γ-IFN; see Refs. 6, 12, and 24). ICAM-1 has previously been implicated in the inflammatory component of several lung diseases, including tuberculosis, asthma, and sarcoidosis (12, 36, 40). However, the expression of ICAM-1 in P. carinii pneumonia has not yet been evaluated.

A number of investigations indicate that TNF-α expression is augmented during P. carinii pneumonia in humans and animal models (9, 18, 19). Studies from our laboratory have demonstrated that P. carinii stimulates release of TNF-α from macrophages through interaction of the cell wall of the organism with cognate macrophage receptors (29). We hypothesized that this enhanced release of TNF-α would act locally to increase ICAM-1 expression during P. carinii pneumonia. Accordingly, this investigation was undertaken to address the following goals: 1) to determine the extent and cellular localization of ICAM-1 protein expression during P. carinii pneumonia and 2) to determine potential mechanisms by which ICAM-1 expression may be regulated during P. carinii infection, particularly whether alterations in ICAM-1 occur through TNF-α-mediated mechanisms.

MATERIALS AND METHODS

Materials. Mouse monoclonal anti-rat ICAM-1 antibody for immunohistochemistry was obtained from Genzyme (Cambridge, MA), and monoclonal mouse anti-human ICAM-1 antibody used in the enzyme-linked immunosorbent assay (ELISA) was from R & D Systems (Minneapolis, MN). Recombinant human TNF-α was purchased from Genzyme, and type I collagen was from Vitrogen (Palo Alto, CA). Sheep anti-mouse IgG conjugated to β-galactosidase and p-nitrophenyl-β-galactoside were from Gibco-BRL Laboratories (Gaithersburg, MD). A human ICAM-1 cDNA was the kind gift of Dr. Michael Dustin (Washington University, St. Louis, MO; 1040-0605/97 $5.00 Copyright © 1997 the American Physiological Society L1103)
see Ref. 37). Ciprofloxacin was provided by Miles Pharmaceuticals (West Haven, CT).

Preparation of P. carinii. All animal studies were approved by the appropriate institutional animal care and utilization committee. P. carinii pneumonia was induced in Harlan Sprague-Dawley rats by immunosuppression with dexamethasone and transtracheal injection with P. carinii as previously described (2, 21). Specific pathogen-free rats were provided freely with drinking water containing 2 mg/ml dexamethasone, 500 mg/l tetracycline, and 200,000 U/l nystatin and were fed an 8% protein diet (Teklad, Madison, WI). On a weekly basis, the animals also received 0.45 g/l oral ciprofloxacin for 2 consecutive days to further reduce the risk of bacterial infections. After 5 days of immunosuppression, rats were transtracheally inoculated with P. carinii (~500,000 cysts) prepared by homogenizing infected rat lungs in a Stomacher microbiological blender (Tekmar, Cincinnati, OH).

After tracheal injection, the rats were immunosuppressed for an additional 6–8 wk and were killed, and whole lung lavage was performed with 50 ml of Hanks' balanced salt solution (HBSS). P. carinii were purified from this lavage by differential centrifugation (21). Lavage fluid was centrifuged (400 g for 10 min), and associated P. carinii cysts were identified in the pellet by Diff-Quik staining. The supernatant containing predominantly suspended P. carinii organisms was centrifuged (1,400 g for 30 min), and the pellet was resuspended in 1 ml of HBSS. Duplicate 10-µl aliquots of suspension were spotted onto glass slides and stained with Diff-Quik, and P. carinii were quantified as previously described (2, 21). If other microorganisms were noted in the lavage smear or on microbiological culture, the material was discarded. P. carinii isolates were found to contain <0.125 U/ml of soluble endotoxin using a sensitive Limulus amoebocyte lysate assay as previously described (29).

Immunolocalization of ICAM-1 in P. carinii-infected rat lung. Lung specimens were obtained from moribund rats with well-established P. carinii pneumonia 6–8 wk after inoculation, fixed with 10% phosphate-buffered Formalin at 15 cmH₂O pressure, embedded in paraffin, and sectioned (20, 31). Five-micrometer sections were deparaffinized with xylene and graded alcohols and were submitted to immunohistochemical examination by an avidin-biotin-mediated immunoperoxidase method (Vectastain ABC method; Vector Laboratories). Endogenous peroxidase activity was quenched with 0.3% H₂O₂ in methanol for 30 min. Nonspecific staining was diminished by incubation with 1% normal goat serum. Monoclonal mouse anti-rat ICAM-1 (10 µg/ml; Genzyme) was applied to the sections overnight. After being washed, the sections were sequentially incubated with biotinylated goat anti-mouse antibody followed by an avidin-biotinylated horse-radish peroxidase macromolecular complex. Bound antibodies were localized with diaminobenzidine tetrahydrochloride and 2% BSA for 1 h at 37°C. After being washed, the cells were blocked with 2% normal goat serum for an additional 24 h. As a positive control of A549 cell ICAM-1 induction, parallel wells were incubated with media containing 1% normal goat serum or nonimmune mouse IgG (1:40 dilution of rabbit sera or 50 µg/ml of mouse IgG) for 30 min before and throughout the subsequent incubation on A549 cells.

Evaluation of cell surface ICAM-1 on A549 cells. After incubation of A549 cells with macrophage-conditioned media or with P. carinii alone, cell surface ICAM-1 was quantified using a cell-based ICAM-1 ELISA (24). A549 cell monolayers were washed with tris(hydroxymethyl)aminomethane-buffered saline containing 0.5% bovine serum albumin (BSA), 1 mM CaCl₂, and 1 mM MgCl₂ and were fixed in 1% paraformaldehyde for 15 min at 25°C. Next, the cells were blocked with 2% BSA for 1 h at 37°C. After being washed, the cells were sequentially incubated with monoclonal anti-human ICAM-1 antibody (100 µl of a 10 µg/ml stock solution) at 37°C for 1 h, followed by a secondary sheep anti-mouse IgG F(ab)₂ conjugated to β-galactosidase (1:200 dilution) over an additional hour. Bound antibodies were detected with 1 mg/ml p-nitrophenyl-β-D-galactoside in 50 mM phosphate buffer with 1.5 mM MgCl₂ (pH 7.2) reacting for 15 min at 37°C, and absorbances were determined at 405 nm. Unless otherwise stated, the amount of basal ICAM-1 expressed by unstimu-
Analysis of steady-state ICAM-1 mRNA content in A549 cells was also conducted to determine expression of ICAM-1 protein. Normal rat lungs, ICAM-1 was detected in epithelial cells but in markedly less quantities than in infected rats (Fig. 1E). In addition, markedly less ICAM-1 staining was detected in endothelial cells and AMs compared with tissues obtained from rats with P. carinii pneumonia. Thus ICAM-1 is present in enhanced quantities in specific lung cell populations during P. carinii pneumonia in infected rats.

As an additional control, ICAM-1 staining was also assessed in lung tissues obtained from rats that were treated with a corticosteroid but that also received trimethoprim and sulfamethoxazole to prevent development of P. carinii pneumonia. Prior studies indicate that corticosteroids may suppress ICAM-1 expression (38). The pattern of ICAM-1 staining in these dexamethasone-treated controls was similar, although less intense, than that observed in uninfected normal lungs, with minimal ICAM-1 being present in epithelial cells, endothelial cells, and AMs. To further quantify the relative expression of ICAM-1 during P. carinii pneumonia, specimens from P. carinii-infected animals and from dexamethasone-treated control animals without P. carinii were stained in parallel, coded, and scored as previously described (20). With the use of this method, lungs with P. carinii pneumonia had a mean ICAM-1 staining score of 2.83 ± 0.31, whereas dexamethasone-immunosuppressed rats without P. carinii pneumonia had a mean score of 0.50 ± 0.29 (P = 0.0008). Thus ICAM-1 expression is also enhanced in the lungs of P. carinii-infected animals compared with dexamethasone-treated rats without P. carinii pneumonia.

To study whether macrophage interaction with P. carinii causes cytokine release, thereby promoting ICAM-1 expression in epithelial cells, AMs were preincubated with P. carinii, and the resulting conditioned medium was removed and recultured on A549 alveolar epithelial cells (Fig. 2). Conditioned media obtained from cultures of control macrophages incubated in the absence of P. carinii mildly stimulated basal ICAM-1 expression in A549 cells. However, culturing AMs with increasing numbers of P. carinii resulted in significant enhancement in immunoreactive ICAM-1 expression in the A549 epithelial cells. Incubating P. carinii with AMs at a P. carinii-to-AM ratio of 1:1 resulted in 292 ± 107% enhanced expression of ICAM-1 in A549 epithelial cells compared with control macrophage-conditioned media incubated without organisms (P = 0.009). Incubations of P. carinii with macrophages at a P. carinii-to-AM ratio of 5:1 resulted in 199 ± 41% enhanced ICAM-1 expression (P = 0.006 compared with control).
data indicate that the interaction of P. carinii with AMs enhances ICAM-1 expression on cultured lung epithelial cells.

P. carinii directly promotes enhanced ICAM-1 surface expression on A549 cells. During the life cycle of the organism, P. carinii trophozoites attach to alveolar epithelial cells by intimate approximation and interdigitation of their surfaces with the cell membranes of host epithelium (21). We postulated that the interaction of P. carinii with A549 lung epithelial cells might directly stimulate surface expression of ICAM-1. To examine this, freshly purified P. carinii were incubated overnight on A549 cell monolayers. The next day, the monolayers were washed, and A549 surface ICAM-1 expression was determined by ELISA with a monoclonal antibody specific for human ICAM-1 (Fig. 3). Increased ICAM-1 expression occurred between a P. carinii-to-A549 ratio of 0:1 compared with 1:1, 2.5:1, 5:1, and 10:1. A P. carinii-to-A549 ratio of 1:1 resulted in significantly increased A549 ICAM-1 surface expression (P = 0.022), whereas a P. carinii-to-A549 ratio of 5:1 yielded maximal enhancement of expression (P =...
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Fig. 2. P. carinii-stimulated AMs enhance surface ICAM-1 expression on A549 lung epithelial cells. Normal AMs were harvested from rats and were incubated with increasing amounts of P. carinii. The next day, conditioned media were removed, clarified by centrifugation, and recultured with A549 lung cells for an additional 24 h. Surface ICAM-1 on A549 cells was subsequently determined by a cell-based enzyme-linked immunosorbent assay (ELISA). Media conditioned by culturing AMs in the presence of increasing numbers of P. carinii resulted in enhancement of A549 surface ICAM-1 expression. Values are means ± SE from 8 determinations (*P < 0.05 compared with media conditioned with AMs alone). OD, optical density.

0.0217 compared with A549 cells incubated without P. carinii). Limitations in P. carinii enumeration and in assay sensitivity made it difficult to reliably assess ICAM-1 differences using ratios of infectivity between 0:1 and 1:1. At the higher ratio of 10:1 P. carinii to A549 cells, a minor reduction in maximal A549 ICAM-1 expression was observed compared with the 5:1 concentration. However, the ratio of P. carinii to A549 cells of 10:1 reliably induced A549 ICAM-1 expression, and this level of ICAM-1 was not statistically different from that induced by the 5:1 concentration of P. carinii. These data indicate that the interaction of P. carinii organisms with A549 lung epithelial cells also exerts a direct stimulation of ICAM-1 expression. It should be noted, however, that the maximal absolute level of ICAM-1 surface expression induced by P. carinii alone was approximately one-third of that induced by P. carinii-exposed AMs in parallel experiments. Therefore, P. carinii directly stimulate cultured A549 lung epithelial cells to express ICAM-1, however, at an apparently lower level than that induced by AM products after P. carinii challenge.

Further experiments were performed using a Transwell coculture system to evaluate whether P. carinii adherence to the A549 cells was required for the ICAM-1 induction rather than release of some soluble factor from the organism. P. carinii cultured directly on A549 cells induced significant ICAM-1 expression by the epithelial cells [optical density (OD) = 0.102 ± 0.014 relative absorbance units (RAU); P = 0.0001 compared with control A549 cell without P. carinii]. However, A549 cells cultured with P. carinii in the upper chamber of a Transwell tissue coculture chamber exhibited no significant increase in A549 cell ICAM-1 surface expression (OD = 0.015 ± 0.013 RAU; P = 0.243 compared with control A549 cells without P. carinii). Thus direct adherence of the organisms to the epithelial cell appears to be necessary for the induction of ICAM-1 expression.

We next sought to determine whether P. carinii and conditioned media from P. carinii-stimulated macrophages exert a synergistic effect on ICAM-1 expression by A549 lung epithelial cells. A small additive increase (30.8 ± 2.8%) in A549 cell ICAM-1 expression was observed in A549 cells incubated with both P. carinii-stimulated macrophage-conditioned media and with freshly isolated P. carinii organisms compared with A549 cells incubated with conditioned media alone. This increase, however, was not statistically greater than expected on an additive basis alone. Therefore, a synergistic effect in A549 cell ICAM-1 expression was not observed by these two stimuli.

P. carinii increase steady-state ICAM-1 mRNA in cultured A549 lung cells. It was conceivable that the P. carinii preparations might contain small amounts of contaminating rat ICAM-1 that, if transferred into the A549 cell cultures, might be detected with the cell-based ELISA assay. This was considered rather unlikely because the A549 cell monolayers were thoroughly washed before analysis and were examined using a monoclonal antibody specific for the human ICAM-1 expressed by A549 cells. However, to further confirm that P. carinii stimulates the expression of ICAM-1 in lung epithelial cells, we examined steady-state ICAM-1 mRNA expression in A549 cells cultured with increasing numbers of P. carinii using a cDNA probe specific for human ICAM-1 (Fig. 4). The human cDNA probe was hybridized with a 1.3-kb RNA species from A549 cells. Incubation of A549 cells in the presence of P. carinii augmented the levels of steady-state ICAM-1 mRNA at P. carinii-to-A549 ratios of 5:1 and 10:1. This was comparable with the pattern of hybridization observed using RNA obtained from A549 cells incubated with TNF-α, a potent inducer of epithelial ICAM-1 expression. No appreciable ICAM-1 mRNA induction was observed in A549 cells incubated in the absence of P. carinii. Equality of RNA loading under the

Fig. 3. P. carinii directly stimulate ICAM-1 surface expression on A549 lung epithelial cells. A549 cells were directly cultured in the presence of increasing numbers of P. carinii. After 24 h, A549 monolayers were washed, and A549 cells surface ICAM-1 was measured by ELISA. Culturing P. carinii with A549 cells directly stimulated expression of ICAM-1 in the A549 lung epithelial cell line. Values are means ± SE from 4 determinations (*P < 0.05 compared with A549 cells cultured in the absence of P. carinii).
Fig. 4. P. carinii enhance ICAM-1 mRNA expression in A549 lung epithelial cells. A549 cells were cultured with purified P. carinii, and total RNA was extracted and separated by electrophoresis (20 µg each lane). A: Northern hybridization performed using a human-specific human ICAM-1 cDNA probe. Lane 1: total RNA from A549 cells cultured in the absence of P. carinii (negative control); lane 2: total A549 RNA from cells cultured in the presence of tumor necrosis factor-α (TNF-α; 5,000 U; positive control); lane 3: RNA from A549 cells cultured in the presence of P. carinii (P. carinii-to-A549 ratio of 3:1); lane 4: RNA from A549 cells cultured with P. carinii at a P. carinii-to-A549 ratio of 5:1; lane 5: total RNA from A549 cells in the presence of P. carinii at a P. carinii-to-A549 ratio of 10:1. B: nitrocellulose membranes were stripped and reprobed with constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to confirm equal loading of total RNA.

Different conditions was initially confirmed by ethidium bromide stains of the 18S and 28S rRNA bands. However, to further verify equivalency of loading, the membranes were rehybridized with GAPDH cDNA, a constitutively expressed target RNA (Fig. 4). Scanning densitometry of these blots (Fig. 5) demonstrated that the ICAM-1-to-GAPDH ratio increased from 0.07 ± 0.02 in A549 cells incubated in the absence of P. carinii to 0.42 ± 0.17 in A549 cells incubated with a P. carinii-to-A549 cell ratio of 5:1 and to 0.86 ± 0.28 in A549 cells cultured with a ratio of 10:1 (P = 0.025 each compared with A549 cells incubated without P. carinii).

Taken together, these data confirm that P. carinii significantly augments ICAM-1 gene expression in A549 lung epithelial cells. In addition, the above findings further confirm that the enhancement of surface ICAM-1 expression on A549 cells infected with P. carinii was due to direct stimulation of the epithelial cells by the organisms and does not likely represent ICAM-1 contamination carried over with P. carinii inocula.

P. carinii enhance ICAM-1 expression in A549 lung cells through TNF-α-mediated mechanisms. To evaluate potential mechanisms by which P. carinii induce ICAM-1 expression in lung epithelial cells, we next studied the role of macrophage-derived TNF-α in our model system of P. carinii infection and ICAM-1 expression. Accordingly, conditioned media derived from AMs cultured with P. carinii (P. carinii-to-AM ratio of 10:1) were clarified by centrifugation and were treated with a neutralizing antibodies. Conditioned media from AMs cultured with P. carinii induced significant expression of ICAM-1 on A549 cells (P. carinii-to-AM ratio of 10:1). Treatment of P. carinii-macrophage-conditioned media with antisera to TNF-α (anti-TNF-α) substantially suppressed ICAM-1 expression by A549 cells. In contrast, nonimmune serum (NI serum) and nonimmune IgG (NI IgG) had minimal effect on P. carinii-macrophage-conditioned media. Furthermore, antibody to γ-interferon (anti-IFN γG) had little effect on A549 ICAM-1 expression. Values are means ± SE from 6 determinations (*P < 0.05 compared with control media from AMs cultured with P. carinii but not treated with antibody).
significant effect on A549 ICAM-1 expression (P = 0.09, not significantly different from control).

Recent studies further indicate that TNF-α binds to P. carinii and fungal cell wall β-glucans through its lectin-binding domain (30, 32). Such lectin binding occurs through a site distinct from the mammalian TNF-α-receptor binding region, leaving this domain potentially accessible to mediate effects on host cells (30). To test the possibility that TNF-α associated with the surface of P. carinii might be causing the observed increases in A549 cells cultured directly with freshly isolated organisms, P. carinii were pretreated with anti-TNF-α before and throughout the subsequent incubation with A549 cells (Fig. 7). Treatment of P. carinii with anti-TNF-α did not significantly impair subsequent ICAM-1 expression by the A549 cells (P = 0.11, not significantly different from A549 cells stimulated with P. carinii in the absence of anti-TNF-α). Nonimmune serum had minimal effect on A549 cell ICAM-1 expression. These experiments indicate that, although P. carinii induces ICAM-1 expression in cultured lung epithelium, this effect is not significantly mediated by surface-bound TNF-α on the organisms.

**DISCUSSION**

This study demonstrates that ICAM-1, a potent leukocyte cell adhesion molecule, is present in enhanced quantities in the lung during P. carinii pneumonia in a rat model. Increased ICAM-1 protein expression was chiefly observed on bronchial and type II epithelial cells, endothelial cells, smooth muscle cells, and AMs in animals with established P. carinii infection. We have further demonstrated that P. carinii induces enhanced ICAM-1 expression in cultured A549 lung epithelial cells through interaction of the organisms with AMs and through the subsequent release of TNF-α. In addition, direct contact of P. carinii with cultured lung epithelial cells also significantly aug-

**Fig. 7.** Effect of antibody to TNF-α on A549 ICAM-1 expression induced by direct culture with P. carinii. To evaluate whether surface-associated TNF-α on P. carinii enhanced A549 ICAM-1 expression, organisms were treated with neutralizing antibodies before and throughout subsequent cultures on A549 lung epithelial cells (P. carinii-to-AM ratio of 10:1). Maximal ICAM-1 (100%) was defined as the amount of A549 surface expression observed on A549 cells cultured with P. carinii in the presence of no added neutralizing antibodies. Treatment of P. carinii with anti-TNF-α did not significantly suppress surface expression of ICAM-1 by A549 cells. NI serum had a minimal effect on ICAM-1 expression by A549 cells. Values are means ± SE from 6 determinations.
expression by A549 lung epithelial cells. However, γ-IFN is predominately produced by large T lymphocytes, and minimal amounts of γ-IFN are released from AMs (17). The current findings do not preclude a role for γ-IFN-mediated expression of ICAM-1 in vivo but likely reflect the small amount of γ-IFN released by macrophages during P. carinii infection in the system employed. Indeed, prior investigations suggest that γ-IFN promotes optimal clearance of P. carinii in vitro (9). It has been proposed that lymphocytic destruction in patients with AIDS results in a relative deficiency of γ-IFN during P. carinii pneumonia (4, 27). Such relative deficiency of γ-IFN during P. carinii pneumonia may reduce ICAM-1 expression in the lung, thus hindering complete clearance of infection in these hosts.

Interestingly, our study revealed that purified P. carinii also directly stimulate ICAM-1 by A549 lung epithelial cells. The mechanisms of this direct ICAM-1 stimulation are not yet clear but may involve either surface antigens of the organism or host proteins bound to the microbe that stimulate the epithelial cells. The interaction of P. carinii with alveolar epithelial cells is a complex process involving glycoprotein A (gpA), a mannose-rich surface protein of P. carinii, as well as host alveolar proteins, including fibronectin, vitronectin, surfactant components, and other host-derived molecules that coat the organisms and promote adherence to the lung epithelium (28, 31, 34). Interestingly, TNF-α also binds to a soluble cell wall fraction of P. carinii and is present on freshly isolated organisms (30, 32). The mechanisms of TNF-α binding to P. carinii are not fully known, but recent investigations demonstrate that TNF-α interacts with fungal cell wall β-glucans through lectin-mediated binding (30). Lectin interactions utilize a region of the TNF-α molecule distinct from that which recognizes mammalian TNF-α receptors (30). Although we postulated that P. carinii-associated TNF-α might mediate enhanced ICAM-1 expression after organism binding to epithelial cells, ICAM-1 expression by A549 cells directly stimulated with freshly isolated P. carinii was not significantly inhibited by TNF-α antisera, suggesting that alternate mechanisms confer this effect. In additional experiments, antibody to γ-IFN also did not reduce ICAM-1 expression in A549 epithelial cells cultured with P. carinii (data not shown). Further studies are necessary to determine the roles of other native and host proteins in mediating this direct stimulation of epithelial ICAM-1 by P. carinii.

Although the absolute level of ICAM-1 induced in epithelial cells directly by P. carinii was less than that evoked by P. carinii-exposed macrophages, our findings do suggest that both mechanisms may occur during infection. For comparison of the response induced by P. carinii alone to a maximal stimulus, we further included TNF-α in selected experiments in which A549 cells were directly stimulated with P. carinii. The maximal surface ICAM-1 expression resulting from direct P. carinii stimulation ranged between 30 and 50% of that observed with TNF-α stimulation alone (1,000 U/ml). Similarly, we observed that P. carinii stimulated roughly one-third of the ICAM-1 mRNA compared with a maximal stimulation of A549 cells with TNF-α (Fig. 5). Our investigation further indicates that P. carinii directly influence epithelial activation of ICAM-1 expression through mechanisms independent of macrophage-derived TNF-α. Additional studies are required to determine the relative extent to which these mechanisms contribute to lung inflammation in vivo during P. carinii pneumonia.

Although ICAM-1 promotes inflammatory cell recruitment necessary for lung defense, exuberant ICAM-1 expression may also be detrimental to the host. Excessive neutrophils in bronchoalveolar lavage from patients with P. carinii pneumonia correlate with enhanced morbidity, increased gas-exchange abnormalities, and poorer survival during infection (22, 35). Marked ICAM-1 expression in P. carinii infection may exacerbate this deleterious neutrophil recruitment. Furthermore, modulation of pulmonary inflammation with corticosteroids improves survival and reduces the occurrence of respiratory failure in patients with moderate to severe P. carinii pneumonia (28). Corticosteroids are known to reduce TNF-α release from stimulated AMs and to directly suppress ICAM-1 expression (16, 25, 38). Recent investigations further indicate that excess pulmonary neutrophil influx may be selectively inhibited with monoclonal antibodies that recognize ICAM-1 (3, 26, 40). Accordingly, selective modulation of ICAM-1 may also have a potential therapeutic benefit during severe P. carinii pneumonia; however, additional animal and predilucine studies are warranted to evaluate the merits of such a strategy.

The mechanisms of lung inflammation that accompany P. carinii infection are only beginning to be defined. This study provides the first evidence that ICAM-1 is upregulated during P. carinii pneumonia. Our data further support a role for macrophage-derived TNF-α in the mediation of increased ICAM-1 expression in cultured lung epithelial cells. In addition, P. carinii organisms also appear to directly augment ICAM-1 protein and mRNA expression by cultured lung cells. Further understanding of lung inflammation during this serious infection should provide additional insights and potential therapeutic strategies for patients with P. carinii pneumonia.

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