

Induction of Fibrinogen Expression in the Lung Epithelium during *Pneumocystis carinii* Pneumonia

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***Pneumocystis carinii* is an important pulmonary pathogen responsible for morbidity and mortality in patients with AIDS. The acute-phase response (APR), the primary mechanism used by the body to restore homeostasis following infection, is characterized by increased levels of circulating fibrinogen (FBG). Although the liver is the primary site of increased FBG synthesis during the APR, we unexpectedly discovered that FBG is synthesized and secreted by lung alveolar epithelial cells in vitro during an inflammatory stimulus. Therefore, we sought to determine whether lung epithelial cells produce FBG in vivo using animal models of *P. carinii* pneumonia (PCP). Inflammation was noted by an influx of macrophages to *P. carinii*-infected alveoli. Northern hybridization revealed that γ -FBG mRNA increased two- to fivefold in *P. carinii*-infected lung tissue, while RNA in situ hybridization demonstrated increased levels of γ -FBG mRNA in the lung epithelium. Immunoelectron microscopy detected lung epithelial cell-specific production of FBG, suggesting induction of a localized inflammatory response resembling the APR. A systemic APR was confirmed by a two- to fivefold upregulation of the levels of hepatic γ -FBG mRNA in animals with PCP, resulting in a corresponding increase in levels of FBG in plasma. Furthermore, immunoelectron microscopy revealed the presence of FBG at the junction of cell membranes of trophic forms of *P. carinii* organisms aggregated along the alveolar epithelium. These results implicate FBG in the pathogenesis of PCP in a manner similar to that of the adhesive glycoproteins fibronectin and vitronectin, which are known to participate in intra-alveolar aggregation of organisms and adherence of *P. carinii* to the lung epithelium.**

Pneumocystis carinii pneumonia (PCP) has been demonstrated in a variety of immunosuppressed animal models with manifestations similar to those observed in afflicted humans (22, 27, 39, 49). Attachment of the organisms to alveolar type I epithelium is an essential step in establishing *P. carinii* infection (27, 30, 31, 41, 42). Ultrastructural studies indicate that *P. carinii* trophic forms attach to the surface of type I alveolar epithelial cells early in infection with limited interaction of organisms with type II cells as PCP progresses (27, 31, 32). The consequences of *P. carinii* attachment to the alveolar epithelium are only beginning to be understood. In the early stages of *P. carinii* infection, mononuclear cell infiltration represents the most prominent feature of the host inflammatory response, while little polymorphonuclear leukocyte or lymphocyte infiltration is observed (27, 29). Osmiophilic material, suggestive of changes in surfactant homeostasis, is observed in areas of marked necrosis (31) and during late stages of infection (32). Levels of surfactant protein-D (SP-D) increase in the lower respiratory tract during PCP (37), and increased amounts of SP-A are recovered in bronchoalveolar lavage fluids (40). Analyses of bronchoalveolar lavage fluids from patients with PCP, both with and without AIDS, revealed an accumulation of extracellular phospholipid surfactant-like material suggestive of an associated proteinosis, which was not observed in immunocompromised patients without PCP (52).

P. carinii infection is complicated by pulmonary fibrin deposition and the development of interstitial fibrosis (54). In a

study of lung biopsies from patients having PCP, marked interstitial fibrosis was observed which was characterized by the absence of intra-alveolar exudate and the atypical clustering of cysts within the connective tissue rather than in the alveolar spaces (54). Focal hyaline membranes, indicative of fibrin deposition are found in PCP (54). Presently it is believed that alveolar fibrin results from leakage of plasma fibrinogen (FBG) followed by its conversion to fibrin in the final stages of coagulation. Fibrin deposition influences inflammatory cell traffic and fibroblast proliferation (24). Furthermore, FBG, fibrin, and fibrin degradation products (FDPs), resulting from plasmin-mediated fibrinolysis, interfere with or inactivate surfactant function (24, 34, 45). Fibrosis in patients with PCP is characterized by fibrous thickening and fibroblastic proliferation within the alveolar septum (54). Patients with fibrosis on the initial biopsy tend to have abnormal pulmonary function tests after recovery from PCP. Thus, *P. carinii* infection clearly results in a disruption of pulmonary function and homeostasis.

FBG is the major blood clotting factor involved in the maintenance of hemostasis. During a systemic inflammatory response, FBG synthesis by hepatocytes is upregulated 2- to 10-fold such that levels of FBG in plasma increased from an average of 3 mg/ml to 6 to 30 mg/ml (10, 53). Increased levels of FBG in plasma and subsequent fibrin formation serve to restore homeostasis by providing a provisional matrix to support the cell interactions of wound healing (7). This upregulation of FBG gene expression during inflammation is one of a myriad of responses associated with the disruption of homeostasis due to infection, tissue injury, or neoplasia, collectively termed the acute-phase response or reaction (APR) (10, 53). Recently, we showed that FBG is synthesized and secreted by lung alveolar epithelial cells in vitro (46). Importantly, FBG

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synthesis by pneumocytes occurs in response to interleukin-6 (IL-6) and dexamethasone (DEX). Our more recent observations indicate that FBG, not fibrin, produced *in vitro* by lung epithelial cells in response to IL-6-DEX treatment, is secreted basolaterally (14) and becomes incorporated into the extracellular matrix (15). Thus, it is likely that locally synthesized FBG functions in the maintenance or restoration of the epithelial barrier during lung injury. We propose that increased synthesis and secretion of lung epithelial cell-derived FBG is one of the physiological events occurring during PCP. In this report, we demonstrate that expression of the γ -FBG gene is elevated in lung epithelial cells during PCP. Because subsequent fibrin deposition can occur during lung injury, these studies suggest that lung cell-type specific expression of FBG contributes to the balance between appropriate wound healing and the development of tissue fibrosis and scarring.

MATERIALS AND METHODS

Animal models and sample preparation. Ferrets were obtained from Marshall Farms (Northrose, N.Y.). PCP was induced in ferrets fed a normal diet by adding DEX (2 mg/liter) and tetracycline (500 mg/liter) to the drinking water (49). Immunosuppression caused by the steroid rendered the animals susceptible to natural infection by *P. carinii*. Control animals were treated with DEX and tetracycline or tetracycline only, as stated above, plus Bactrim (20 ml/liter) in the drinking water to prevent *P. carinii* infection. C.B-17 *scid/scid* mice, 8 to 10 weeks old, were obtained from the Trudeau Animal Breeding Facility (22). These SCID mice spontaneously develop detectable *P. carinii* infection at about 4 weeks of age. All forms of *P. carinii* were visualized by light microscopy after various staining procedures; the cyst form of *P. carinii* was visualized in lung sections after Gomori's methenamine silver (GMS) staining (19). To assess *P. carinii*-infected lungs for fungal contamination and to confirm the presence of *P. carinii* cysts and trophozoites, cytospin preparations of *P. carinii*-infected lung homogenates deposited by cytocentrifugation onto slides were stained with modified Diff-Quik (Difco, Detroit, Mich.) (22).

Lungs and livers were harvested after sacrificing the animals with an intraperitoneal injection of a lethal dose of Beuthanasia-D (390-mg/ml pentobarbital-50-mg/ml phenytoin; 1 ml per animal). At times, ferrets were exsanguinated by intracardiac puncture after the animals reached a plane of anesthesia but before death as determined by toe pinch reflex. Blood was anticoagulated with heparin for preparation of plasma. To retain airway macrophages (3), infected or control tissues used for histology and *in situ* hybridizations were sliced into 2- to 3-mm sections, fixed by immersion in formalin, and then embedded in paraffin blocks.

RNA isolation and Northern analysis. Molecular biology reagents were obtained from Life Technologies (Gaithersburg, Md.), chemical reagents were obtained from Sigma (St. Louis, Mo.), radionucleotides were purchased from DuPont New England Nuclear (Boston, Mass.), and the Zetaprobe nylon membrane was from Bio-Rad (Centerville, N.Y.). Total RNA and poly(A)⁺ mRNA were isolated as previously described (20), and Northern blot hybridization was carried out with ³²P-labeled ferret lung-specific γ -FBG cDNA, pFLG γ 3 (47), or the rat liver-specific γ -FBG cDNA, pR γ BP18 (17).

In situ RNA:RNA hybridization. *In situ* hybridization was performed essentially as previously described (9, 16, 17, 19, 47, 51). The pFLG γ 3 cDNA was cloned from ferret lung mRNA as previously described (47); the sequence is deposited with GenBank under accession no. U28494. The sense and antisense pFLG γ 3, γ -actin, and *P. carinii* surface glycoprotein A (gpA) riboprobes were labeled to a specific activity of 2.93×10^7 cpm/ μ g by using [³H]CTP and [³H]UTP. The riboprobe specific activity was calculated from the specific activity of the input ribonucleotides as previously described (1). Thus, probes generated from the same batches of radionucleotides will have the same specific activity, while the total mass synthesized is dependent on the efficiency of *in vitro* transcription, which varies from probe to probe. The antisense orientation of the riboprobe detects the positive expression of the mRNA, whereas the sense orientation of the riboprobe is used to detect background levels of silver grains in the absence of specific mRNA expression. The slides probed *in situ* were exposed to NTB-2 emulsion (Kodak, Rochester, N.Y.) for 8 weeks at 4°C. After photographic development, slides were counterstained with Mayer's hematoxylin and eosin (H&E) to visualize tissue and cell morphology by brightfield microscopy; silver grains were visualized under darkfield microscopy. Some slides upon which the *in situ* hybridization procedure was carried out were counterstained with GMS as previously described (19).

SDS-PAGE and Western blot analysis of human and ferret plasma FBG. Human FBG was purchased from KabiVitrum (Franklin, Ohio), and mouse and rat FBG were from Sigma. Ferret FBG was quantitatively precipitated from plasma (13) or purified as previously described (27). Commercially obtained FBGs were purified further to remove contaminating plasminogen and fibronectin (FN) (46). For Western blotting, 20 μ g of protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-

blotted to nitrocellulose. Immunoblotting was performed with monoclonal antibody (MAb) H9B7 generated against the γ chain of human FBG (18) and shown to cross-react with ferret FBG (47). Secondary antibodies and substrate color development were performed as previously described (18).

IEM. Polyclonal antiserum was raised in rabbits against purified mouse FBG, and the immunoglobulin G (IgG) fraction was affinity purified (28, 46). *P. carinii*-infected SCID mice were sacrificed, and the lungs were inflation fixed *in situ* with 4% paraformaldehyde-0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, as previously described (57). Fixed lungs were removed and processed into embedding resin, and immunoelectron microscopy (IEM) was performed as previously described (14).

RESULTS

Establishment of *P. carinii* infection and inflammatory cell response. *P. carinii* infection was documented after 4 to 6 weeks of DEX treatment by staining of tissue sections with GMS (Fig. 1A) and H&E (Fig. 1B), cytocentrifugation and Diff Quik staining of organisms from infected lung homogenates (Fig. 1C), and *in situ* hybridization of ferret lung tissue sections for expression of *P. carinii*-specific surface glycoprotein A (gpA) mRNA (Fig. 1B and D). Positive detection of mRNA is denoted under darkfield microscopy as abundant, white silver grains localized to organisms. Note that not all alveoli were uniformly infected. Panels A and B of Fig. 1 were also hybridized with the gpA antisense riboprobe. Under brightfield illumination, the silver grains denoting a positive signal for gpA mRNA in *P. carinii* appear as black dots. In addition, *P. carinii*-infected ferrets demonstrated the characteristic inflammatory cell response of alveolar monocyte/macrophage accumulation with little detection of polymorphonuclear leukocytes (Fig. 1A to C). The course of *P. carinii* infection was similar in SCID mice (not shown).

PCP induces an acute-phase inflammatory reactant in the lung. Because cultured lung epithelial cells synthesize and secrete FBG, and hepatic FBG gene expression is upregulated during an inflammatory response, we determined whether *P. carinii* infection induced FBG gene expression in the lung. The results of Northern hybridization indicated that the expression of γ -FBG mRNA was elevated four- to fivefold in the lungs of *P. carinii*-infected animals compared to the level in normal animals (Fig. 2B), indicative of a localized inflammatory response. Equal loading of host mRNA was indicated by similar staining of the 28S rRNA by acridine orange-stained gels run in parallel (not shown). Figure 2 shows autoradiographs of liver and lung tissues exposed at room temperature for 15 min and at -70°C for 6 h, respectively, indicating that, while the overall relative abundance of γ -FBG mRNA per microgram of RNA is much lower in the lung compared to the liver, the relative induction of γ -FBG mRNA was four- to fivefold higher in both the lung and liver of a *P. carinii*-infected animal (Fig. 2A and B, lanes 2) than in those of a control. To determine the cell-type specificity of γ -FBG mRNA expression in the infected lung, RNA:RNA *in situ* hybridization was performed. In both uninfected and infected lungs, γ -FBG mRNA expression was observed in the epithelium. The levels of γ -FBG mRNA were increased in airway epithelial cells of infected lung tissue (Fig. 3).

Alveolar epithelium produces FBG protein during PCP. To determine whether lung epithelial cells synthesize FBG protein *in vivo*, IEM of *P. carinii*-infected lung tissue was performed. The results showed the intracellular presence of FBG in secretory granules of epithelium lining the fused basement membrane of the septa between adjacent alveoli (Fig. 4A). *P. carinii* organisms were observed attached to alveolar epithelial cells where intracellular FBG protein was detected (Fig. 4B). While the complete life cycle of *P. carinii* has not been fully elucidated due to the lack of a continuous *in vitro* culture system, several developmental stages of the organism have been identified by light and electron microscopy (23, 27, 31, 32, 59). The two

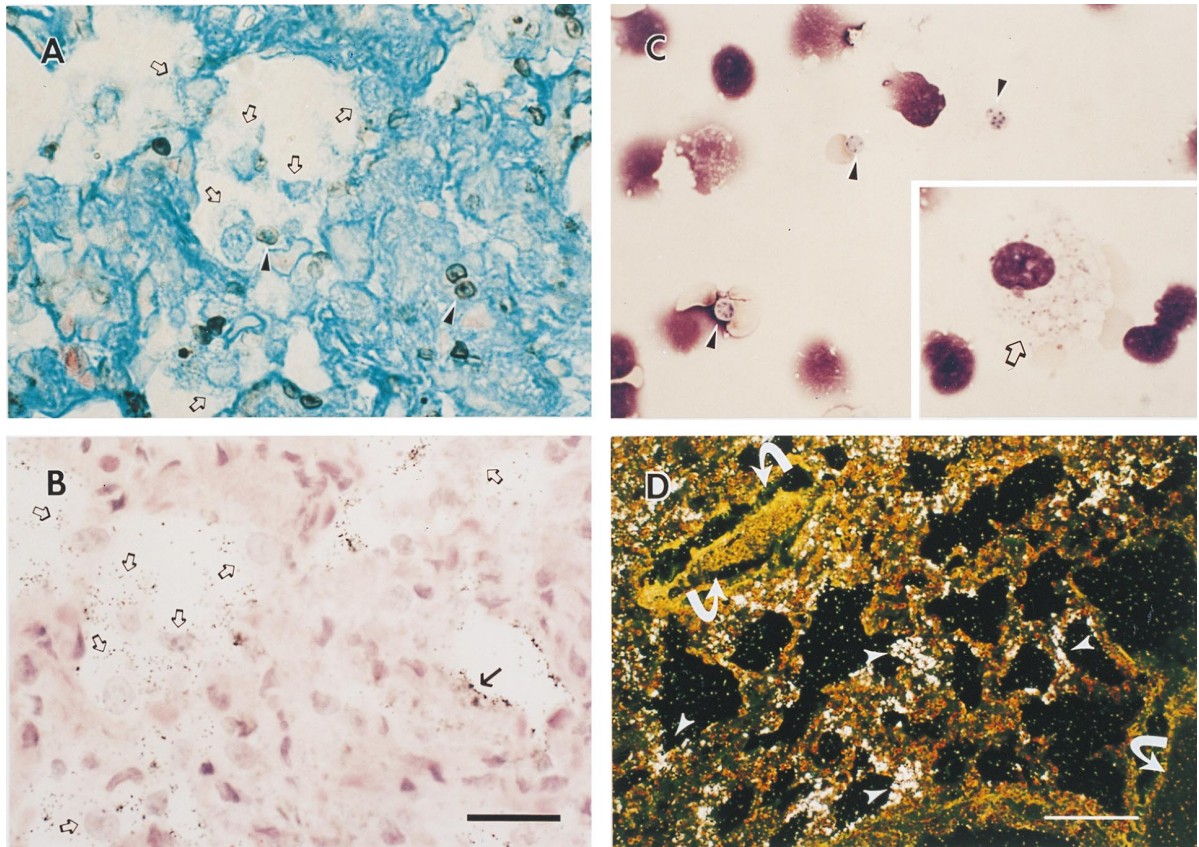


FIG. 1. Pathology of *P. carinii*-infected lung tissue. Alveolar macrophages (open arrows) were detected in *P. carinii*-infected ferret lung tissue by GMS (A) and H&E (B) staining (cysts, arrowheads; trophs, arrows). Diff Quik staining demonstrates both trophic (inset, open arrow) and cyst (arrowheads) nuclei in a cytospin preparation of infected ferret lung homogenate (C). Note that the cyst forms of *P. carinii* shown in this field (arrowheads) contain between four and eight nuclei representative of intracystic bodies that correspond to the individual nuclei of the trophic forms released upon excystation during a stage in the *P. carinii* life cycle. The bar in panel B represents 20 μ m for panels A to C. Trophic nuclei within a macrophage cytoplasm were occasionally detected (C, inset). (D) A low-power darkfield exposure of *P. carinii*-infected lung probed with the gpA antisense riboprobe specific for *P. carinii* gpA mRNA. Bar, 70 μ m. Arrows indicate blood vessels.

major developmental forms of the organism, the amorphous trophic form (also known as troph, trophozoite, or intracystic body) and the rigid cyst form differ in the composition of their cell walls (59). During the putative life cycle, the trophozoite matures into a precyst which develops up to 8 intracystic bodies. Upon excystation, these "daughter" trophs are released to renew the cycle. In this study, the characteristic trophic and cyst forms of *P. carinii* were observed by both light (Fig. 1) and transmission electron microscopy (Fig. 4). Furthermore, intra-alveolar FBG was detected at the junction of cell membranes of trophic forms of *P. carinii* aggregated at the apical face of the alveolar epithelium (Fig. 4D), suggesting that FBG may participate in aggregation and adherence of the organisms to type I epithelium.

PCP induces a systemic APR. Northern hybridization revealed that *P. carinii* infection induced upregulation of the γ -FBG gene as determined by increases in the steady-state levels of liver γ -FBG mRNA (Fig. 2A). RNA in situ hybridization studies demonstrated that the levels of γ -FBG mRNA were significantly upregulated in all hepatocytes during PCP, while the levels of γ -actin remained the same (Fig. 5). This observation is consistent with a systemic APR to the infection. The induction of systemic inflammation in the ferret model of PCP was further supported by increased levels of FBG in plasma (Fig. 6A), also detected by Western blot identification of the ferret FBG γ -chain with the cross-reacting MAbs, H9B7 (Fig. 6B).

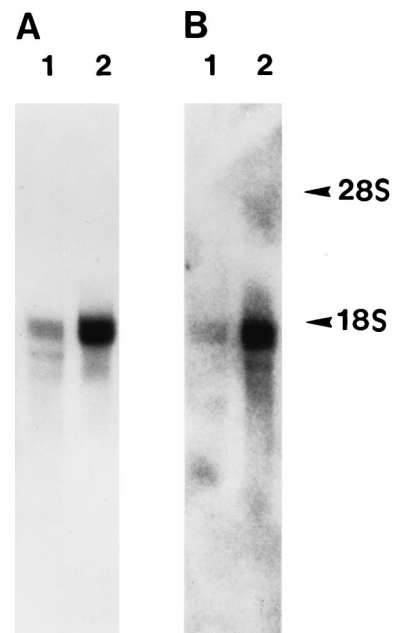


FIG. 2. Northern blot analysis of normal and PCP ferret liver and lung mRNAs. Poly(A)⁺ mRNA (5 μ g) from normal (lanes 1) and *P. carinii*-infected (lanes 2) ferret liver (A) and lung (B) probed with pFLG γ 3. The positions of the host rRNAs are indicated.

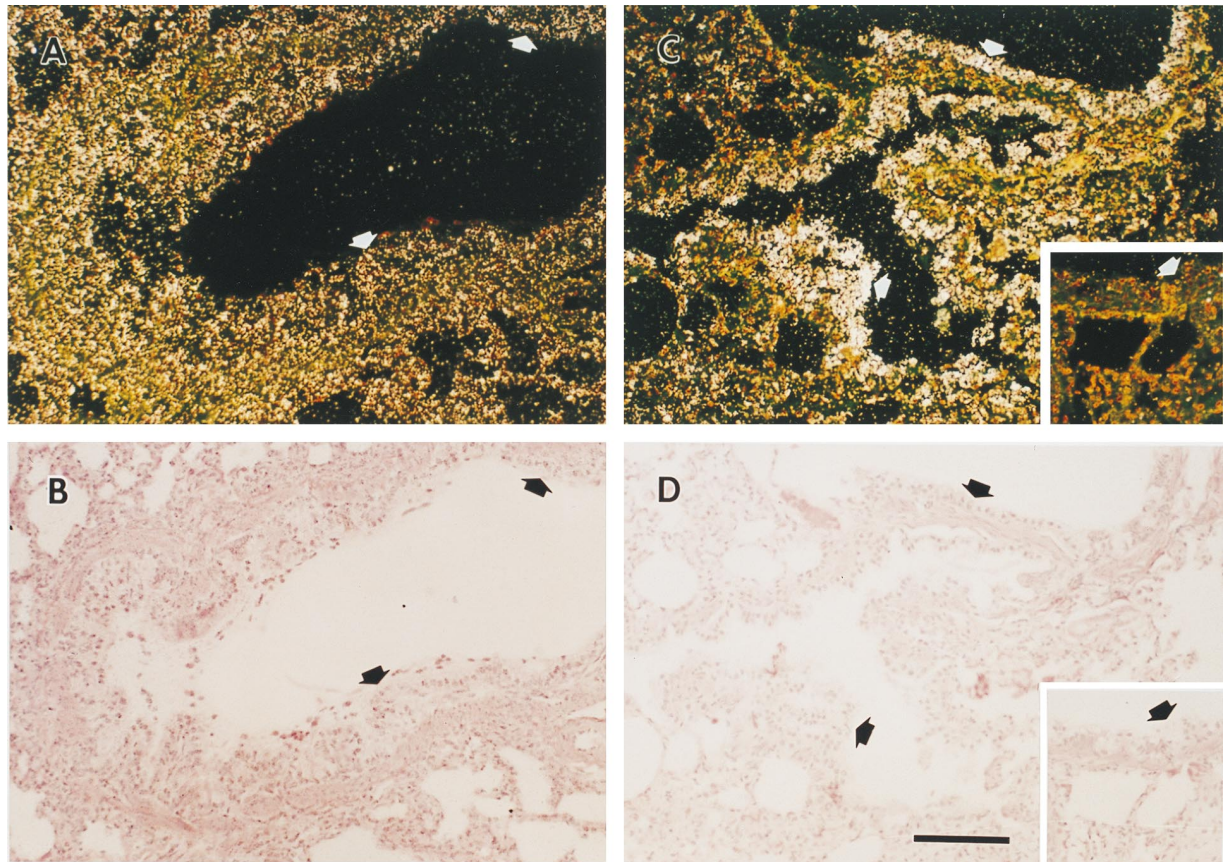


FIG. 3. In situ hybridization of lung tissues from normal and *P. carinii*-infected ferrets. Tissue sections shown in panels A to D were probed with pFLG γ 3 antisense probe; insets were probed with pFLG γ 3 sense probe. The arrows indicate bronchial epithelial cells in *P. carinii*-infected (C) and normal (A) ferret lung tissues. H&E stains of the same fields are also shown (B and D, respectively). Positive detection of mRNA is denoted under darkfield microscopy as abundant, white silver grains localized to the cytoplasm of cells (panels A and C).

The role of *P. carinii* infection in the absence of DEX immunosuppression. To determine whether *P. carinii* infection in the absence of DEX immunosuppression resulted in increased lung expression of γ -FBG, the SCID mouse model of PCP was utilized. The levels of γ -FBG mRNA were increased twofold in *P. carinii*-infected SCID mouse liver consistent with the level of induction of FBG gene expression in a variety of in vivo and in vitro models of inflammation. While the basal level of γ -FBG mRNA was undetectable in control lung total RNA, γ -FBG mRNA was detectable in *P. carinii*-infected lung total RNA, indicating an increase in steady-state levels of γ -FBG mRNA during *P. carinii* infection (Fig. 7). These results indicate that *P. carinii* infection alone in the absence of DEX immunosuppression is sufficient to induce the mediator(s) of γ -FBG expression locally in the lung and systemically in the liver.

The role of DEX immunosuppression in the absence of *P. carinii* infection. To determine whether DEX treatment alone, in the absence of *P. carinii* infection, resulted in elevated γ -FBG gene expression, ferrets were treated with tetracycline, Bactrim, and DEX to achieve immunosuppression but prevent PCP; control animals were treated with tetracycline and Bactrim but not with DEX. RNA was prepared from the lungs and livers of matched pairs of animals 3 and 4 weeks after initiation of DEX immunosuppression. Microscopic examination of GMS-stained histological sections cut from the Bactrim-treated immunosuppressed and control ferret lungs indicated that Bactrim was successful in preventing *P. carinii*

infection (not shown). Total RNAs (20 μ g) from 4-week DEX-immunosuppressed tissues and normal tissues were probed with the ferret specific γ -FBG cDNA, pFLG γ 3. Equivalent amounts of γ -FBG mRNA were found in DEX-treated compared to normal ferret liver, indicating that prolonged exposure of the animal to DEX immunosuppression did not result in induction of γ -FBG gene expression (not shown). To demonstrate basal amounts of γ -FBG mRNA in lung tissues of the control and DEX-treated ferrets, reverse transcriptase (RT) PCR amplification of total RNA (10 μ g) from *P. carinii*-infected liver and lung tissues was carried out. The results demonstrated that the lung tissue mRNA was intact and that basal amounts of steady-state γ -FBG mRNA were present in normal lung tissue (Fig. 8A), as shown above by Northern (Fig. 2) and in situ hybridizations (Fig. 3). The primer pairs used for amplification of the RNA:DNA heteroduplex resulted in a 200-bp product for γ -FBG and a 450-bp product for β -actin (Fig. 8A). To confirm the absence of *P. carinii* organisms at the microscopic level, RT-PCR was performed using primer pairs specific for ferret *P. carinii* gpA. The results indicated that none of the Bactrim-treated animals were susceptible to breakthrough infection with *P. carinii*, as the characteristic 220-bp product for ferret-derived *P. carinii* gpA was not obtained (Fig. 8B). Taken together, these results confirm that the observed increase in γ -FBG gene expression in both liver and lung tissues occurs only during *P. carinii* infection and is not a result of prolonged DEX immunosuppression.

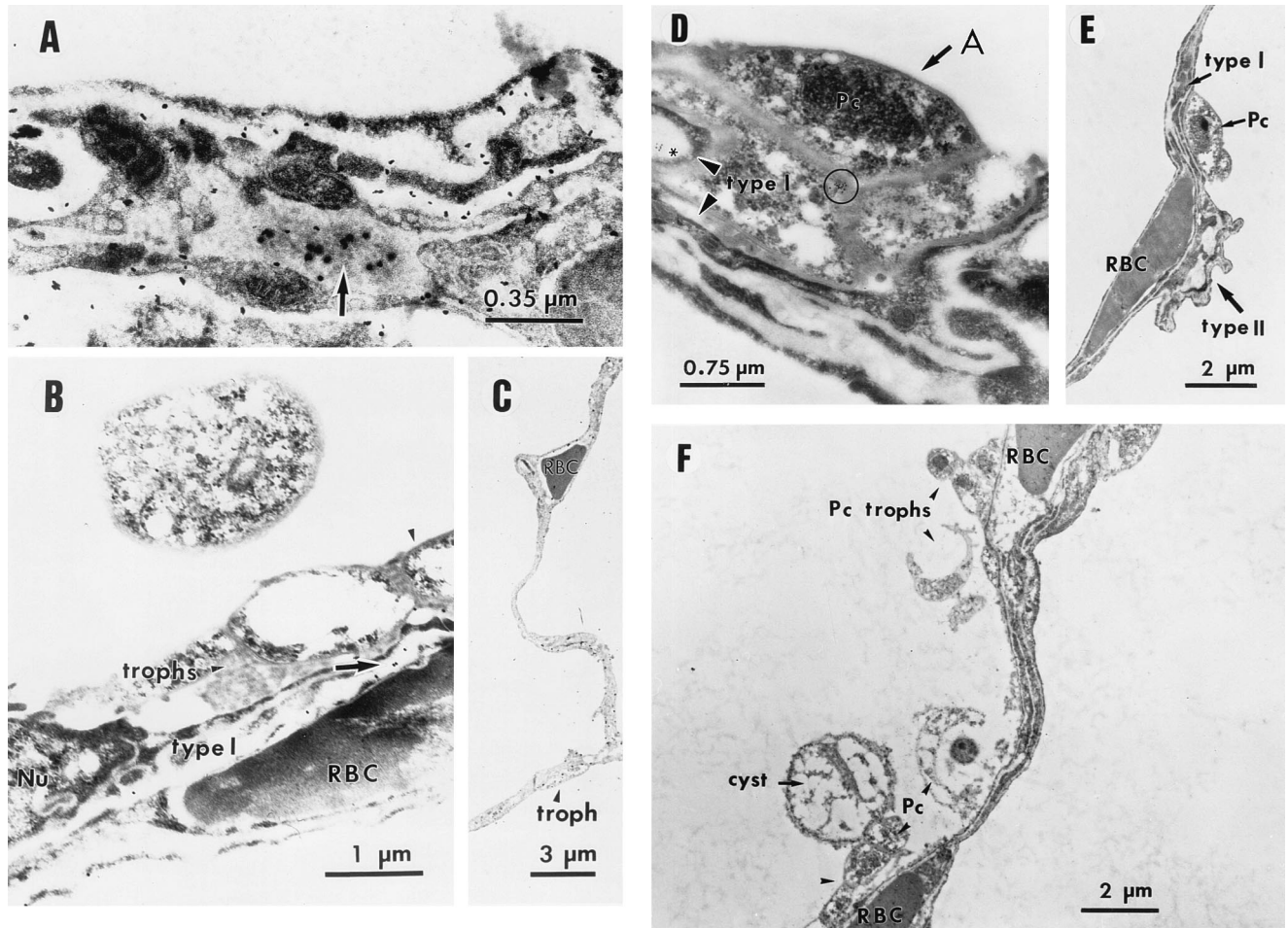


FIG. 4. IEM localization of FBG in the lung epithelium. The intracellular presence of FBG in *P. carinii*-infected lung tissue was determined by IEM by using 100 μ g of anti-FBG IgG/ml detected with Protein-A-gold (diameter, 20 nm) conjugate (panels A and B, arrows; panel D, asterisk). FBG was detected at the junction of cell membranes of trophic forms of *P. carinii* organisms (panel D, circle) aggregated (panel D, arrow) along the alveolar epithelium (panel D, arrowheads). Purified rabbit IgG (100 μ g/ml) was used as the negative control in the primary antibody step (F). The morphologically rigid cyst and pleomorphic trophic forms of *P. carinii* organisms attached to type I epithelium and aggregated in the alveolar spaces are shown in panels B to F. Lower magnifications show the integrity of septal walls between alveoli (C and F). Alveolar capillaries are indicated by the presence of the erythrocytes (RBC). The appropriate magnification for each field is indicated. A, alveolar lumen; Nu, nucleus; Pc, *P. carinii*.

DISCUSSION

Our data show clearly that *P. carinii* infection results in local and systemic inflammatory responses as measured by increased levels of γ -FBG mRNA in lung and liver tissues, respectively. Prolonged immunosuppression of animals with DEX, in the absence of *P. carinii* infection, did not result in an increase in γ -FBG mRNA expression in either the lung or liver. In light of these results, the induction of FBG mRNAs in the ferret is not likely the result of nonspecific upregulation secondary to DEX administration but is a specific consequence of PCP. Finally, we showed the in vivo production of FBG by the lung alveolar epithelium by IEM and have previously demonstrated that IL-6 and DEX, known positive regulators in the induction of hepatic FBG gene expression (10, 53), induce the synthesis and secretion of intact FBG from a human lung epithelial cell line (46). Furthermore, elevated expression of γ FBG mRNA in the liver and lung during PCP suggested that, in addition to a systemic (liver) inflammatory reaction, a tissue-specific (i.e., lung) inflammatory reaction akin to the APR was induced. Haptoglobin, another APR protein whose expression was considered liver specific, is also expressed in the lung epithelium during inflammation (58).

Until recently, little was known about the induction of an APR during PCP. Syrjala et al. (50) monitored the levels of C-reactive protein (CRP) in human immunodeficiency virus-infected and immunocompromised patients with or without PCP; CRP is upregulated several hundredfold in a systemic APR (10, 53). The levels of CRP were elevated over 200-fold in patients with a fatal outcome of PCP, while human immunodeficiency virus infection itself did not significantly increase CRP levels (50). The erythrocyte sedimentation rate was increased in PCP patients as well, indicative of elevated levels of FBG in plasma in response to a systemic inflammation (10, 53). A review of case histories examining AIDS patients with PCP revealed that 7 of 37 cases had hypoalbuminemia (8); albumin is downregulated during the APR (10, 53). The peripheral blood monocyte/macrophage accessory cell population represents the body's major source of IL-6, the cytokine which plays a central role in regulating the coordinated cytokine response to injury or infection during the host response to acute or chronic inflammation (53); lung cells express elevated levels of IL-6 during PCP (2, 57). Alveolar macrophages were the predominant inflammatory cell type observed during PCP in ferrets, as was also shown in other animal models of PCP (29, 57).

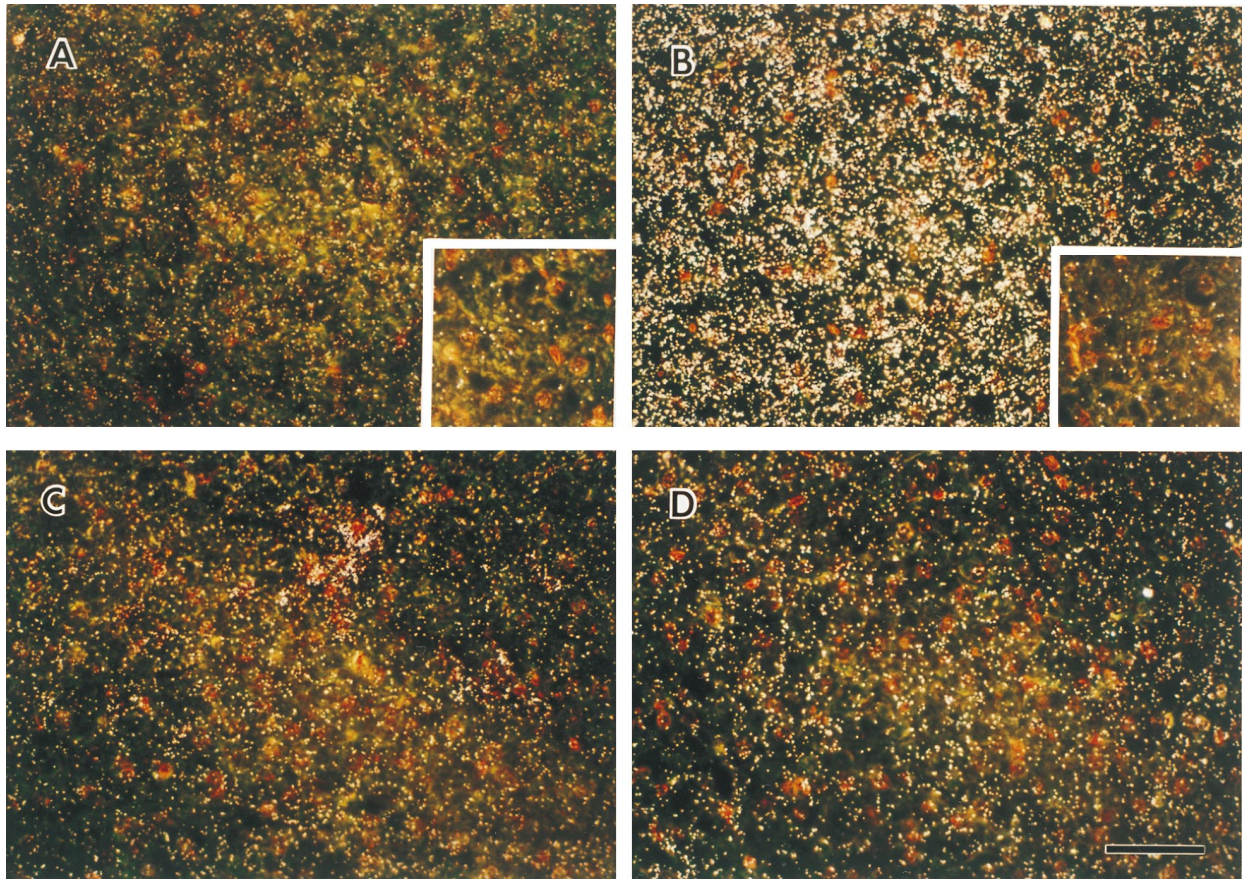


FIG. 5. In situ hybridization of liver tissue from normal and *P. carinii*-infected ferrets. Normal (A and C) and *P. carinii*-infected (B and D) livers were probed with pFLG γ 3⁺ (panels A and B) or γ -actin antisense probes (panels C and D); insets were probed with pFLG γ 3⁻ sense probe.

Macrophages contribute to the control of *P. carinii* infection by the production of the proinflammatory cytokines IL-1 β , IL-6 and tumor necrosis factor alpha (TNF- α). During PCP, IL-1 and TNF- α are present only in the lungs (5, 6, 21), whereas IL-6 is found in both the lungs and circulation (4, 57). These findings suggest that TNF- α and IL-1 function principally at the site of infection, whereas IL-6 acts both at the site of infection and systemically in the upregulation of FBG gene expression in the lung and liver, respectively, as shown in this

study. Together, these observations indicate that *P. carinii* infection induces a systemic inflammatory response. Moreover, production of FBG by the lung epithelium is indicative of a local inflammatory response during infection, providing the first demonstration of the extrahepatic expression of FBG during an acute or chronic inflammatory response in vivo.

A growing body of literature has emerged elucidating interrelationships among inflammatory cells, cytokines, and FBG and its related products, fibrin and FDPs, that is applicable to

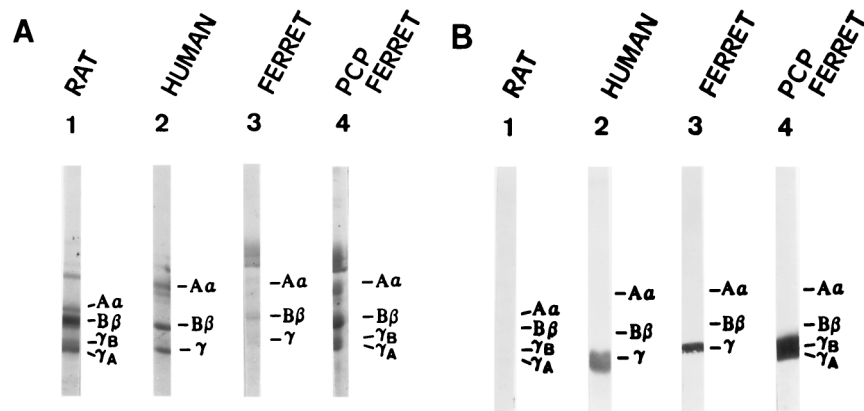


FIG. 6. SDS-PAGE and Western blot of plasma FBG. Ferret plasma FBG was quantitatively precipitated from 8 μ l of plasma and resolved by SDS-7.5% PAGE. (A) Coomassie blue stain; (B) immunoblot with cross-reacting anti-human γ chain MAb, H9B7.

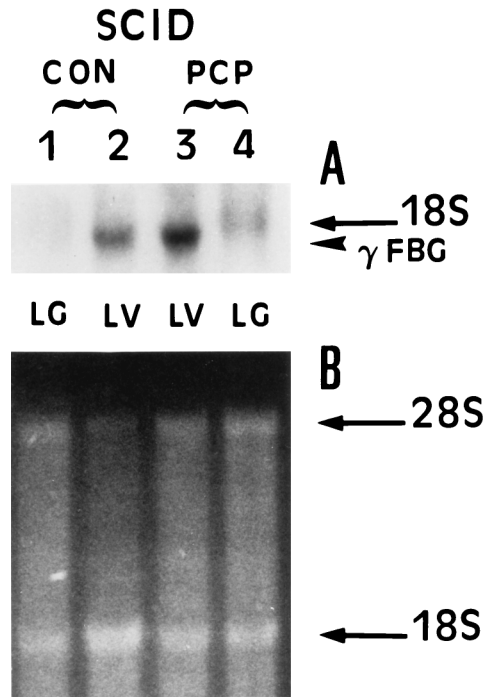


FIG. 7. Northern blot analysis of γ -FBG mRNA in *P. carinii*-infected SCID mouse liver and lung. (A) Total RNA (20 μ g) from uninfected (CON, lanes 1 and 2) and *P. carinii*-infected (PCP, lanes 3 and 4) SCID mouse liver (LV, lanes 2 and 3) and lung (LG, lanes 1 and 4) was probed with γ -FBG cDNA pR- γ BP18. (B) Acridine orange-stained gel of duplicate set of denatured RNA.

understanding the progression of PCP. During *P. carinii* infection, elevated levels of TNF- α may induce pulmonary expression of intercellular adhesion molecule 1 (ICAM-1, CD54) (60), which is involved in cell-cell interactions (48). It is known that the γ -chain of FBG can enhance leukocyte binding to cells through a bridging mechanism involving either two ICAM-1s on opposing cells or through CD11b-CD18 (CR3, Mac-1) and ICAM-1 (26, 56). Additionally, the presence of FDPs may adversely affect functional CD4⁺ T lymphocytes (24, 43), which are essential for host immunity to *P. carinii* infection (39). Furthermore, FDPs induce monocytes to express both procoagulant (tissue factor) and antifibrinolytic activities (tissue plasminogen activator inhibitor 1) (44). These studies demonstrate that lung-specific injury induces a systemic inflammatory response and suggest that excessive fibrin(ogen) in lung tissue poses a serious threat to adequate resolution of lung infection and injury.

The mechanism of *P. carinii* attachment and how the lung epithelium responds to this attachment has been investigated extensively. *P. carinii* surface gpA (12) mediates attachment of the organism to the lung epithelium (30, 41, 42). Several host proteins bind to *P. carinii* gpA, including SP-A (61), SP-D (37), mannose binding protein (11, 38), and the extracellular matrix components FN (41, 42) and vitronectin (VN) (30, 55). Aside from its known functions in fibrin formation and platelet aggregation, the γ chain has been shown to mediate the adhesion of microorganisms to FBG and fibrin (2, 25). Adhesion of microorganisms to surfaces is the critical early event in colonization. Adherence of host proteins to the surface of microorganisms may allow them to escape immune surveillance by disguising themselves as "self" (25). FN (41, 42) and VN (30, 55) also affect attachment of *P. carinii* to the lung epithelium by functioning as bridging molecules. Adherence of *P. carinii* to

host molecules takes advantage of the well-described RGD-adhesive domains and heparin binding domains of FN or VN (41, 55). Levels of FN, VN (33), and now FBG have been shown to increase in the lung during PCP. FBG also contains RGD and heparin binding domains (35, 36) and functions as a bridging molecule in known cell-cell interactions (26, 56). Thus, it is likely that FBG synthesized by the lung epithelium may promote adhesion of *P. carinii* and/or adhesion of inflammatory cells to sites of infection. In support of this hypothesis, we observed FBG immunogold staining that appeared to bridge between *P. carinii* organisms adhered to the alveolar epithelium. We postulate that the FBG RGD and heparin binding domains participate in such intra-alveolar clumping of the organisms.

Tissue response to injury involves the activation of repair mechanisms that promote removal of debris and tissue remodeling to restore normal architecture and function. Extensive or prolonged lung injury is likely to result in excessive tissue destruction and development of fibrosis (24). Synthesis of FBG in lung may serve to restore homeostasis by aiding in wound repair or extracellular matrix remodeling after injury, similar to the function of circulating hepatic FBG produced in response to acute or chronic inflammation. Indeed, neutralization of IL-6 during PCP results in more severe inflammation and delayed clearance of the organism from the lung (4). However, elevated levels of FBG at the sites of inflammation, particularly associated with lung injury, may result in pathogenesis caused by overactive repair mechanisms. The association of high levels of FBG and fibrin deposition in lung disease becomes of greater concern when considering the possibility that local synthesis of FBG could contribute to rearranged lung architecture. Moreover, formation of fibrin and subsequent fibrinolysis in lung tissue at sites of inflammation may further enhance production of proinflammatory cytokines and thereby lead to pathophysiologic events. An imbalance of procoagulant and fibrinolytic activities of resident and infiltrating mononuclear cells may also lead to abnormalities in surfactant function

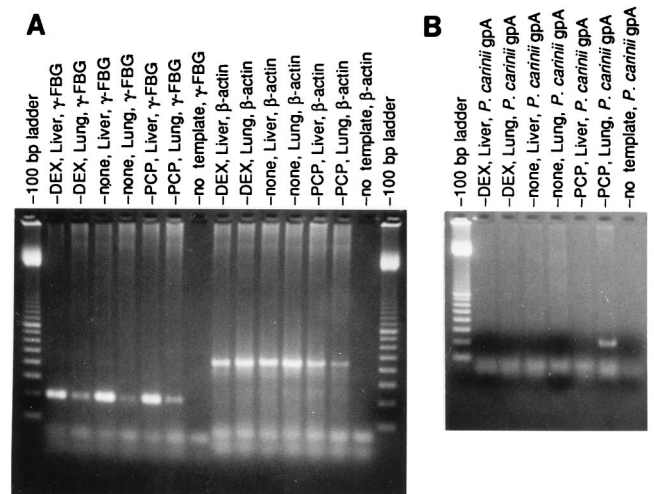


FIG. 8. RT-PCR of RNA isolated from ferrets treated in the presence or absence of DEX and Bactrim. Bactrim-treated DEX-immunosuppressed (DEX), control (none), and *P. carinii*-infected (PCP) ferret lungs and livers were amplified with γ -FBG and β -actin primer pairs (A). Total RNAs from the livers and lungs of *P. carinii*-infected ferrets were amplified with a gpA-specific primer pair as positive controls for the RT-PCR (B). In both panels, no template served as the negative control reaction. The molecular size markers are in 100-bp increments.

(34, 45). When surfactant production is reduced or surfactant is damaged, alveoli collapse, causing hypoxia and the conditions that favor edema formation, resulting in a potentially fatal outcome in patients suffering from PCP or other severe lung diseases. Therefore, synthesis of FBG upon induction by proinflammatory mediators in the pulmonary epithelium is a significant new finding that may have dramatic implications for improving the management of patients with lung diseases or infections.

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