Fibrinogen Synthesis in the Lung in Response to Particulate Air Pollution

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The mechanisms by which elevated levels of particulate air pollution increase the risk of cardiovascular (CV)-related deaths remain unclear. A role for plasma fibrinogen has been proposed with the suggestion that particle induced inflammation in the lung may stimulate an hepatic acute phase response and thereby increase plasma fibrinogen concentration, a known risk factor for CV disease. Human alveolar epithelial cells have been reported to synthesize fibrinogen and we therefore investigated the possibility that particle deposition may, through direct and indirect mechanisms, induce local alveolar production of fibrinogen. Here we present data to suggest that PM$_{10}$ exposure could stimulate alveolar epithelial cell synthesis of fibrinogen indirectly, through the induction of inflammatory cytokines such as interleukin-6. Locally produced fibrinogen may exacerbate pulmonary inflammation and potentially contribute to systemic levels of plasma fibrinogen, thereby increasing the risk of CV responses in susceptible, PM$_{10}$ exposed individuals.

**Keywords:** pollution; cardiovascular disease; fibrinogen

**INTRODUCTION**

A number of epidemiological studies have reported a rise in the number of cardiovascular (CV)-related deaths in association with elevated levels of particulate air pollution (Burnett *et al.*, 1995; Schwartz, 1999). Seaton *et al.* (1995) suggested that this association may involve a rise in plasma fibrinogen, a known risk factor for CV disease, in response to deposition of particulate matter in the lung. They proposed that cytokines, released during alveolar inflammatory responses to particle deposition, could stimulate an hepatic acute phase response with a resultant rise in plasma fibrinogen concentration. Following a report that human alveolar epithelial cells can both synthesize and secrete fibrinogen (Haidaris, 1997), we investigated whether PM$_{10}$ particles could stimulate alveolar epithelial cell expression of fibrinogen either directly, by particle–epithelial cell interactions, or indirectly, through induction of inflammatory cytokine release by neighbouring cells. The ability of particles to induce cytokine release by epithelial cells and macrophages has previously been demonstrated in vitro (Becker *et al.*, 1996; Quay *et al.*, 1998).

Fibrinogen is the precursor of fibrin, the basis of the blood clot, and is considered primarily for its role in haemostasis. The proteolytic degradation of fibrinogen and fibrin, a mechanism to regulate clot formation, leads to the generation of a number of low-molecular-weight peptides termed fibrin(ogen) degradation products (FDPs). A number of inflammatory properties have been described for both fibrinogen and FDPs, including phagocyte activation (Sitrin *et al.*, 1998) and neutrophil chemotaxis (Senior *et al.*, 1986), respectively. It is plausible, therefore, that induction of fibrinogen synthesis in the lung could exacerbate the pro-inflammatory effects of particle exposure. Alveolar inflammation has been shown to induce hepatic synthesis of fibrinogen (Simpson-Haidaris *et al.*, 1998), and so through exacerbation of pulmonary inflammation, lung-derived fibrinogen may therefore induce a chronic acute phase response, with a resultant rise in plasma fibrinogen concentration and an increased risk of CV disease in response to particulate exposure.

**MATERIALS AND METHODS**

The human alveolar type II epithelial cell line, A549, was treated with either interleukin (IL)-6 (0,
0.1, 1 or 10 ng/ml), IL-8 (10 ng/ml), interferon-gamma (IFNγ) (10 ng/ml), tumour necrosis factor-alpha (TNFα) (10 ng/ml) or PM10 (25 µg/ml) for 24 h, in order to determine the effects of inflammatory cytokine and direct particle exposure on fibrinogen expression. All treatments were carried out under serum-free culture conditions. Fibrinogen expression by A549 cells was studied by indirect immunofluorescence using a monoclonal anti-fibrinogen antibody (Sigma, Poole, UK) and fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin (Sigma). FITC fluorescence was visualized by digitally enhanced UV video microscopy (Coolsnap fx, Phototonics, Roper Scientific, Hemel Hempstead, UK). Image analysis software (Metamorph, Roper Scientific) was then used to analyse the fluorescence intensity of individual cells. Fluorescence intensities of 300 or 900 cells were analysed per treatment group. Each value represents the mean intensity of 100 cells. Statistical analysis was carried out using one-way analysis of variance with Tukey’s multiple comparison.

RESULTS
A subpopulation of A549 cells was found to constitutively express fibrinogen in the cytoplasm. IL-6 stimulated a dose dependent upregulation of fibrinogen expression in A549 (Fig. 1), with a significant increase at both 1 and 10 ng/ml (P < 0.01 and P < 0.001, respectively, n = 9). In contrast, neither IL-8, TNFα nor IFNγ had any significant effect on A549 cell expression of fibrinogen (Fig. 2). In preliminary experiments, PM10 treatment (25 µg, 24 h) of the epithelial cells induced a slight up-regulation of fibrinogen expression (P < 0.05, n = 3) (Fig. 3), although subsequent treatments have produced variable findings.

DISCUSSION
These studies demonstrate the synthesis of fibrinogen by the human alveolar type II epithelial cell line, A549, as previously reported (Haidaris, 1997). We found that IL-6, reported to be produced by lung cells in response to particle exposure (Becker et al., 1996; Quay et al., 1998), was able to up-regulate fibrinogen expression by A549 cells. In contrast, IL-8, TNFα and IFNγ had no significant effect on A549 cell expression of fibrinogen. TNFα has been shown to stimulate the release of other cytokines, such as IL-6 (Baumann and Gauldie, 1994) and may therefore play an indirect role in the stimulation of fibrinogen synthesis. The direct involvement of IL-6 in alveolar fibrinogen synthesis would be expected as its ability to induce hepatic synthesis of fibrinogen is well established (Baumann and Gauldie, 1994). These findings suggest that the regulation of alveolar fibrinogen production may involve specific mediators within the local cytokine network.

In this study, the direct treatment of epithelial cells with PM10 led to a slight, but less pronounced
increase in fibrinogen expression than that observed for IL-6. This would suggest that PM$_{10}$-mediated stimulation of alveolar fibrinogen production is most likely to involve an indirect mechanism through local induction of inflammatory cytokine release, rather than by direct stimulation of epithelial cells by particles themselves.

On the basis of reports suggesting an involvement of coagulation peptides in inflammatory responses (McGilvray and Rotstein, 1998), it is plausible to suggest that the production of fibrinogen in the lung, in response to particulate air pollution, may exacerbate both local and systemic particle-mediated effects (Fig. 4). Activation of mononuclear phagocytes by fibrinogen (Sitrin et al., 1998), for example, could stimulate further cytokine release in the lung during particle-induced inflammation. Highly inflammogenic FDPs are also known to stimulate neutrophil chemotaxis (Seniot et al., 1986). Through such induction of cytokine release and attraction of inflammatory cells, increased concentrations of fibrinogen in the lung could exacerbate particle-mediated pulmonary inflammation.

Although this study has only demonstrated in vitro production of fibrinogen in response to inflammatory cytokine stimulation, in vivo synthesis in response to pulmonary inflammation has also been reported. Increases in plasma fibrinogen and serum IL-6 have been reported in response to dust inhalation (Sjogren et al., 1999). Simpson-Haidaris et al. (1998) reported...
induction of both lung cell-specific and hepatocyte production of fibrinogen in response to *Pneumocystis carinii* infection in the lung. These studies suggest a role for alveolar inflammation in the induction of both local and systemic fibrinogen synthesis. Through exacerbation of pulmonary inflammation, lung-derived fibrinogen could contribute to systemic levels of cytokines and thereby lead to further stimulation of hepatic fibrinogen synthesis. Fibrinogen produced in the lung could also contribute directly to plasma fibrinogen concentration by transfer from the alveolar space to the circulation. The ability of high-molecular-weight molecules to gain access to the blood from the lung has been demonstrated recently in studies of the delivery of therapeutic peptides by inhalation (Sanjar and Matthews, 2001). By these two mechanisms, particle-mediated stimulation of fibrinogen production in the lung could result in a rise in plasma fibrinogen and thereby an increase in the risk of CV-related disease in susceptible individuals exposed to elevated levels of particulate air pollution.

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**REFERENCES**


