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Review

Idiopathic Pulmonary Fibrosis: Molecular Mechanisms and Possible Therapeutic Strategies

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Abstract. Idiopathic pulmonary fibrosis (IPF) is a devastating disease with an almost universally terminal outcome. In recent years much insight has been gained into the pathogenesis of IPF from both a bleomycin mice-model as well as *ex vivo* human tissue studies. Alveolar damage and inflammation of unknown etiology, eventually leading to interstitial fibrosis, characterize IPF. Apoptosis has emerged as an important factor in the pathogenesis of IPF. This review will outline the current understanding of the immunological and molecular mechanisms underlying IPF and discuss new therapeutic strategies.

Key words: apoptosis; fibrosis; Fas; inflammation; bleomycin.

Introduction

Idiopathic pulmorary fibrosis (IPF) is a devastating, chronic progressive disease with an almost invariably terminal outcome. IPF is characterized, in the end-stage, by impaired gas exchange and a restricted lung-volume due to interstitial alveolar fibrosis. Pulmonary fibrosis may result from diverse insults to the lung: exogenous factors which include infections, radiation damage and inhalation of dusts, and endogenous factors, which include connective tissue disorders and sarcoidosis. In up to 50% of cases no known predisposing factor has been found, and this disease is known as IPF or cryptogenic fibrosing alveolitis in the United Kingdom. Patients suffering from IPF, most of them over 50 years of age, initially suffer from dyspnoe on exertion, non-productive coughing or both. On clinical examination of the chest, end-inspiratory crackles at the bases of the lungs are noted and approximately 25% of patients have finger clubbing. On chest radiographs, reticular shadowing and honeycombing are found. The histopathology of clinical IPF comprises four distinct interstitial pneumonias: usual interstitial pneumonia, the most common form, desquamitive interstitial pneumonia, acute interstitial pneumonia and nonspecific interstitial pneumonia[33].

Due to the relatively low occurrence of IPF and case definition differences between epidemiological reports, an accurate incidence of IPF is hard to establish. The incidence of IPF was believed to be 5 persons in every

Abbreviations used: BAL – broncho-alveolar lavage, EBV – Epstein-Barr virus, ET-1 – endothelin-1, FasL – Fas ligand, G-CSF – granulocyte colony-stimulating factor, IFN-γ – interferon γ, IL – interleukin, IPF – idiopathic pulmonary fibrosis, MAPK – mitogen-activated protein kinase, MCP – monocyte-chemoattractant protein, MHC – major histocompatibility complex, MIP – macrophage inflammatory protein, TGF-β – transforming growth factor beta, Th – T helper, TNF-α – tumor necrosis factor α, PAI-1 – plasminogen activator inhibitor-1, SCID – severe combined immunodeficiency.

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100 000; however, more recently the incidence has been reported to be 18 persons in every 100 000 in the United States[13]. The effects from therapeutical protocols, comprising corticosteroids and immunosuppressants/cytotoxics (e.g. cyclophosphamide), are disappointing and side-effects are severe. The median survival from diagnosis is approximately 4 year[49] or, in a survival study where prevalent cases were excluded, even 2.9 year[27]. Single lung-transplantation is currently the only therapeutic option conferring survival benefit in end-stage IPF[25]. Annual mortality due to IPF has doubled over the last two decades[32], and is still rising in the UK, Canada and the United States[28], highlighting the need for a better understanding of and improved therapeutic strategies for IPF.

In recent years, studies in rodent-bleomycin models and *ex vivo* studies of human material have yielded considerable advances with regard to the molecular mechanisms underlying IPF and this review will outline the insights gained. Possible new avenues for therapy emerging from this enhanced insight into IPF will be discussed.

Pathogenesis

Although alveolar epithelial damage *per se* does not lead to IPF, notably the earliest recognizable event in IPF is pulmonary epithelial damage. The subsequent alveolitis and fibroblast proliferation eventually lead to extensive interstitial fibrosis. The reason, however, for the precipitating injury of alveolar cells, marking the onset of IPF, has remained obscure. A variety of risk factors have been suggested, including exposure to several exogenous factors, especially wood, metal dusts, smoking and some therapeutic drugs[4, 29–31, 61]. However, not all subjects equally exposed to these factors develop IPF. The relation, therefore, between these factors and disease initiation remains unclear.

Viral involvement

IPF is sometimes preceded by viral disease-like symptoms and a relationship between infection with Epstein-Barr virus (EBV) and IPF has been suggested since 1984[70]. More recently, this association was apparently corroborated by STEWART et al.[65], who showed that 11 out of 27 patients with IPF and none of the control subjects (total of 28) were EBV positive, both by immunohistochemistry and PCR. This observation, however, contrasts the results obtained by WANGOO et al.[73]. Investigations into possible adenovirus E1A involvement have shown no association with IPF[41]. A trial using the antiviral agent ribavarin in advanced cases of IPF yielded no benefits, suggesting there may be no involvement of viral factors in late-stage IPF[1]. Hence, it is still doubtful whether a viral infection is really a causative event in IPF.

Genetic predisposition

Several familial occurrences of IPF have been identified[5, 46, 66, 68], strongly suggesting that a genetic predisposition may be important in IPF development. In agreement, different inbred strains of mice show different susceptibilities to the fibrogenic agent bleomycin. Early genetic research was focused on polymorphisms in the MHC, but the results obtained were contradictory[43, 69] and this research was not continued. Technical possibilities for genetic research have been vastly improved in recent years and it is to be hoped that further research into the genetic basis of IPF will be performed.

Role for apoptosis

Recently, exciting advances have been made in unraveling the earliest recognizable change in IPF: the loss of alveolar epithelial cells, which seems to involve apoptosis. One of the key pathways leading to apoptosis is the Fas/FasL-pathway. Fas (CD95/Apo-1), a member of the TNF-receptor family, is expressed on a variety of cells and forms the receptor for FasL. Upon binding of FasL, a member of the TNF family, apoptosis is induced in the Fas-bearing cell. FasL is expressed predominantly on activated T lymphocytes and natural killer cells. Cytotoxic T cells, for example, exert their cytotoxic effect partly through inducing apoptosis via the Fas/FasL-pathway[45]. Apoptosis can also play a role in disease. Fas mutations have been reported to cause lymphoproliferative disorders with autoimmune manifestations[60, 62]. Excessive Fas activity, on the other hand, may cause tissue destruction: an intra-peritoneal injection of an anti-Fas antibody, which mimics Fas-FasL cross-linking, into adult mice caused severe liver damage by apoptosis[56].

Recently, Fas-induced apoptosis has been implicated in the pathogenesis of IPF. KUWANO et al.[39] demonstrated apoptosis in bronchiolar and alveolar epithelial cells from patients with IPF, using the TUNEL method. Furthermore, it was shown, by means of immunohistochemistry on lung-tissue samples from IPF patients, that the expression of Fas on bronchiolar and alveolar epithelial cells as well as the expression of FasL on infiltrating lymphocytes and granulocytes is

up-regulated in patients suffering from this disease[40]. Supporting data were obtained using the bleomycin model, a widely used and reproducible murine model for IPF. In this model, bleomycin induces pulmonary toxicity, including DNA damage consistent with apoptosis, leading to pulmonary fibrosis similar to that in humans[22]. Although extrapolation to the human situation must be done with care, it is fair to say this model has contributed to our insight into IPF. Using the murine bleomycin model, FasL mRNA was shown to be up-regulated in infiltrating lymphocytes and Fas was shown to be up-regulated on alveolar and bronchial epithelial cells, in which enhanced apoptosis was detected[21]. Furthermore, in mice that were repeatedly given inhalations of aerosolized anti-Fas antibody, excessive apoptosis and inflammation was observed, which resulted in pulmonary fibrosis[20]. Finally, it was demonstrated that mice deficient in Fas (*lpr* mutants) or FasL (*gld* mutants) were protected from bleomycin-induced pulmonary fibrosis and that with a soluble form of the Fas antigen or anti-FasL antibody similar protection was achieved[38]. Several confounding factors in these studies should be mentioned. As has been noted by CHAPMAN[8], Fas/FasL pathway disruption could lead to altered cytokine release by lymphocytes, possibly conferring protection from bleomycin-induced pulmonary fibrosis. Furthermore, protection from bleomycin-induced pulmonary fibrosis in the *lpr* and *gld* mutants may be an indirect consequence of the phenotype. An answer to this question could be obtained by performing experiments using Fas/FasL knock-in mice. Nonetheless, the reported observations provide strong evidence for the hypothesis that excessive FasL-Fas-mediated apoptosis of alveolar epithelial cells is crucial in IPF development.

Although Fas-mediated apoptosis appears to be a key event in the etiology of IPF, the identity of the events initiating this apoptotic reaction remains obscure. Several of the risk factors proposed for IPF, such as viruses, toxins (e.g. bleomycin) and aberrant MHC T cell receptor interaction, can initiate apoptosis. A genetic predisposition for aberrant epithelial cell apoptosis after exposure to an exogenous apoptosis-inducing agent seems a plausible causative mechanism for IPF, but, obviously, further research into this field, focussing on genetic abnormalities in the apoptosis elements involved, is required.

Apoptosis and inflammation

Apoptosis avoids the inflammation associated with necrotic cell death. In general, the Fas pathway is implicated in containing inflammatory reactions by inducing apoptosis of inflammatory cells, which would apparently be at odds with the observed inflammatory reaction during IPF. However, it has been reported that Fas ligation can induce proinflammatory cytokine release, for example of IL-8, indicating that the Fas pathway can also lead to inflammation[10, 19, 50]. In agreement Fas ligation in the lung in experimental rodents *in vivo* produces an inflammatory reaction[20].

T cell involvement

The role of T cells in the pathogenesis of IPF is unclear. IPF exhibits a Th2 cytokine pattern[71], characterised by IL-4 (suggested to be a fibrogenic cytokine[58]) and IL-5, and a relative deficiency of IFN-γ (an anti-fibrotic cytokine[18]) and Th1 inhibitors in the bleomycin model did not ameliorate fibrosis[36]. As we discussed, FasL-expressing, infiltrating T cells are implicated in the initiation of IPF[40]. However, *in vitro* apoptosis of alveolar epithelial-derived cells induced by $CD8^+$ T cells was reported to be FasL-independent and TNF-α-mediated[44]. Furthermore, SCID variants of bleomycin-susceptible mice strains showed no different development of pulmonary fibrosis than their wild-type controls, showing that T cells are not required in this respect[23], prompting alternative explanations as to the identity of the FasL-presenting cells responsible for epithelial apoptosis.

Inflammation

Although apoptosis seems essential for initiating IPF, the inflammatory reaction is involved in the actual development of fibrotic tissue. Hence, the inflammatory cell response, long considered to be the pivotal phase in the initiation of IPF, seems to be a later stage in the reaction to the alveolar damage. This alveolar damage may cause activation of resident macrophages and, in turn, an influx of granulocytes, but also monocytes and lymphocytes. In conjunction with the residential cells, these cells thus mobilized release, amongst others, the profibrotic TGF-β and TNF-α as well as the anti-fibrotic IFN-γ. The resultant effect of these persistent inflammatory signals on fibroblasts eventually leads to extracellular collagen deposition and fibrosis. We shall now discuss the role of different pro- and anti-fibrotic cytokines that may become interesting targets for therapy in IPF.

Fibrogenic cytokines

TGF-β is believed to play a crucial role in the deposition of extracellular matrix. TGF-β stimulates col-

lagen synthesis[59]. In experimental pulmonary fibrosis, predominantly the TGF-β_1 isoform is enhanced[12] and, also in human pulmonary fibrosis, TGF-β_1 gene and protein expression is enhanced[6]. Furthermore, a TGF-β antibody, the TGF-β binding proteoglycan decorin and a neutralising TGF-β soluble receptor, reduced bleomycin-induced pulmonary fibrosis in animal experiments[16, 17, 72]. Intracellular signal transduction of the TGF-β signal utilizes Smad proteins and, recently, Smad 7 has been identified as an intracellular antagonist of TGF-β signalling[54]. Gene transfer of Smad 7, using a viral vector, protected mice from bleomycin-induced pulmonary fibrosis[55] and may thus become interesting for anti-fibrotic gene therapy.

TNF-α may also be an interesting target for anti-cytokine therapy in IPF. ORTIZ et al.[57] showed that, in experimental pulmonary fibrosis, TNF mRNA levels in macrophages correlated with inflammation after exposure to bleomycin. Moreover, TNF-receptor knock-out mice were protected from the development of fibrosis in this model. Furthermore, TNF-α antibody reduced bleomycin-induced pulmonary fibrosis, as well as suppressing lung TGF-β_1 and IL-5 mRNA expression and lowering eosinophilia. In this study it was suggested that TNF-α is involved in IL-5-mediated recruitment of eosinophils which, in turn, release fibrinogenic cytokines[75]. Mice over-expressing TNF-α in pulmonary epithelium develop a pulmonary pathology with a striking resemblance to human idiopathic pulmonary fibrosis[51]. Hence, the role of TNF-α in IPF is undisputed and novel TNF inhibitors such as thalidomide may become important for treating IPF.

In addition to TGF-β and TNF-α, several other cytokines stimulate fibrosis in the lung. TGF-α has been shown to amplify the fibrotic response to lung injury. Pulmonary collagen accumulation and lung fibrosis were significantly lower after bleomycin injury in TGF--α-deficient mice than in wild-type mice. No difference in lung inflammation was observed between the two groups[48]. Furthermore, over-expression of TGF-α in pulmonary epithelial cells in transgenic mice resulted in severe pulmonary fibrosis[35]. Recently, ET-1 was suggested to play a role in the pathogenesis of IPF. Enhanced levels of ET-1 were reported in the lungs of patients with pulmonary fibrosis due to systemic sclerosis[7], and *in vitro* ET-1 has a profibrotic effect[14]. In bleomycin-induced pulmonary fibrosis, enhanced ET-1 levels were reported[52]. However, bosentan, an ET-1 receptor antagonist, could not prevent collagen deposition in experimental pulmonary fibrosis[53], and so the exact role of ET-1 in the pathogenesis of IPF is unclear.

Anti-fibrotic cytokines

As well as pro-fibrotic cytokines, anti-fibrotic cytokines have also been reported. In the healthy lung there is supposedly a balance between pro- and anti-fibrotic signals. In IPF this balance is apparently tilted towards collagen production[11] and, therefore, anti-fibrotic cytokines may become therapeutically important in IPF. One of the prime candidates for such anti-fibrotic cytokine therapy is IFN-γ: IFN-γ inhibits collagen production *in vitro*, and IFN-γ treatment of bleomycin-challenged mice reduces fibrosis by inhibiting TGF-β over-expression and subsequent collagen deposition[18]. In a preliminary study by ZIESCHE et al.[77], long-term treatment of IPF patients with IFN-γ-1b and low-dose prednisolone was reported to improve lung function considerably compared to treatment with prednisolone alone.

Chemokines

Chemotactic cytokines are also involved in pulmonary fibrosis, and not exclusively in the recruitment of inflammatory cells. G-CSF and IL-8 can regulate the influx of neutrophils. Both G-CSF and IL-8 levels were enhanced in the BAL fluid from IPF patients, correlating with the numbers of neutrophils in the BAL fluid[3, 76]. The number of neutrophils in the BAL fluid, in turn, shows an inverse correlation with the percentage forced vital capacity of patients with IPF[3]. MIP-1α antibodies reduce mononuclear phagocyte recruitment and attenuate bleomycin-induced pulmonary fibrosis in mice[63], apparently mediated by TNF-α and IL-6[64]. MIP-2, a chemokine with angiogenic properties, seems involved in neovascularisation in bleomycin-induced pulmonary fibrosis. MIP-2 antibodies attenuated angiogenesis and pulmonary fibrosis, while neutrophil influx and fibroblast proliferation were not affected[34]. Furthermore, TGF-β and procollagen production by fibroblasts from a murine Th2-type pulmonary fibrosis-model was MCP-1-dependent[24], further supporting an important role for chemokines in IPF.

Fibrinolysis

Enhancing alveolar fibrinolytic activity may become an interesting therapeutic avenue. A decreased fibrinolytic activity has been found in the BAL fluid of patients with IPF[9]. Urokinase-type plasminogen activator is one of the important mediators of fibrinolysis in the healthy lung and can be inhibited by PAI-1. In bleomycin-induced pulmonary fibrosis, the expression of

PAI-1 in genetically modified animals positively correlated with the extent of collagen accumulation in the lung[15].

Anti-Apoptotic Therapeutic Avenues

Current therapies aimed at interfering with the inflammatory component, such as corticosteroids (believed to exert their function by inducing apoptosis of inflammatory cells), have shown little objective effectiveness[47]. Currently, several new therapeutic strategies are being developed. Cytokine therapies, targeting IFN-γ, TGF-β or TNF-α have already been discussed. However, they interfere in a relatively late phase of the pathogenesis of fibrosis and targeting the apoptotic initiation may prove to be more effective. Captopril, an angiotensin-converting enzyme inhibitor, has been reported to inhibit collagen accumulation in radiated rat lungs[74] and was shown to inhibit Fas-induced apoptosis of human lung epithelial cells in culture[67]. p38 MAPK is an intracellular signaling pathway element important in transducing inflammatory signals and apoptosis[37, 42]. p38 MAPK inhibition impairs activation-induced T cell death and spontaneous apoptosis of human neutrophils[2, 26]. Moreover, expression of FasL was reported to be dependent on p38 MAPK activity[26] and p38 MAPK inhibitors, therefore, hold promise as new agents in IPF-treatment.

In conclusion, current evidence suggests that IPF is caused by Fas-dependent alveolar apoptosis with the resulting inflammatory reaction finally leading to interstitial fibrosis. At present, no satisfactory treatment is available, but as the molecular basis of both apoptosis and inflammation is rapidly being elucidated, novel therapeutic options are coming within reach.

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