Thalidomide reduces IL-18, IL-8 and TNF- α release from alveolar macrophages in interstitial lung disease

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ABSTRACT: Thalidomide exhibits diverse actions of anti-inflammation, immunomodulation and anti-angiogenesis. The efficacy of thalidomide treatment in sarcoidosis with lupus pernio is thought to be due to inhibition of tumour necrosis factor (TNF)- α . The mechanisms that underlie the properties of thalidomide are still unclear in interstitial lung disease.

The current authors investigated the potential inhibitory effects of thalidomide at concentrations of 0.1, 0.01 and 0.001 mM on the production of transforming growth factor- β , TNF- α , interleukin (IL)-1 β , IL-6, IL-8, IL-10, IL-12p70, IL-12p40 and IL-18 by alveolar macrophages from bronchoalveolar lavage in patients with sarcoidosis (n=8), hypersensitivity pneumonitis (HP; n=8) and idiopathic pulmonary fibrosis (IPF; n=12).

In sarcoidosis and HP patients, thalidomide induced a dose-dependent, partial suppression of lipopolysaccharide (LPS)-stimulated TNF- α , IL-12p40 and IL-18 release. At the highest thalidomide concentration (0.1 mM), LPS-stimulated IL-8 production was also suppressed. In IPF patients, although spontaneous production of TNF- α , IL-12p40, IL-18 and IL-8 was lower than in sarcoidosis and HP patients, with LPS stimulation the cytokines were significantly elevated and also partially inhibited by thalidomide.

In conclusion, thalidomide has the potential to improve the therapeutic regimens for sarcoidosis, hypersensitivity pneumonitis and idiopathic pulmonary fibrosis by reducing tumour necrosis factor- α , interleukin-12p40, interleukin-18 and interleukin-8 production.

KEYWORDS: Alveolar macrophage, cytokine, interstitial lung disease, thalidomide

Thalidomide, a derivative of glutamic acid, was synthesised originally as a sedative and an anti-emetic. Subsequently the drug was withdrawn because of its teratogenic effects [1]. However, thalidomide shows anti-inflammatory activities, such as blocking tumour necrosis factor (TNF)- α production by stimulated human peripheral blood mononuclear cells (PBMCs) [2] and by alveolar macrophages (AMs) [3, 4], and inhibiting interleukin (IL)-12 release [5]. Thalidomide also shows immunomodulatory properties, inhibiting the production of T helper cell type (Th)1 cytokines and inducing Th2 cytokine production by stimulated human PBMCs [6–8]. Thalidomide was found effective first in erythema nodosum leprosum lesions [9], and later in rheumatological diseases [10], Crohn's disease [11], tuberculosis [12], cutaneous sarcoidosis [13] and lung transplantation [14]. In addition, the ability of thalidomide to inhibit angiogenesis has led to its use in malignancy, particularly multiple myeloma [15].

Sarcoidosis and hypersensitivity pneumonitis (HP) are granulomatous diseases usually classified as a Th1 response [16, 17]. IL-12, IL-18 and TNF- α , possibly playing a central role in granuloma formation, may represent a target for therapeutic interventions [18–20]. Thalidomide has shown a beneficial effect in chronic cutaneous sarcoidosis [13]. Lupus pernio is a unique disfiguring skin involvement of the face characteristic of sarcoidosis [16]. Skin lesions persisting despite corticosteroid therapy have been effectively controlled by thalidomide [13].

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease of unknown cause, characterised by epithelial injury and fibroblast proliferation [21]. In IPF, TNF- α plays a role as a cytokine that bridges inflammation, repair responses and fibrosis and may be involved in extracellular matrix remodelling. Recent reports have shown that in IPF patients angiogenic cytokines are elevated and capillary density is increased in the least fibrotic lung regions [22,

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23], suggesting that the cytokine-inhibitory effect and anti-angiogenic activity of thalidomide might be beneficial in IPF.

Previous studies have shown that thalidomide can inhibit the production of inflammatory cytokines such as TNF- α and IL-12 from PBMCs and AMs [2–5]. However, the mechanisms underlying the effects of thalidomide in interstitial lung disease are still unclear. The current authors set out to investigate further potential inhibitory effects of thalidomide on a panel of cytokines (transforming growth factor (TGF)- β 1, TNF- α , IL-1 β , IL-6, IL-8, IL-10, IL-12p70, IL-12p40 and IL-18) from AMs recovered by bronchoalveolar lavage (BAL) in patients with sarcoidosis, HP or IPF.

MATERIALS AND METHODS

Study population

Consecutive patients with active pulmonary sarcoidosis (n=8), chronic HP (n=8), or IPF (n=12) were investigated (table 1). No patient was receiving corticosteroid and/or immunosuppressant treatment at the time of BAL. Written informed consent was obtained according to institutional guidelines.

All sarcoidosis patients were diagnosed on the basis of compatible clinical and radiographic features, histological evidence of noncaseating granuloma on transbronchial biopsy specimens or an increased CD4/CD8 ratio in BAL fluid, and the exclusion of other granulomatous lung diseases [16]. The criteria of disease activity were as follows: 1) recently developed symptoms or increasing symptoms such as cough, dyspnoea, weakness, fever, and arthralgia; and/or 2) chest radiographic evidence of progressive disease; and/or 3) deterioration of lung function. According to chest radiographic staging, two patients had stage I disease, five patients had stage II disease and one patient had stage III disease.

The eight HP patients fulfilled the following diagnostic criteria: 1) a history of exposure to organic antigens; 2) clinical signs and symptoms consistent with HP; 3) radiologic features and/or functional abnormalities characteristic of interstitial lung

disease; 4) evidence of serum precipitins against one or more organic antigen; and 5) increased lymphocytes in BAL fluid. All eight patients presented with the chronic form of insidious onset HP. On high-resolution computed tomography (HRCT), they all showed widespread and dominant ground-glass densities, with only minor reticulation and no honeycombing. Late-stage cases with extensive fibrosis were not investigated in this study. Four HP patients were budgerigar fanciers, three were pigeon breeders and one had humidifier's lung.

A total of 12 IPF patients were diagnosed according to American Thoracic Society/European Respiratory Society criteria, including the HRCT characteristics of IPF [24]. In three of the patients, a surgical biopsy showed histological evidence of usual interstitial pneumonia. No patients had left ventricular cardiac failure or a history of chronic pulmonary infections.

BAL procedure

BAL was performed *via* a fiberoptic bronchoscope. Sterile isotonic saline solution was instilled into the right middle or left lingular lobe in 10 $\times$ 20 mL aliquots to a total volume of 200 mL, with immediate aspiration by gentle suction after the instillation of each aliquot. A volume of >50% was retrieved. The recovered BAL fluid was filtered through two layers of sterile gauze and subsequently centrifuged at 500 $\times$ g for 10 min at 4°C. The cells were counted in a haemocytometer. Cell viability was assessed by Trypan blue exclusion. Cell differentials were made on smears stained with May–Grünwald–Giemsa, by counting 600 cells. Immunocytochemical staining was performed to obtain a CD4/CD8 count.

Cell culture

AM cultures were performed as previously described [20]. After the cell pellet was washed three times with PBS, the BAL cells were resuspended to a final concentration of 1 $\times$ 10⁶ cells·mL⁻¹ in RPMI 1640 medium supplemented with 10% heat-inactivated foetal calf serum, 2 mM L-glutamine, 200 U·mL⁻¹ penicillin and 200 mg·mL⁻¹ streptomycin (Seromed; Biochrom KG, Berlin, Germany). The cell suspension was added at 1 $\times$ 10⁶ cells·well⁻¹ to a 24-well plastic tissue culture plate (Falcon; Becton Dickinson, Franklin Lakes, NJ, USA) and was incubated at 37°C in a 5% CO₂ humidified atmosphere for 1 h to permit the adherence of AMs. Nonadherent cells were removed by three washes with RPMI 1640 medium. The purity of adherent AMs was measured at >95% by morphology and nonspecific esterase staining. The purified AMs were incubated for an additional 24 h with either: 1 mL RPMI 1640 medium alone; 1 mL RPMI 1640 medium and 100 ng·mL⁻¹ lipopolysaccharide (LPS; Sigma-Aldrich, St Louis, MO, USA); or 1 mL RPMI 1640 in the absence and presence of 100 ng·mL⁻¹ LPS together with thalidomide (ICN Biomedicals, Solon, OH, USA) at concentrations of 0.001, 0.01 and 0.1 mM (0.25, 2.5 and 25 μ g·mL⁻¹, respectively). The culture supernatants were harvested and centrifuged, before being stored in fractions at -80°C until analysis.

ELISA assays for TGF- β , TNF- α and interleukins

The concentrations of TGF- β , TNF- α , IL-1 β , IL-6, IL-8, IL-10, IL-12p70, IL-12p40 and IL-18 in cultured supernatants were

TABLE 1 Demographics of patients

	Sarcoidosis or HP	IPF
Subjects (female/male)	16 (9/7)	12 (3/9)
Age yrs	55 $\pm$ 3	70 $\pm$ 2**
Never-smokers	11	5
Current smokers	0	0
Ex-smokers	5	7
VC % pred	73 $\pm$ 4	78 $\pm$ 4
TLC % pred	76 $\pm$ 4	67 $\pm$ 3
FEV₁/VC %	76 $\pm$ 2	76 $\pm$ 3
P_aO₂ mmHg	71 $\pm$ 3	73 $\pm$ 3
A_aDO₂ mmHg	30 $\pm$ 3	28 $\pm$ 3

Data are presented as n or mean $\pm$ SEM. HP: hypersensitivity pneumonitis; IPF: idiopathic pulmonary fibrosis; VC: vital capacity; % pred: percentage of predicted value; TLC: total lung capacity; FEV₁: forced expiratory volume in one second; P_aO₂: arterial oxygen tension; A_aDO₂: alveolo-arterial oxygen difference. **: p<0.01 (sarcoidosis and HP versus IPF). 1 mmHg=0.133 kPa.

quantified using commercially available human ELISA kits (Pierce Biotechnology, Rockford, IL, USA; R&D Systems Inc, Minneapolis, MN, USA; MBL, Nagoya, Japan) with sensitivities (in $\text{pg}\cdot\text{mL}^{-1}$) of 1.9, 2, 1, 1, 2, 3, 0.5, 15 and 12.5 respectively. All the ELISA kits used were specific for the measurement of natural and recombinant human specific cytokines without cross-reactivity with other cytokines. Cytokine concentrations were expressed in $\text{pg}\cdot\text{mL}^{-1}\cdot 10^6\text{ AMs}^{-1}$ or $\text{ng}\cdot\text{mL}^{-1}\cdot 10^6\text{ AMs}^{-1}$ after correction for the proportion of AMs.

Statistical analysis

Data are presented as mean $\pm$ SEM. Within each group, the data were analysed using Kruskal–Wallis one-way ANOVA on ranks. A p -value <0.05 was regarded as statistically significant.

Spontaneous IL-12p40 production was very low and hardly detectable by the ELISA assay in all patients (data not shown).

RESULTS

Effects of thalidomide on spontaneous and LPS-stimulated cytokine production from AMs in patients with sarcoidosis or HP

Since the number of BAL samples with sufficient cells for AM culture was small, patients with sarcoidosis ($n=8$) and HP ($n=8$) were grouped together as patients with granulomatous lung disorders to allow statistically meaningful comparisons.

As shown in table 2, the spontaneous production of cytokines was not significantly inhibited by thalidomide at any tested concentration (all $p>0.05$). LPS-stimulated production of all cytokines except TGF- β and IL-12p70 was significantly higher than spontaneous cytokine release (table 3; $p<0.05$ and $p<0.01$, variously). Thalidomide produced a dose-dependent suppression of LPS-stimulated TNF- α , IL-12p40 and IL-18 release ($p<0.05$ and $p<0.01$, variously). At the highest thalidomide concentration studied (0.1 mM), LPS-stimulated TNF- α , IL-8, IL-12p40 and IL-18 production was reduced by 42,

FIGURE 1. The effect of thalidomide (Thali.) on lipopolysaccharide (LPS)-stimulated cytokine production in alveolar macrophages (AMs) from patients with sarcoidosis or hypersensitivity pneumonitis. a) Tumour necrosis factor (TNF)- α , b) interleukin (IL)-8, c) IL-12p40, and d) IL-18. Data are presented as mean $\pm$ SEM. *: $p<0.05$ versus spontaneous cytokine production; **: $p<0.01$ versus spontaneous cytokine production; #: $p<0.05$ versus LPS-stimulated cytokine production; ##: $p<0.01$ versus LPS-stimulated cytokine production.

FIGURE 2. The effect of thalidomide (Thali.) on lipopolysaccharide (LPS)-stimulated cytokine production in alveolar macrophages (AMs) from patients with idiopathic pulmonary fibrosis. a) Tumour necrosis factor (TNF)- α , b) interleukin (IL)-8, c) IL-12p40, and d) IL-18. Data are presented as mean+SEM. **: $p < 0.01$ versus spontaneous cytokine production; #: $p < 0.01$ versus LPS-stimulated cytokine production.

21, 52 and 39%, respectively, in comparison with LPS-stimulated baseline production (fig. 1; all $p < 0.05$).

Effects of thalidomide on spontaneous and LPS-stimulated cytokine production from AMs in patients with IPF

As shown in table 3, thalidomide at all tested concentrations did not show significant inhibition of spontaneous cytokine production in patients with IPF (all $p > 0.05$). Although spontaneous production of TNF- α , IL-18 and IL-8 was lower in IPF patients than in sarcoidosis or HP patients, with LPS treatment, cytokine levels were significantly elevated in a manner that was inhibited by thalidomide. Thalidomide at the highest concentration tested (0.1 mM) reduced LPS-stimulated TNF- α , IL-8, IL-12p40 and IL-18 production by 38, 26, 64 and 32%, respectively, in comparison with LPS-stimulated baseline production (fig. 2; all $p < 0.01$).

DISCUSSION

The present study demonstrates that thalidomide can induce a dose-dependent partial suppression of LPS-stimulated TNF- α ,

IL-12p40 and IL-18 release by AMs *in vitro*. At the highest concentration studied, thalidomide also reduced the LPS-stimulated release of IL-8 from the AMs of patients with sarcoidosis, HP or IPF. The inhibitory effect of thalidomide on TNF- α in the current study is corroborated by other research. Thalidomide has been found to inhibit both the production and mRNA expression of TNF- α from LPS-stimulated human monocytes and mouse macrophages *in vitro* by enhancing the degradation of TNF- α mRNA [3, 4].

The present study is the first to report an inhibition of AM IL-18 production by thalidomide in interstitial lung diseases. IL-18 is a member of the IL-1 cytokine family [25]. It functions as a pro-inflammatory cytokine with potent interferon (IFN)- γ -inducing activity. IL-18 can cause a rapid activation of the monocyte/macrophage system, up-regulating these cells' innate immune capabilities and promoting the differentiation of T-cells to the Th1 subset [25]. IL-18 plays a crucial role in sarcoidosis and HP, which are Th1 diseases [19, 20]; sarcoidosis patients show significantly elevated levels of

TABLE 2 The effect of thalidomide on spontaneous cytokine release from alveolar macrophages (AMs) in patients with interstitial lung diseases

	Spontaneous	Thalidomide mM		
		0.001	0.01	0.1
Sarcoidosis or HP				
TGF-β1 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	40±5	39±4 (98)	40±5 (100)	38±5 (95)
TNF-α pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	692±298	472±154 (68)	477±165 (69)	411±135 (60)
IL-1β pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	41±18	42±21 (102)	47±27 (114)	37±22 (90)
IL-6 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	924±402	776±407 (84)	893±442 (97)	781±379 (85)
IL-8 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	111±20	114±23 (103)	112±25 (101)	94±20 (85)
IL-10 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	33±13	18±6 (55)	20±7 (61)	17±6 (52)
IL-12p70 pg·mL ⁻¹ ·(10 ⁶ AMs) ⁻¹	14±8	13±8 (93)	13±8 (93)	9±4 (64)
IL-18 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	182±44	112±23 (62)	103±21 (57)	97±17 (53)
IPF				
TGF-β1 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	9.5±1.9	10.6±1.6 (111)	10.6±1.8 (111)	9.6±1.7 (101)
TNF-α pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	123±33	102±26 (83)	100±28 (81)	93±26 (76)
IL-1β pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	6±2	4.5±1.4 (75)	5±1.8 (83)	5.1±2 (85)
IL-6 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	219±107	160±75 (73)	203±104 (93)	254±153 (112)
IL-8 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	24.9±3.6	20.6±3.1 (83)	20.5±3.6 (82)	19.4±3.4 (78)
IL-10 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	3.9±1.8	1.9±0.8 (49)	1.9±0.9 (49)	1.6±0.8 (41)
IL-12p70 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	1.0±0.2	0.3±0.1 (30)	0.2±0.07 (20)	0.3±0.1 (30)
IL-18 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	42.4±6.7	30.3±6.5 (71)	26.2±7.3 (62)	39.1±9.4 (92)

Data are presented as mean ± SEM (% of spontaneous production). HP: hypersensitivity pneumonitis; TGF: transforming growth factor; TNF: tumour necrosis factor; IL: interleukin; IPF: idiopathic pulmonary fibrosis.

IL-18 in BAL fluid compared with healthy subjects [20]. IL-18 has been found to be increased in the airway epithelial cells of sarcoidosis patients [26]. A previous study showed that the production of IL-18 by AMs is significantly elevated in patients with HP in the absence or presence of LPS stimulation [20]. IL-18, TNF- α and other cytokines are implicated in orchestrating inflammatory cell accumulation, granuloma formation and fibrogenesis in granulomatous diseases including sarcoidosis and HP [20, 26, 27]. The therapeutic action of thalidomide in cutaneous sarcoidosis is probably mediated by down-regulation of TNF- α , IL-12, IL-18 and possibly other cytokines [28, 29]. In a mouse model of allergic asthma, Th2 cytokine (IL-4 and IL-5) production, as well as an increase in immunoglobulin E levels, was promoted by intrapulmonary administration of IL-18 [30], indicating that IL-18 may contribute to the pathogenesis of allergic asthma. However, the role of IL-18 in Th2 diseases such as pulmonary fibrosis is still unclear.

IL-12 is a 70 kDa (p70) heterodimeric cytokine comprising a 40 kDa (p40) subunit and a 35 kDa (p35) subunit linked by a disulfide bond. IL-12p70 activity shifts T-cell reactivity toward a Th1 immune response [31]. In the present study, thalidomide did not inhibit the production of IL-12p70 from AMs. The spontaneous release of IL-12p70 from the AMs of patients with interstitial lung disease was relatively low, and LPS stimulated IL-12p70 release only poorly, in agreement with published data [5]. The current study confirmed the inhibitory effect of thalidomide on LPS-stimulated IL-12p40 production shown by MOLLER *et al.* [5] in PBMCs from healthy controls.

Likewise, a dose-dependent reduction in IL-6 production caused by thalidomide has been reported in experimental murine tuberculosis, as an animal model of the pulmonary granulomatous response [32]. However, two other reports did not show an effect of thalidomide on IL-6 [2, 5]. Similarly, thalidomide failed to inhibit IL-6 production by human AM from interstitial lung disease patients in the current study, suggesting different modulating effects of thalidomide in different experimental conditions and clinical situations. In addition, the current authors have shown that LPS-stimulated IL-8 production by AMs is partially inhibited by thalidomide at a concentration of 0.1 mM, in both IPF patients and patients with granulomatous diseases. This result is supported by another study using different cells [33], in which thalidomide reduced TNF- α -induced IL-8 protein production, as well as mRNA expression, in endometrial stromal cells *in vitro*, possibly by reducing nuclear factor- κ B activation.

In IPF, TNF- α is associated with pulmonary inflammation and/or fibrosis and is present in the lungs of patients with usual interstitial pneumonia, possibly leading to up-regulation and over-expression of other fibrogenic cytokines. In TNF- α transgenic mice, histopathology of the lung has revealed changes similar to those seen in IPF [34]. Recently, anti-TNF- α -directed therapy has been suggested as possible treatment for IPF.

IPF is characterised by epithelial injury, fibroproliferation and excessive extracellular matrix deposition [21]. There is also growing evidence in support of the concept that angiogenesis supports fibroblast proliferation and deposition of extracellular

TABLE 3 The effect of thalidomide on lipopolysaccharide (LPS)-stimulated cytokine release from alveolar macrophages (AMs) in patients with interstitial lung diseases

	Spontaneous	LPS	Thalidomide mM		
			0.001	0.01	0.1
Sarcoidosis or HP					
TGF-β1 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	40 ± 5	39 ± 5	38 ± 4 (97)	40 ± 4 (102)	41 ± 5 (105)
TNF-α pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	692 ± 298	12108 ± 2989**	9894 ± 2848 (82) ^{##}	8451 ± 2317 (70) ^{##}	7115 ± 1657 (58) [#]
IL-1β pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	41 ± 18	311 ± 72*	339 ± 80 (109)	346 ± 103 (111)	241 ± 68 (77)
IL-6 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	924 ± 402	22443 ± 5839**	22913 ± 677 5 (102)	22870 ± 6109 (102)	21932 ± 6361 (98)
IL-8 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	111 ± 20	633 ± 158**	572 ± 130 (90)	562 ± 148 (89)	501 ± 119 (79) [#]
IL-10 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	33 ± 13	489 ± 113*	404 ± 90 (83)	447 ± 114 (91)	366 ± 73 (75)
IL-12p70 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	14 ± 8	23 ± 9	18 ± 10 (78)	20 ± 9 (87)	19 ± 11 (83)
IL-12p40 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹		2223 ± 1127	1345 ± 720 (61) [#]	1168 ± 598 (53) [#]	1060 ± 624 (48) [#]
IL-18 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	182 ± 44	343 ± 65*	249 ± 45 (73) [#]	235 ± 38 (68) [#]	210 ± 35 (61) [#]
IPF					
TGF-β1 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	9.5 ± 1.9	10.6 ± 1.7(100)	10.1 ± 1.6 (95)	7.9 ± 2.0 (75)	9.2 ± 1.8 (87)
TNF-α pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	123 ± 33	5606 ± 1222**	3931 ± 1036 (70) ^{##}	3834 ± 1143 (68) ^{##}	3508 ± 993 (62) ^{##}
IL-1β pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	6 ± 2	86 ± 13*	84 ± 14 (98)	79 ± 9 (92)	74 ± 14 (86)
IL-6 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	219 ± 107	17121 ± 4214*	16496 ± 3829 (96)	15059 ± 4110 (88)	15860 ± 4890 (92)
IL-8 ng·mL ⁻¹ ·10 ⁶ AMs ⁻¹	24.9 ± 3.6	106 ± 14**	116 ± 19(109)	80 ± 16 (75) ^{##}	78 ± 15 (74) ^{##}
IL-10 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	3.9 ± 1.8	130 ± 29**	139 ± 44 (107)	151 ± 45 (116)	143 ± 41 (110)
IL-12p70 pg·mL ⁻¹ ·(10 ⁶ AMs) ⁻¹	1.0 ± 0.2	4.0 ± 2.1	1.4 ± 0.6 (35)	1.2 ± 0.6 (30)	1.2 ± 0.6 (30)
IL-12p40 pg·mL ⁻¹ ·(10 ⁶ AMs) ⁻¹		1037 ± 291	482 ± 160 (46) ^{##}	378 ± 133 (38) ^{##}	374 ± 137 (36) ^{##}
IL-18 pg·mL ⁻¹ ·10 ⁶ AMs ⁻¹	42.4 ± 6.7	112 ± 12**	79 ± 11 (71) ^{##}	77 ± 8.7 (69) ^{##}	76 ± 10 (68) ^{##}

Data are presented as mean ± SEM (% of LPS-stimulated production). HP: hypersensitivity pneumonitis; TGF: transforming growth factor; TNF: tumour necrosis factor; IL: interleukin; IPF: idiopathic pulmonary fibrosis. *: p<0.05 versus spontaneous cytokine production; **: p<0.01 versus spontaneous cytokine production; #: p<0.05 versus LPS-stimulated cytokine production; ##: p<0.01 versus LPS-stimulated cytokine production.

matrix in the lungs during the repair process. Evidence of neovascularisation has been identified both in animal models of lung fibrosis and in patients with IPF [22, 23, 34, 35]. SIMLER *et al.* [36] reported that patients with idiopathic interstitial pneumonia had significantly higher plasma concentrations of angiogenic cytokines including IL-8 and endothelin-1. These results strengthen the hypothesis that angiogenesis is involved in the fibrotic pathway. Along these lines, thalidomide-induced IL-8 inhibition, as shown in the present study, may be beneficial in patients with lung fibrosis. Thalidomide has already shown potent anti-angiogenic properties in malignant diseases, especially in multiple myeloma [15]. The thalidomide-mediated inhibitions of immune responses and of angiogenesis are probably inter-related: some affected cytokines, such as TNF- α and IL-8, function in both processes. Therefore, thalidomide may be a promising drug in the treatment of IPF, acting through at least two mechanisms that include TNF- α inhibition and anti-angiogenic activity.

Increased IFN- γ levels are characteristic of both sarcoidosis and HP [16, 37]. Different studies have yielded divergent results of the effect of thalidomide on IFN- γ . A placebo-controlled pilot study of thalidomide in patients with active tuberculosis showed that TNF- α production was significantly reduced during thalidomide treatment, while IFN- γ production was enhanced [12]. A single oral dose of thalidomide

given to six healthy volunteers induced an increase in the capacity of PBMCs to secrete the Th1 cytokine IFN- γ upon stimulation, while their ability to release the Th2 cytokine IL-5 decreased [6]. However, in experimental murine tuberculosis, thalidomide treatment (30 mg·kg⁻¹) resulted in a significant reduction in TNF- α , IL-6 and IL-10 production in blood, and mRNA expression in lungs, while IL-12 and IFN- γ were unaffected [32]. In further contrast to these findings, thalidomide has been shown to suppress the production of IL-12 and IFN- γ and to enhance the production of IL-4 and IL-5 by stimulated PBMCs [5, 7, 8], indicating that thalidomide is able to switch a Th1 to a Th2 response, which may be favourable in sarcoidosis and HP.

A limitation of the present study is that a significant inhibition by thalidomide was found only for the LPS-induced, not the spontaneous, release of cytokines, although the spontaneous release of TNF- α , IL-8 and IL-18 tended to be reduced by thalidomide as well. The functional significance of blocking only LPS-induced cytokine release in patients with interstitial lung disease remains unclear.

In conclusion, the results of the current study suggest that thalidomide inhibits the production of tumour necrosis factor- α , interleukin-12p40, interleukin-18 and interleukin-8 by alveolar macrophages in interstitial lung disease. Thalidomide has potential as a drug to improve the therapeutic

regimens for sarcoidosis, hypersensitivity pneumonitis and idiopathic pulmonary fibrosis by reducing production of these cytokines, but its clinical utility is likely to be limited, owing to its unfavourable side-effect profile. Further research is warranted to validate the clinical value of thalidomide, and its analogues with lower toxicities, in the treatment of interstitial lung diseases.

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